ABSENCE OF POSITIVE FEEDBACK EFFECT OF OESTROGEN ON LH RELEASE IN PATIENTS WITH TESTICULAR FEMINIZATION SYNDROME

By Toshihiro Aono, Akira Miyake, Takayuki Kinugasa, Keiichi Kurachi and Keishi Matsumoto

ABSTRACT

The response of serum LH to exogenous oestrogen administration was studied in 5 patients with testicular feminization syndrome (TFS). The serum LH levels were elevated in all the patients, while serum testosterone levels were within the normal male range. Serum FSH levels were elevated in 4 patients and normal in one patient. Intravenous administration of 100 μg of LH-RH provoked a further increase in both LH and FSH. Following intravenous injection of 20 mg of conjugated oestrogen (Premarin®), the LH levels were serially determined until 120 h in TFS patients, 5 normal males, and 10 normal females during the mid-follicular phase (D7–9). Both TFS patients and normal males showed no LH release following oestrogen injection in contrast to normal females who displayed a significant increase in LH with a peak at 48 to 56 h after the injection.

These results seem to suggest that the insensitivity of the hypothalamus to androgen in TFS patients do not affect the sex differentiation of the hypothalamus. The possible role of oestradiol conversion from testosterone in the hypothalamus is discussed.

It has been well documented that adult female rats are characterized by a cyclic gonadotrophin secretion, whereas male rats show a tonic gonadotrophin secretion (Harris 1964; Gorski & Wagner 1965). This sex difference in gonadotrophin secretion is regulated by the hypothalamus, and is dependent on androgen exposure of animals during the early post-natal life (Pfeiffer 1936; Shay et al. 1939; Barraclough 1961). Yamaji et al. (1971) reported that oestrogen administration did not provoke LH release in male rhesus monkeys. Dörner et al.
(1972) also reported that no LH release was found after conjugated oestrogen administration in normal males, whereas homosexual men displayed LH increase above the initial values. On the other hand, Leyendecker et al. (1971) reported that a positive feedback effect of oestrogen on LH release was observed in normally menstruating women, oligomenorrhoeic women, post-menopausal women, amenorrhoeic women, and XY gonadal dysgenesis in contrast to normal males. Our previous studies (Aono et al. 1976, 1977) also demonstrated that conjugated oestrogen administration induced LH release in normal cyclic women, patients with hypothalamic amenorrhoea, and women with polycystic ovaries.

The patients with testicular feminization syndrome (TFS) display feminine phenotype because of end-organ insensitivity to normal male levels of serum testosterone (French et al. 1966; Tremblay et al. 1972; Judd et al. 1972). Increased level of LH despite a normal testosterone levels also suggests the insensitivity of hypothalamus to androgens.

The present studies were conducted to assess the sex differentiation of the hypothalamus in TFS by measuring the serum LH response to iv oestrogen in patients with TFS.

**MATERIALS AND METHODS**

1. **Subjects**

Five TFS patients aged from 18 to 24 years with karyotype of 46,XY were studied (Table 1). There were 3 patients with complete form of TFS and 2 patients with incomplete form as judged by the degree of clitoromegaly and sexual hair growth. All subjects had not been gonadectomized and had not received any sex steroid hormones prior to the studies. Subsequent histological examinations of gonads in patients K. H., M. K., and H. A. revealed immature seminiferous tubules without mature germ cells and slight hyperplasia of Leydig cells. Serum levels of testosterone determined by competitive protein binding assay (CPBA) (Mayes & Nugent 1968) were 2.4 to 11.8 ng/ml which were within the normal range. Serum levels of oestradiol determined by RIA method (Wu & Lundy 1971) were normal or slightly higher than the normal male level.

Five normal males aged from 25 to 37 years volunteered for the control study. Ten normal cyclic women during the mid-follicular phase (D7–9) aged from 21 to 38 years also participated in the oestrogen provocation test.

2. **Methods**

   a) **LH–RH test.** – One hundred \( \mu \)g of synthetic LH–RH (Daiichi Pharmaceutical Co.) was administered intravenously in the morning after overnight fasting (Aono et al. 1973). Blood samples were collected 0, 15, 30, 60, 120, and 180 min after the injection, and serum levels of LH and FSH were determined by double antibody radioimmunoassay as described previously (Aono et al. 1972). The Second International Reference Preparation of Human Menopausal Gonadotrophin was used as standard material and the results were expressed as ng/ml of LER 907. The mean immunological potencies of LER 907 assayed in our laboratory were 255 mIU/\( \mu \)g for LH and 40 mIU/\( \mu \)g for FSH and these values were used as conversion factors.
Table 1.
Clinical and laboratory data on patients with TFS.

