A CASE OF MALE PSEUDOHERMAPHRODITISM
ASSOCIATED WITH ELEVATED LH,
NORMAL FSH AND LOW TESTOSTERONE POSSIBLY DUE TO
THE SECRETION OF AN ABNORMAL LH MOLECULE

By

I. James Park, Lonnie S. Burnett,
Howard W. Jones, Jr., Claude J. Migeon and Robert M. Blizzard

ABSTRACT

A 27 year old female is described who had 46,XY chromosome complement, ambiguous external genitalia with elevated LH, slightly above normal FSH and low testosterone. Her plasma testosterone level increased 15–20 fold after HCG stimulation (5000 IU × 3), then returned to pre-stimulation level 3 months later. This was possibly due to the secretion of an abnormal LH molecule which is immunoreactive but biologically inactive in the human.

Abnormal external genitalia in karyotypic males with testes is of multiple aetiology (Saez et al. 1971; Keenan et al. 1974; New & Suvannakul 1970; Parks et al. 1971). The purpose of this paper is to describe a hitherto unreported form of male pseudohermaphroditism which may have resulted from the production of immunologically reactive, but biologically inactive gonadotrophins in the human.

METHODS

The levels of serum leutinizing hormone (LH) were determined by a modification (Rodbard et al. 1968; Johanson et al. 1969) of the double antibody radioimmunoassay method (Midgley 1966). Methodological details, including the use of logit transformation,
have been previously reported (Johanson et al. 1969). All values were determined in triplicate, and the standard was the 2nd International Reference preparation of human menopausal gonadotrophin (2nd IRP-HMG). Inter-assay variability ranged from ± 8% at the level of 40 mIU/ml to 12% at the level of 2.5 mIU/ml of serum.

The levels of serum follicle stimulating hormone (FSH) were determined by a modification (Raitt et al. 1969) of the technique of Midgley (1967). All values were determined in triplicate and the standard was the 2nd International Reference preparation of human menopausal gonadotrophin (2nd IRP-HMG). Inter-assay variability range from 9.1% at the level of 35 mIU/ml to 18% at the level 7.1 mIU/ml serum.

On a subsequent date, LH and FSH levels were repeated on plasma samples which had been stored frozen. Although the assay technique was similar, the standard used was LER-907. The equivalence in our laboratory has been: for LH 1 mIU (2nd IRP-HMG) = 5.4 ng (LER-907) and for FSH 1 mIU (2nd IRP-HMG) = 21 ng (LER-907).

The methods used for the determination of urinary steroids are as follows: 17-ketosteroids (17-KS) by modification of the method of Callow et al. (1938), 17-hydroxycorticosteroid (17-OHCS) by modification of the Glenn-Nelson method (Glenn & Nelson 1953), pregnanediol by the technique of Kloppper et al. (1955), pregnanetriol by modification of the technique of Bongiovanni & Eberlein (1958), oestrogen fractionation by the method of Brown et al. (1957).

The urinary gonadotrophins were measured by bioassay using the uterine weight of the immature mouse as end point. The technique used was a modification of that of Klinefelter et al. (1943). In our laboratory, gonadotrophins of normal adult males can be positive up to 25 mouse units/24 h. It must be noted that the kaolin extraction of our method results in the loss of a fraction of urinary LH, so that the assay measures mainly FSH activity.

Plasma testosterone determinations were made using a radioimmunoassay (de Larcerda et al. 1973) which was a variation of the technique of Furuyama et al. (1970). Androstenedione was also measured by radioimmunoassay using antibodies prepared against androstenedione-3-oxime.

**CASE REPORT AND RESULTS**

A 27 year old white female presented because of amenorrhoea and absent vagina. At birth the abnormality of the genitalia was not noted and the child was raised as a girl. Axillary and pubic hair developed at 13 years of age. Breast development was absent and no menstruation occurred. At age 16 years she was treated with Enovid cyclically for 6 months without effect on menses but with small breast development.

