The role of oestrogens on gonadotrophin secretion in the testicular feminization syndrome

Martha Medina, Alfredo Ulloa-Aguirre, María A. Fernández, and Gregorio Pérez-Palacios

Department of Reproductive Biology, Instituto Nacional de la Nutrición, México 22, D. F.

Abstract. The role of oestrogens on gonadotrophin secretion was assessed in three related patients with the complete form of testicular feminization syndrome. Serum LH and FSH levels were measured before and after LH stimulation as well as before, during and after chronic clomiphene citrate administration. Moderately elevated LH basal levels with a significant LH rise following LRH were observed. Normal or even low FSH level with poor response to LRH were found in all subjects. Administration of clomiphene citrate resulted in a significant serum LH increase without any change of FSH. Following castration both LH and FSH rose and a normal response to LRH was observed.

These results were interpreted as demonstrating that, while endogenous oestrogens modulate LH secretion in patients with androgen unresponsiveness, it plays no role in regulating FSH secretion and suggested that a factor of testicular origin without androgenic or oestrogenic activity is responsible for FSH regulation.

The hormonal profile of the testicular feminization syndrome (TFS) has been well established (Judd et al. 1972; Tremblay et al. 1972; Faiman & Winter 1974; Addison et al. 1976; Boyar et al. 1978).

Moderately elevated LH and low or normal FSH circulating levels have been extensively reported (Judd et al. 1972; Faiman & Winter 1974; Addison et al. 1976) in spite of the well recognized complete androgen resistance and an arrest of spermatogenesis in these patients. The modulating effect of endogenous oestrogens has been suggested as an explanation for this finding (Judd et al. 1972; Pérez-Palacios & Jaffe 1972; Faiman & Winter 1974) since oestrogen production rate is elevated in TFS patients (Wilson et al. 1974).

We have studied the effect of chronic administration of a non-steroidal anti-oestrogenic compound on the serum LH and FSH levels in three related patients with testicular feminization syndrome to elucidate the role of oestrogens in the regulation of gonadotrophins in this syndrome.

Materials and Methods

1. Subjects

Three related phenotypic females with the complete form of testicular feminization syndrome were studied. All patients were post-pubertal (Subject A: 15 year old, Subject B: 18 year old, and Subject C: 38 year old). Diagnosis was established on the following basis: a) 46 XY karyotype, b) female external genitalia, c) adequate breast development and lack of sexual hair, d) absence of Mullerian structures, and e) bilateral cryptorchidic testes. Testicular histological examination disclosed an arrest of spermatogenesis at the spermatogonial stage. Seminiferous tubules contained Sertoli cells and spermatogonia and interstitial cells had undergone a marked hyper-
plasia. Written consent from the patients as well as approval of the Institute's ethical committee were obtained.

2. Methods

Serum LH and FSH were measured by specific radioimmunoassay (RIA) as previously described (Scaglia et al. 1976). Results were expressed as mIU/ml according to the 2nd IRP-HMG. Inter- and intra-assay coefficients of variation were 9.1 and 5.0% for LH and 11.5 and 10.1% for FSH respectively.

Serum testosterone (T) was measured by RIA as previously described (Febres et al. 1975). Normal values for LH, FSH and T have been reported previously (Pérez-Palacios et al. 1973; Febres et al. 1975). LRH was a kind gift from Hoechst Farbwerke, A.G., and clomiphene citrate was supplied by Merrell de México, S.A. The reagents for the LH and FSH radioimmunoassays were obtained from NIAMDD, NIH. Androgen resistance was assessed by determination of 4S cytosol androgen receptors in cultured genital skin-derived fibroblasts according to the procedure described by Griffin et al. (1976) with minor modifications. In brief, fibroblasts were grown from genital skin of subjects A, B and C. Following five consecutive sub-cultures fibroblasts were seeded in roller bottles and incubated at 37°C with 1 nM (1,2,4,5,6,7-3H) dihydrotestosterone (New England Nuclear, Co) for 30 min. The cells were harvested and washed, and then suspended in tris-EDTA-trisylol buffer with 0.5 mM KCl, and disrupted using a Sonicator cell disruptor (Heat Systems, Ultrasonics, Inc.). The sonicate was submitted to centrifugation at 105 000 x g for 1 h, and 200 µl samples of the supernates were applied on the top of a 5—20% linear sucrose gradient containing 10% glycerol, 20 mM Tris, and 1.5 mM EDTA, pH 7.4 with 0.5 mM KCl. The tubes were centrifuged for 18 h at 250 000 x g for 20 h using a SW 50.1 Beckman rotor. Sixty fractions were collected and submitted to radioactive counting. Cytosol protein concentration was determined by the method of Lowry et al. (1951). [14C]-ovalbumin (New England Nuclear Co) was used as an internal marker for the gradient. Normal foreskin fibroblasts were used as experimental control.