<table>
<thead>
<tr>
<th>No. Patient</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Breast development</th>
<th>Pubic &amp; axillary hair</th>
<th>Clitoromegaly</th>
<th>Vagina (cm)</th>
<th>Sex chromosome</th>
<th>Testosterone (ng/ml)</th>
<th>Oestradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. K. H.</td>
<td>24</td>
<td>153</td>
<td>48</td>
<td>Tanner 5</td>
<td>very scanty</td>
<td>no</td>
<td>2.5</td>
<td>XY</td>
<td>10.7</td>
<td>31.0</td>
</tr>
<tr>
<td>2. M. K.</td>
<td>19</td>
<td>159</td>
<td>50</td>
<td>Tanner 4</td>
<td>very scanty</td>
<td>no</td>
<td>4.0</td>
<td>XY</td>
<td>11.8</td>
<td>33.9</td>
</tr>
<tr>
<td>3. H. A.</td>
<td>18</td>
<td>159</td>
<td>47</td>
<td>Tanner 4</td>
<td>very scanty</td>
<td>no</td>
<td>7.0</td>
<td>XY</td>
<td>2.4</td>
<td>19.3</td>
</tr>
<tr>
<td>4. M. T.</td>
<td>24</td>
<td>166</td>
<td>48</td>
<td>Tanner 5</td>
<td>scanty</td>
<td>yes</td>
<td>0</td>
<td>XY</td>
<td>6.4</td>
<td>11.0</td>
</tr>
<tr>
<td>5. K. M.</td>
<td>20</td>
<td>157</td>
<td>43</td>
<td>Tanner 3</td>
<td>moderate</td>
<td>yes</td>
<td>2.5</td>
<td>XY</td>
<td>4.3</td>
<td>63.0</td>
</tr>
</tbody>
</table>

Normal range

3.0–11.0  15–25
b) Oestrogen provocation test. – In order to assess the LH-release following acute administration of oestrogen, 20 mg of conjugated oestrogens, (Premarin®, Ayerst Laboratories) was administered intravenously between 9 and 10 a.m. and serum levels of LH were assayed 0, 8, 24, 32, 48, 56, 72, 96, and 120 h after the injection (Aono et al. 1976).

Statistical analyses were performed by paired and unpaired Student’s t-test.

RESULTS

1. LH-RH test

The individual responses of LH and FSH following LH-RH injection in 5 patients with TFS are shown in Fig. 1. Shaded areas represent the range of response in normal males. Basal LH levels were elevated in all patients (150.2 to 941.2 ng/ml), and further increased with a peak level between 15 to 60 min after LH-RH injection. The mean (± se) maximum fold-increase in TFS patients (4.8 ± 1.0) is significantly lower (P < 0.05) than that in normal males (9.7 ± 1.4). In four patients, basal FSH levels were elevated (900 to 2688 ng/ml) and peaks occurred 30 to 60 min after LH-RH injection, while the remaining one patient (H. A.) showed normal FSH levels with a delayed response. The mean maximum fold-increase in TFS patients (2.2 ± 0.3) was not significantly different from that in normal males (2.0 ± 0.2). There were no remarkable

![Graph](image1)

**Fig. 1.**

Individual responses of LH and FSH to single iv injection of 100 µg of LH-RH in patients with testicular feminization syndrome. Shaded areas represent ranges of response of normal males. Numbers represent patient number in Table 1.
Table 2.
Serum LH levels before and after iv injection of conjugated oestrogen in patients with TFS, normal males and normal cyclic women during mid-follicular phase (D7-9).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Serum LH level (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TFS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. K. H.</td>
<td>24</td>
<td>500.0</td>
</tr>
<tr>
<td>2. M. K.</td>
<td>19</td>
<td>427.5</td>
</tr>
<tr>
<td>3. H. A.</td>
<td>18</td>
<td>166.7</td>
</tr>
<tr>
<td>4. M. T.</td>
<td>24</td>
<td>549.0</td>
</tr>
<tr>
<td>5. K. M.</td>
<td>20</td>
<td>112.5</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>351.0</td>
</tr>
<tr>
<td>se</td>
<td></td>
<td>39.0</td>
</tr>
<tr>
<td>Normal males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. A. M.</td>
<td>29</td>
<td>37.6</td>
</tr>
<tr>
<td>2. T. S.</td>
<td>25</td>
<td>30.6</td>
</tr>
<tr>
<td>3. T. K.</td>
<td>29</td>
<td>35.3</td>
</tr>
<tr>
<td>4. O. A.</td>
<td>37</td>
<td>32.2</td>
</tr>
<tr>
<td>5. J. M.</td>
<td>31</td>
<td>42.4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>35.7</td>
</tr>
<tr>
<td>se</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>10 Normal cyclic women (D7-9)</td>
<td>21–38</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>38.4</td>
</tr>
<tr>
<td>se</td>
<td></td>
<td>5.5</td>
</tr>
</tbody>
</table>

Differences from before administration (P) * < 0.05  ** < 0.01.
differences in LH or FSH responses to LH-RH as well in the basal levels between complete and incomplete forms of TFS. These data suggest the existence of hypothalamic insensitivity to androgens and that a reserve of gonadotrophins in the pituitary is present in TFS patients.

