INFUSIONS OF hFSH AND hLH IN NORMAL MEN

II. Serum testosterone response to infused hLH and hFSH

By

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ABSTRACT

Standardized 4 h intravenous infusions of human follicle stimulating hormone (hFSH) and/or human luteinizing hormone (hLH) were given either separately or combined to 7 normal male volunteers. The infusions raised the serum gonadotrophin levels at least 10 (FSH) and 18 (LH) times above the basal ones. Serum testosterone (T) levels were measured serially before, during and after the infusions and, in 4 subjects, during a corresponding period of another normal day. During a normal or basal 24 h period fluctuations were seen and also a circadian rhythm with lower levels in the evening. The infusion of hFSH alone (3 subjects) did not alter the serum T levels. The infusion of hLH alone in 2 subjects raised serum T levels by 17% and 55% over those of the basal day. The combined FSH/LH infusion caused a significant rise (35–68%) in 4 subjects and greater rise in 2 of them than after infusion of the hLH alone. The serum T responses were gradual, reaching a maximum 7–8 h after the end of the infusion.

Factors governing serum testosterone (T) levels or testosterone production by the testis are little known. Recent methodological progress (Christensen & Mason 1965) has made it possible to study separately the effect of the trophic hormones in vitro on the two components of the testis, the interstitial tissue (Leydig cells) and the tubules (Sertoli cells and spermatogenic epithelial cells). Investigators using these techniques have confirmed the specific effect (cyclic AMP rise) of LH on the interstitial tissue and of FSH on the tubular tissue (van der Molen...
et al. 1973). While in vitro experiments have proven that the Leydig cells produce T, there is still controversy regarding T production by the tubules (Lacy 1973). Hafiez et al. (1972) have shown that, in vitro, prolactin and LH have a synergistic action in stimulating testicular T production by the rat testis. Data are still lacking, however, on the synergistic action of the various trophic hormones on the separated testicular components with regard to T production.

In vitro experiments have been hampered by a lack of sufficiently pure pituitary gonadotrophin preparations. However, animal experiments have indicated that FSH on its own does not increase T production in the intact male rat (van der Molen et al. 1973) but does augment the response of the rabbit testis produced by LH (Johnson & Ewing 1971).

Few data are available on the effect of pituitary gonadotrophins on serum T levels in man. It has nevertheless been long accepted from animal experiments that LH is the main trophic hormone promoting the production of T by the Leydig cells of the human testis. While good responses are seen in serum T levels after intramuscular and intravenous administration of human chorionic gonadotrophin (HCG) (Nieschlag et al. 1971; Anderson et al. 1972; Maurer et al. 1973; Vivanco et al. 1973), LH itself can only be inferred to have the same effect as it has not been rigorously proven to do so (Hall 1970). Little is known about the effect of FSH on the serum T levels in man but it has been associated with circadian variations in serum T levels (Faiman & Winter 1971).

A circadian rhythm in plasma T levels is now generally accepted (Faiman & Winter 1971; Rose et al. 1972; Barberia et al. 1973). It has however been debated (Boon et al. 1972) and superimposed episodic fluctuation described (Schiavi et al. 1974).

An earlier study in this laboratory on the plasma 17β-hydroxyandrogen (Anderson 1970) response to infused hLH in man (Marshall et al. 1973) showed unexpectedly that only one out of four male subjects gave a definite response. Using the same preparation of hLH (Medical Research Council: MRC) we have extended this study with identical infusion procedure now using, in addition, an MRC preparation of hFSH suitable for intravenous administration. To account for circadian and episodic changes we have besides measured basal serum T levels on a separate control day, either in the morning and evening, or with frequent sampling throughout the day.

SUBJECTS AND METHODS

1. Subjects

Seven healthy male subjects, members of the hospital staff, aged 26 to 37, volunteered for this study. They were not fasting and remained recumbent during the infusions which all lasted for 4 h. The FSH infusions were begun at 10.00 hours, the others between 12.00 and 14.00 hours. During the basal control period the subjects carried on with their
normal activities. In subjects 1, 2 and 3 basal T values were obtained on two occasions 12 h apart on the day previous to the hFSH infusion. Serum T levels were measured before, during and up to 60 h after the hFSH infusion in these three subjects. In subjects 4 and 5 basal T values were obtained 13 times on a separate control day. These two subjects also had serial serum T level determinations during a day of a combined infusion of hFSH and hLH and a day of infusion of hLH alone. In subjects 6 and 7 basal T values were similarly obtained 8 times on a control day and serum T levels were measured serially before, during and until 16 h after a combined hFSH and hLH infusion.

2. **Infusion procedures**

From one arm, using an indwelling catheter, serum samples were taken before, during and after the infusions as shown in Figs. 1–3. On the other arm the infusion was administered intravenously using an indwelling needle connected to a 20 cm polypropylene tubing.