From the family history it was learned that there was no consanguinity, no similar disorder in the family, no family history of infertility or amenorrhoea, and that there was one normal brother and one normal sister. In 1966 at 22 years of age an augmentation mammoplasty was undertaken. Unfortunately, infection occurred, drainage was necessary and transverse lateral scars resulted. She has been living as a female and no hormonal treatment has been taken since the age of 16 years.

On physical examination her height was 170 cm, her weight 57.7 kg and the
blood pressure 110/80. Except for the abnormalities related to the breasts and the genitalia, the entire physical examination was normal. The presence of minimal breast tissue was noted probably as a result of mammoplasty. The pattern of pubic hair was of female type. The external genitalia were moderately ambiguous (Fig. 1). The clitoris was enlarged to $3 \times 1$ cm and consisted primarily of a fleshy prepuce. The corpora measured $1 \times 0.5$ cm. The urethra opened under the clitoris and was normal for a female. This was separated from the genital pouch which was blind and 3 to 4 cm in depth. The labioscrotal folds were almost completely fused, although the urethra could be envisioned at the anterior portion of the fusion. By rectal examination no uterus was palpable. In the right inguinal area a gonad was felt. No left gonad was palpable.

On the basis of the evidence obtained by the physical examination and prior to receiving the laboratory data, the patient was thought to present with the syndrome of androgen insensitivity. For this reason, she was placed on a research protocol aimed at the determination of the response of the nitrogen balance to androgen administration. After 10 days of constant diet, 100 mg of testosterone propionate was given im for 5 days. After another 5 day control period, 50 mg of dihydrotestosterone was given im for 5 days. Following a third control period of 5 days, 5000 IU of HCG (follutein) was given for 3 days.

Although the results of the nitrogen balance were inconclusive, they suggested
Table 1.
Endocrine laboratory determinations.

<table>
<thead>
<tr>
<th></th>
<th>07-28-71</th>
<th>08-08-71</th>
<th>08-18-71</th>
<th>08-28-71</th>
<th>08-31-71</th>
<th>11-26-71</th>
<th>11-27-71</th>
<th>01-16-72</th>
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<tbody>
<tr>
<td>Urinary 17-KS (mg/24 h)</td>
<td>4.2</td>
<td>28.9</td>
<td>17.6</td>
<td>14.4</td>
<td>18.5</td>
<td>6.1</td>
<td>5.5</td>
<td></td>
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<tr>
<td>Total urinary oestrogen (mg/24 h)</td>
<td>24</td>
<td></td>
<td>26</td>
<td>34</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary gonadotrophins (mouse U/24 h)</td>
<td>50 Pos.</td>
<td></td>
<td>50 Pos.</td>
<td></td>
<td>25 Pos.</td>
<td>50 Pos.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum LH* (mIU/ml)</td>
<td>43</td>
<td></td>
<td>&gt;66</td>
<td>43</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Serum FSH* (mIU/ml)</td>
<td>247</td>
<td></td>
<td>916</td>
<td>398</td>
<td>406</td>
<td>350</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma T (ng/100 ml)</td>
<td>18</td>
<td></td>
<td>218</td>
<td>211</td>
<td>315</td>
<td>295</td>
<td>290</td>
<td></td>
</tr>
<tr>
<td>Plasma Δ (ng/100 ml)</td>
<td>218</td>
<td></td>
<td>789</td>
<td>836</td>
<td>44 (PM)</td>
<td>33 (AM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>116</td>
<td>169</td>
<td></td>
<td>210</td>
<td></td>
<td>143</td>
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Normal values in adult males: Urinary 17-KS: 6 to 18 mg/24 h. Urinary 17-OHCS: 3 to 9 mg/24 h. Urinary pregnanediol: 0.5 to 2.0 mg/24 h. Urinary pregnanetriol: < 2.5 mg/24 h.
Total urinary oestrogens – 5 to 20 mg/24 h.
Urinary gonadotrophins: Positive up to 25 mouse U, but negative for 50 mouse U/24 h.
Serum LH: 10.9 ± 4.0 (sd) mIU/ml; 44 to 96 ng/ml.
Serum FSH: 7.4 ± 1.9 (sd) mIU/ml; 100 to 275 ng/ml.
Plasma T: 575 ± 150 (sd) ng/100 ml.
Plasma Δ: 109 ± 20 (sd) ng/100 ml.
a positive balance and body weight went from 57.7 kg prior to the study to 60.5 kg at the end of the protocol, a gain of 2.8 kg over a 20 day period.