Fig. 1.

Serum FSH and LH levels before and after iv LRH stimulation (100 µg) in patients with complete testicular feminization syndrome before (upper panel) and after (lower panel) gonadectomy.
3. Experimental
Serum LH, FSH, and T were measured in five blood samples drawn at 15 min interval. A 100 μg LRH bolus was given intravenously and blood samples were drawn at 30 and 60 min. Clomiphene citrate 200 mg, was administered by mouth daily for 28 consecutive days. Serum gonadotrophin was measured in five samples drawn at 15 min interval before and after clomiphene administration. During anti-oestrogen administration gonadotrophin was measured at weekly interval (3 samples each).

After completion of these studies all patients underwent surgical bilateral gonadectomy. After two months of castration, serum LH and FSH were measured before and after a LRH stimulus.

All samples were analyzed on the same assay in order to avoid interassay variability.

Results
Basal serum LH and FSH levels (x ± sd) were:
Patient A: 14.5 ± 1.9 and 8.2 ± 0.37; Patient B: 17.5 ± 1.6 and 4.0 ± 0.7; Patient C: 20.0 ± 2.2 and 2.6 ± 0.2 mIU/ml. A normal pituitary response to synthetic LRH in terms of LH and a poor response in terms of FSH were observed in all cases, as shown in Fig. 1 (upper panel). Mean serum testosterone levels in patients A, B, and C were 21.1, 37.5 and 30.0 nmol/l, respectively.

Fig. 2 depicts the serum LH and FSH levels before, during, and after clomiphene administration. As can be seen a significant rise (P < 0.01, Student’s paired t-test) of the serum LH levels was observed during the anti-oestrogen administration in all cases, however no significant changes were noted of the FSH levels in either subject.

Following surgical gonadal removal a significant rise on both LH and FSH basal levels was observed (Fig. 1, lower panel) and a significant LRH pituitary response in terms of both LH and FSH was also noticed in all subjects.

As shown in Fig. 3 a lack of 4.6 S androgen cytosol receptor molecule on skin fibroblasts from all patients, as compared with normal fibroblasts used as experimental control, was demonstrated.
Discussion

The regulation of gonadotrophin secretion in men is not well understood. It is recognized that pituitary LH secretion is primarily modulated by testosterone and its neutral, reduced, and phenolic derivatives, while FSH secretion is regulated either by oestrogens, or by a testicular produced non-steroidal factor. (Judd et al. 1972; Lee et al. 1972; Kulin & Reiter 1972; Franchimont et al. 1972; Sherins & Loriaux 1973; Santen 1975; Lasley et al. 1976). Therefore it might be expected that individuals with androgen resistance and arrest of spermatogenesis at the spermatogonial stage as in TFS, would have elevated circulating levels of LH and FSH.

The present study shows that in three related patients with complete absence of cytosol androgen receptor molecules (TFS), serum LH was moderately elevated with a normal response to LRH, while FSH was low with a poor response to LRH. This confirms and extends previous reports (Fairman & Winter 1974; Addison et al. 1976; Lacroix et al. 1979).

The result of chronic clomiphene administration was a significant rise in serum LH, indicating that endogenous oestrogens are important in the regulation of LH in this syndrome.

Interestingly, the administration of clomiphene was unable to modify the FSH levels, demonstrating that endogenous oestrogens do not participate in FSH regulation in TFS, and suggesting that a testicular factor without androgenic or oestrogenic activity is involved in FSH regulation. Whether this FSH regulating factor is produced by Sertoli cell and/or spermatogonia cannot be ascertained.

Recently Lacroix et al. (1979) reported an increase in serum FSH during short-term (6 days) administration of clomiphene to TFS patients. However, our studies disclosed that even after 28 consecutive days of clomiphene administration FSH levels remained unchanged.

The fact that after castration a significant increase on both LH and FSH was noticed is in line with our observation that oestrogens and an uncertain testicular factor are involved in gonadotrophin regulation in complete androgen resistance syndrome.

The overall data are interpreted as demonstrating the important role of oestrogens on LH, but not upon FSH, regulation and suggest different regulatory mechanisms for LH and FSH in this inherited disorder.

Fig. 3.
Sucrose-gradient plots of [3H]dihydrotestosterone binding in fibroblasts from subjects A, B and C and from a normal control.
References


Received on November 9th, 1979.