The hFSH preparation used was the MRC's Human Pituitary FSH Clinical - 70/10, batch F1. The biological activity of each ampoule was stated as 113.6 IU against the 2nd IRP HMG. Measured by our radioimmunoassay against the MRC 69/104 (see below) each ampoule contained 70 IU. Four ampoules were given in each infusion, 40% as an initial loading dose injection and 56% infused at a constant rate over 4 h.

The hLH preparation was the MRC's Human Pituitary LH Clinical - 70/67, Batch L1. The biological activity of each ampoule was approximately 600 IU against the 2nd IRP HMG. The LH content of each ampoule measured by our radioimmunoassay was 25.2 IU. Two ampoules were given in each infusion, 25% as an initial loading injection and 75% at a constant rate over 4 h.

The same doses of each gonadotrophin were given in the combined infusions.

Further data on the gonadotrophin preparations and more detailed description of the infusion procedures are given in the preceding paper (Kjeld et al. 1976).

3. **Radioimmunoassays (RIA)**

(i) *Serum testosterone (T)* was measured by a RIA method using antiserum raised in rabbits against T-3-oxime-bovine serum albumin. The antigen was prepared by Professor P. Shahbasi in collaboration with one of us by the method of Erlanger et al. (1957). The antiserum was tested against structurally similar steroids and showed a cross-reactivity of less than 1% for all those tested except 5α-dihydrotestosterone which cross-reacted 39%. Serum samples were extracted with diethyl ether, and the extraction checked with internal standards of tritiated T (Amersham) which was the label used in the assay. There was no purification step used beyond the ether extraction and the slight interference by physiological levels of 5α-dihydrotestosterone (∼0.2 ng/ml) accepted. Dextran-coated charcoal was employed to separate bound and free testosterone. Intra- and inter-assay coefficients of variation were 6 and 9% respectively. Normal range in male adults, aged 26–38 (n = 16) was: 11.8–32.6 nmoles/l (3.5–9.3 ng/ml) at 9–11 a.m. and 6.9–27.8 nmoles/l (2.0–7.3 ng/ml) at 6–8 p.m.

(ii) *Serum LH and FSH* were measured by double antibody RIA (Marshall et al. 1972, 1973) results for both gonadotrophins being expressed as U/l compared with the MRC Pituitary Standard, 69/104 (Bangham et al. 1973). Normal ranges for adult men were 2.0–8.0 U/l and 1.6–8.6 U/l for LH and FSH respectively.
4. Analysis of results

In order to assess mathematically the effect of the gonadotrophins, serum T levels after the infusions have been compared with the diurnally corresponding basal levels, where these have been obtained. This has been done by comparing the mean T levels over 0–16 h commencing with the end of the infusions. Because of episodic fluctuations and the inability to obtain samples at the same time of day the levels were derived by measuring the areas below the curves in mm² and dividing it by the time scale (mm) and the number of mm per concentration unit on the ordinate.

RESULTS

(1) Changes induced in serum levels of FSH and LH

The basal gonadotrophin levels immediately before the infusions were 2.5–3.9 U/l for FSH and 1.5–3.0 U/l for LH. In all cases equivalently raised levels were attained during the infusions or a rise to more than 10 (for FSH) and 18 (for LH) times the basal level. Serum levels of LH rose by about 4 U/l during the FSH infusion but FSH did not show a significant rise during the LH infusion. Changes of gonadotrophin levels during and after the infusions are further described in the previous paper (Kjeld et al. 1976).

Fig. 1.
Serum T levels before, during and after intravenous infusion of hFSH into 3 normal male subjects. The first two values are from the previous day.
Serum T levels in subjects 4 and 5 (i) during a basal day, (ii) during a hLH-infusion day and (iii) a combined hLH/hFSH-infusion day. The vertical arrows indicate 16 h from the end of the infusion (Table 1).

(2) Serum T levels

(a) The basal serum T values during the 24 h control periods. – Subjects 1–3 who had basal T levels measured only once in the morning and evening all showed lower values in the evening (Fig. 1). Subjects 4–7 who had several measurements throughout the control period showed episodic fluctuations superimposed on a circadian rhythm with high nocturnal and morning values (Figs. 2 and 3). The greatest deviation from the mean value was 55% in subject 4 in whom the highest levels were approximately three times the lowest ones.

(b) The FSH infusion. – In subjects 1–3 no response in serum T levels was seen after the FSH infusion beyond the changes to be expected from episodic fluctuations, while the subjects continued to show the usual circadian rhythm (Fig. 1).
(c) The LH infusion. – After the LH infusion the mean serum T rose by 55.5 and 16.9% over the basal day in subjects 4 and 5 respectively (Figs. 2 and 3, Table 1). This rise did not start until after the infusion, was gradual and abolished the circadian depression in the evening.