2. Oestrogen provocation test

Individual LH responses to conjugated oestrogen in patients with TFS and normal males, and the mean LH response to conjugated oestrogen in normal cyclic women are summarized in Table 2. Normal males showed an initial suppression of LH between 8 and 32 h after the injection, followed by a recovery to the initial level. The mean LH level in normal cyclic women was suppressed 8 h after the injection, then showed a rebound increase between 32 and 120 h. The mean LH level in TFS patients showed an initial suppression between 8 and 32 h after the injection, but did not show any rebound increase.

In order to compare the LH responses to conjugated oestrogen in TFS patients with those in normal males and normal cyclic women, the per cent changes in LH from pre-injection levels were calculated and shown in Fig. 2. The initial per cent decrease of LH in TFS patients was significantly greater than that in normal cyclic women between 8 to 32 h after the injection, and was also lower than in normal males 8 and 32 h after the injection. The mean per cent increase of LH in TFS patients was significantly lower than in normal

![Fig. 2.](image)

Mean per cent change of LH following iv injection of conjugated oestrogen (Premarin® 20 mg) in normal women at the midfollicular phase (D7–9) (O—O), normal males (●—●) and patients with testicular feminization syndrome (▲—▲). Vertical bars denote standard error of mean. Difference from TFS patients (P) * < 0.05, ** < 0.01.
cyclic women between 48 to 96 h after the injection, but not significantly different from that in normal males. These data indicate that the response of LH to exogenously administered oestrogen in TFS patients is shown to be the male type but not the female type.

**DISCUSSION**

Our data on the elevated levels of serum LH in TFS patients are consistent with the previous reports (Zárate *et al.* 1974; Tremblay *et al.* 1972; Judd *et al.* 1972; Faiman & Winter 1974). Since the LH secretion is regulated by the circulating testosterone level, elevated LH levels despite normal testosterone concentrations in TFS patients suggest the existence of insensitivity of the hypothalamus to androgens. FSH levels in the present series are also mostly elevated. Our results on the lower maximum fold-increase in LH after LH-RH stimulation in TFS patients compare with normal males and comparable maximum fold-increase in FSH to normal males are consistent with previous results by Zárate *et al.* (1974).

The present data confirm the concept that positive oestrogen feedback on LH release occur in normal females but not in normal males (Leyendecker *et al.* 1971; Dörner *et al.* 1972; Aono *et al.* 1976). Pfeiffer (1936) first showed that the sex-specific differentiation of tonic or cyclic secretion of gonadotrophin in rats was induced by the presence or absence of testicular tissue during early post-natal life. Barraclough (1961) demonstrated that the testosterone administered during first few days of life was responsible for permanent sterility with tonic gonadotrophin secretion in female rats and mice. It may be said that the critical period for the hypothalamic sex differentiation is present during the peri-natal period in mammals with a short gestation period, while this exists during pre-natal period in mammals with a long gestation period, e.g. dogs (Neumann *et al.* 1970) and monkeys (Goy 1970). Dörner (1976) speculated that the critical period for the sex-related differentiation of the hypothalamus in the human may be present in the 4th to 5th month of foetal life according to the following findings. 1) Chronological sequence of sex hormone dependent differentiation processes are in the order of gonaducts, external genitalia and hypothalamus. 2) Histological changes indicating maturation of hypothalamic cells occur during the 2nd trimester of pregnancy. 3) Serum testosterone level in male foetuses are found to be high at 11 to 18 weeks of foetal age.

It could be assumed that the androgen insensitivity of the hypothalamus during foetal life in TFS patients might cause cyclic gonadotrophin secretory conditions. But, the present patients with TFS displayed no LH release after oestrogen administration. The discrepancy between this hypothesis and the present findings could be explained by the following mechanism; testosterone
secreted from the testes of TFS patients can be readily converted to oestradiol in the hypothalamus and this oestrogen may induce a male type gonadotrophin secretion. Reddy et al. (1974) reported an increased aromatizing activity in the hypothalamus of newborn male rats compared with that of adult rats. Moreover, it is reported that administration of a large amount of oestrogen to newborn female rats leads to a persistent oestrus (Takasugi 1963). In these situations, administration of only aromatizable androgens can produce persistent oestrus but un-aromatizable androgens such as dihydrotestosterone do not have this effect (Whalen & Luttge 1971). These findings seem to suggest that oestrogens formed from androgens in the hypothalamus may play a role in hypothalamic sex differentiation in TFS patients.

Although TFS patients offer a model for the study on hypothalamic differentiation in the human, further studies would be necessary in order to establish this hypothesis.

ACKNOWLEDGMENTS

We wish to express appreciation to the National Institute for Biological Standards and Control, England and the National Institute of Arthritis, Metabolism and Digestive Diseases, USA for their generous gift of standard materials. We gratefully acknowledge supply of synthetic LH-RH from the Daiichi Pharmaceutical Co., Japan.

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Received on August 5th, 1977.