Following this experimental protocol, a deepening of the voice was noted approximately one week after the last injection of HCG. Increasing pubic hair allegedly occurred during this time which extended to the umbilicus. Hair also appeared on the upper lip and chin and acne was difficult to control. This virilization lasted several weeks after the HCG was discontinued and then gradually regressed. Re-examination two months later revealed that the voice was somewhat deeper than before the balance studies and that both gonads were now palpable in the inguinal areas. The buccal smear was chromatin negative and the chromosome complement was 46,XY. At the distal part of the long arm of the Y chromosome fluorescense was demonstrated using quinacrine mustard.

At laparoscopy no uterus or Fallopian tubes were demonstrated. A fold of peritonium grew across both pelves. In January 1972 a gonadectomy was performed, both gonads being removed from the inguinal areas.

The endocrine laboratory determinations are presented in Table 1. The basal urinary 17-ketosteroids were at early adolescent levels. After three days of human chorionic gonadotrophin administration, the 17-ketosteroid excretion rose to 18.5 mg/24 h. Plasma testosterone was 53 ng/100 ml before stimulation with HCG and rose to 789 and 836 ng/100 ml at the end of HCG stimulation. After discontinuing HCG, but before gonadectomy, the testosterone values were 44 and 33 ng/100 ml. The urinary excretions of 17-hydroxycorticosteroid, pregnanediol and pregnanetriol were in the normal range and did not change significantly under HCG stimulation. Prior to the administration of HCG the total urinary gonadotrophin determination done by the mouse uterine weight method revealed a positive test at 50 mouse units and a negative test at 100 mouse units. These determinations are at the upper limit of normal for adult males and females. During human chorionic gonadotrophin stimulation the total urinary gonadotrophins were positive at 100 mouse units, the highest level tested. Determinations done 3 and 5 months following HCG administration showed positive tests at 25 and 50 mouse units, respectively. Serum LH values were at menopausal levels before HCG was administered and serum FSH was in the high normal female range (Table 1). The day after discontinuing HCG the serum LH was further elevated without change in serum FSH (Table 1). Three months after stopping HCG and while the gonads were still intact, serum LH levels had returned to pre-HCG values. It must be noted that good parallelism of LH and FSH from the patient’s plasma and from the LER-907 standard was observed. The total urinary oestrogen determinations were at the normal adult female range for the luteal phase but higher than normal male levels before, during, and after HCG administration. The HCG stimulation did not increase oestrogen production to the level seen in subjects with normal testes (Weinstein et al. 1974).
Pathology report (Fig. 2)

The gonads measured 3.5 x 2 x 1.2 and 3 x 1.5 x 1 cm. At one end there was a crescent-like structure which resembled an epididymis.

Microscopically, there were abundant seminiferous tubules with a basement membrane which was thickened in some areas and normal appearing in others. There were some germ cells present but no spermatocytes were seen. Sertoli cells were present and appeared to be normal in number and morphology. There were scanty interstitial cells and no Reinke's crystaloids were seen. An epididymis was present on each testis and appeared normal. On the right side an adrenal rest was observed. The general impression was that of infantile testes.

DISCUSSION

Differentiation of the external genitalia along male lines is due to their responsiveness to androgenic substances whatever the source (Jones & Scott 1971). In the male such androgens originate from the foetal testes. Stimulation of the testes to secrete androgens in utero is produced by gonadotrophins which cross the placenta and/or by foetal pituitary gonadotrophins. Abnormal differentiation in the male can therefore result from either end organ unresponsiveness, or primary testicular failure or inappropriate gonadotrophin stimulation of the testes.
Development of the Müllerian ducts is prevented in the normal male foetus by a “Müllerian inhibiting factor” which is produced by the testes, possibly the Sertoli cells (Josso 1971). The production of this substance is probably not controlled by gonadotrophin and is not a steroid.