(d) The combined FSH/LH infusion. – In subjects 4–7 after the combined infusion, there was a significant response in serum T levels by all subjects (Figs. 2 and 3, Table 1). The rise was sluggish, started only after the end of the infusion and reached a maximum after approximately 7 h, then levelling off at a raised level, the whole response lasting for at least 16 h and abolishing the circadian evening depression. In subject 4 the response looked similar to the response after LH alone but had the higher mean level of 6.39 or 68.3% rise above the basal day value (Table 1). Subject 5 had a rise of 46.6% above the basal day values,

Serum T levels in subjects 6 and 7 during (i) a basal day and (ii) a combined hLH/hFSH-infusion day. The vertical dotted lines indicate 16 h from the end of the infusions.
**Table 1.**
Mean* serum testosterone (T) levels during 0–16 h after 4-h infusions of hLH and hLH + hFSH compared with basal levels over the corresponding period of another day (from data of Figs. 2 and 3).

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Basal day T. ng/ml</th>
<th>LH infusion day T. ng/ml</th>
<th>% rise over basal levels</th>
<th>(LH + FSH) infusion day T. ng/ml</th>
<th>% rise over basal levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3.80</td>
<td>5.91</td>
<td>55.5 (P &lt; 0.001)</td>
<td>6.39</td>
<td>68.3 (P &lt; 0.001)</td>
</tr>
<tr>
<td>5</td>
<td>5.94</td>
<td>6.94</td>
<td>16.9 (P &lt; 0.01)</td>
<td>8.70</td>
<td>46.6 (P &lt; 0.001)</td>
</tr>
<tr>
<td>6</td>
<td>6.56</td>
<td>–</td>
<td>–</td>
<td>8.86</td>
<td>35.2 (P &lt; 0.02)</td>
</tr>
<tr>
<td>7</td>
<td>4.56</td>
<td>–</td>
<td>–</td>
<td>6.38</td>
<td>39.7 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Mean</td>
<td>5.22</td>
<td>6.42</td>
<td>36.2</td>
<td>7.58</td>
<td>47.45</td>
</tr>
</tbody>
</table>

* Mean values were derived by measuring the area under the serum T. Curves in mm² and then dividing by the appropriate time scale (mm) and the number of mm per concentration unit.

a response significantly (P < 0.001) higher than after the infusion of LH alone. Subjects 6 and 7 had rises of 35.2 % and 39.7 % respectively above their basal day values.

**DISCUSSION**

Our measurements of the basal serum T levels show a definite circadian rhythm with higher levels in the late morning and the lowest generally in the early evening at about 10 p.m. This is in agreement with the majority of reported studies (Anderson 1970; Faiman & Winter 1971; Rose et al. 1972; Barberia et al. 1973; Schiavi et al. 1974). The cause of this diurnal rhythm remains unknown as is the cause of the episodic fluctuations which were so marked in subjects 4 and 5. Similar fluctuations have been observed by other investigators (Evans & MacLean 1971; Boon et al. 1972; Alford et al. 1973; Schiavi et al. 1974). Faiman & Winter (1971) showed that there is probably no adrenal involvement in this circadian rhythm, and while they found a correlation between serum FSH and plasma T changes, Alford et al. (1973) could not find any such relationship.

While FSH did not elevate T levels on its own, LH seems to be able to do so to a varying extent. Our findings in the two LH infusions may be regarded as in reasonable agreement with our earlier findings (Marshall et al. 1973) where only one response was found after 4 identical LH infusions. This, together with
our higher and consistent responses after the combined infusions, strongly suggests a synergism in action between the two gonadotrophins with regard to T production.

Because of the sluggish and prolonged response seen after these infusions the abrupt episodic fluctuations seen in normal subjects are unlikely to depend on fluctuations in gonadotrophin secretion. Further, levels such as those reached in the infusions have not been described in normal man although the gonadotrophin levels are known to fluctuate episodically. One can not, however, rule out some ratio (FSH/LH) sensitive mechanism not reflected in the presently attained blood levels of LH and FSH.

Maurer et al. (1973), infusing HCG intravenously over 3 h in men observed a serum T response after 30 min from the start of the infusion when using either 100 or 500 IU total dose. When they used only 50 IU an initial depression and no overall rise was observed. Kley et al. (1974) reported various responses in serum T levels after 8 h intravenous infusion of luteinizing hormone releasing hormone (LH-RH) where plasma LH rose 3 to 4 times above basal values and FSH to a lesser extent.

We have in this study been limited by the quantities of gonadotrophins available, and larger doses administered might have elicited greater and earlier responses. However, the gonadotrophin levels attained during the infusions are higher than those seen in normal males and still higher levels would be grossly unphysiological. In conclusion, the results presented point to a multifactorial control mechanism of serum T levels in man where FSH plays a definite if permissive role. LH is probably the main factor but needs some co-factors for its effect to take place and, besides, has its T secretory effects modified by other hormones or agents still unknown.

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