Male pseudohermaphroditism in patients with androgen insensitivity (testicular feminization syndrome) is characterized by normally suppressed Müllerian elements as the “inhibiting factor” appears to be produced normally and by female external genitalia and rudimentary Wolffian elements as there is no end organ response to androgens. It has been shown recently that the androgen insensitivity is due to the absence of a 5α-dihydrotestosterone binding protein in the cells of the end organs (Keenan et al. 1974). There is no evidence that our patient had androgen insensitivity since she responded clinically to the administration of exogenous androgens and HCG. Furthermore, her control levels of plasma testosterone were those of a normal female, although they could be raised to the male range following HCG stimulation.

In certain types of male pseudohermaphroditism the testes are defective in their production of androgen and/or Müllerian inhibiting factor. The defect can be either anatomical, such as in dysgenesis and in atrophy of the foetal testes, or enzymatic such as 17-ketosteroid reductase (Saez et al. 1971), 17-hydroxylase (New & Suvannakul 1970), 3β-hydroxysteroid dehydrogenase (Parks et al. 1971), or due to other unexplained causes. Our patient did not have testicular agenesis or atrophy, and there was no evidence for inability to synthesize androgens when HCG was given. Particularly there was no evidence for a deficiency of 17-ketosteroid reductase since testosterone and androstenedione were in the normal male range following HCG. The fact that, under control conditions, androstenedione was normal and testosterone low for a normal male but in the range for a normal female is probably the reflection that androstenedione in both sexes is mainly of adrenal origin. Therefore, her pseudohermaphroditism was not due to a testicular defect.

Patients with deficient gonadotrophin production by the pituitary, such as Kallmann’s syndrome, anencephaly, idiopathic hypopituitarism, or pituitary aplasia, often present with cryptorchidism and/or microphallus, but to our knowledge have not presented with ambiguity of the external genitalia. Although such patients may produce small amounts of gonadotrophin, this amount is inadequate to produce virilization at adolescence. In any event the low LH levels detected in these various syndromes are in contrast with our patient who had normal to high gonadotrophin values by urinary bioassay along the post-menopausal levels of LH and borderline elevated or normal levels of FSH by radioimmunoassay before gonadectomy was performed. The testes were capable of responding to exogenously administered HCG but not to endogenously produced LH. Consequently, one might conclude that the gonadotrophins in her blood and urine were radioimmunologically reactive but biologically inactive. Urinary gonadotrophins
determined by bioassay measure mainly FSH activity and therefore the normal to slightly elevated levels in the patient may be the reflection of FSH secretion rather than LH output.

An alternative but less likely explanation is that the Leydig cells in the testes of this patient were relatively insensitive to LH but could respond to HCG when given in large doses. The response of plasma testosterone to three days of HCG (5000 IU per day) is comparable to that observed in pre-adolescent males (Saez et al. 1971) and, therefore, a partial LH insensitivity is not a likely possibility.

Our patient had normal size testes but scanty Leydig cells. This contrasts with the findings in androgen insensitivity patients who have normal appearing Leydig cells. The sparsity of the interstitial cells in our patient could be explained in several ways. There could be an agenesis of the cells. In this case, however, HCG administration would not have elicited a normal testosterone response. If on the other hand, the interstitial cells were present but not visible as is the situation prior to puberty, this would have to be due to absent or incomplete gonadotrophic stimulation. Since the levels of immunoreactive LH were elevated rather than low, it could be that this peptide had an abnormal configuration. Such a structural abnormality would have to be reconciled with the normal immunoreactivity. It is possible that there was an abnormality in subunit secretion; only the knowledge of the chemical structure of the LH secreted by our patient could answer this question.

REFERENCES


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