Testosterone and 17 OH-progesterone responses
in men to 3 h LH infusions

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Abstract. LH is recognized as the Leydig cell stimulus for testosterone production. Yet earlier studies of bolus administered human pituitary LH did not result in testosterone elevations and spontaneous LH elevations infrequently cause recognizable parallel rises of testosterone in adult males. Therefore, 10 normal adult volunteers were given 3 h infusions of human pituitary LH A-3 (2 IU/kg) and sampling was continued for 3 h after the gonadotrophin was stopped. Testosterone, the precursor 17 OH-progesterone and LH were determined in blood samples. There was a mean 5.2-fold LH elevation. From a group baseline mean of 698 ng/100 ml ± 72 (± SE) testosterone gradually rose and was significantly elevated at 90, 120 and 150 min (787 ± 67 P < 0.03, 767 ± 60 P < 0.04, and 804 ± 69 P < 0.001, respectively). 17 OH-progesterone was determined on 9 subjects and averaged 142 ng/100 ml ± 16 before LH and gradually rose to 178 ± 18 at 120 min (P < 0.05) and then declined slightly at 150 and 180 min. Both steroids significantly declined between 180 min (end of LH infusion) and 240 min with no further significant concentration changes. The two men with the highest basal testosterone titers showed the smallest testosterone increases, but in both 17 OH-progesterone rose. In conclusion tonic LH stimulation caused mean testosterone and 17 OH-progesterone to peak at 150 min and at 120 min, respectively. Factors within the Leydig cell appear to limit increases of testosterone and 17 OH-progesterone during the first few hours of enhanced gonadotrophin stimulation.

A wide variety of in vivo and in vitro studies in laboratory animals have demonstrated that the normal Leydig cell produces testosterone after stimulation with LH in the appropriate milieu. Arterial infusion of the dog testis with gonadotrophin increased testosterone in the venous effluent in 3–6 min (Eik-Nes 1975). LH is released from the pituitary as pulsatile elevations superimposed on tonic discharge in normal men (Nankin & Troen 1971, 1972). When normal men underwent frequent blood sampling studies and both LH and testosterone titers were determined on the same specimens, about 1/3 of LH elevations were followed by recognizable testosterone rises 45 to 80 and 20 to 140 min later (Naftolin et al. 1973; Judd et al. 1973). Using integrated sampling a significant positive correlation was found between LH and testosterone levels in the succeeding sampling period (Alford et al. 1973a). In order to study the relationship between LH pulses and testosterone responses, 4 normal men were given human pituitary LH A-3 one unit/kg iv over 1 min. This resulted in a pulsatile nine-fold mean rise in circulating LH, which was about three-fold greater than spontaneous elevations (Nankin & Troen 1971, 1972) but there were no significant increases
in circulating testosterone over the next 6 h (Nankin et al. 1975). In order to examine Leydig cell responses in men to more prolonged LH stimulation circulating concentrations of testosterone and the precursor steroid 17 OH-progesterone were determined before, during and after a 3-h infusion of human pituitary LH. The results are reported here.

**Materials and Methods**

The participants were 10 healthy male volunteers (aged 20 to 28 years) who were clinically normal and who had normal semen analyses (Table 1). The techniques used for hormone sampling have been described previously (Nankin et al. 1975). Briefly, the subjects arrived after breakfast and indwelling catheters were inserted into the antecubital veins of both arms. In the left arm the catheters were connected to three-way stopcocks, and normal saline (with 1 U heparin/ml) was infused at 25 ml/h, to keep the tubing patent for obtaining blood samples. Tubing was cleared of saline before each specimen was drawn. Between 08.30 and 09.00 h four baseline blood samples were obtained.

In the right arms normal saline was infused in preparation for gonadotrophin administration. At 09.00 h, the subjects were given human pituitary LH LER-1549 (A-3) in a dose of 2 IU/kg (bioassay) of body weight (Table 1). One-third was given intravenously over 1 min and the remaining two-thirds were given over the next 3 h by continuous infusion. At the end of 3 h the catheters were removed from the right arms. Blood samples were obtained every 30 min during the infusions and for 3 h after the infusions were completed. Midway through the final 30 min an extra sample was obtained from each volunteer. Blood specimens were allowed to clot, promptly centrifuged and sera stored at −20°C. The subjects were seated or ambulatory. Smoking was not permitted. Lunch was eaten at 12.00 h. No side-effects were noted. Each volunteer signed an informed consent and the study was approved by appropriate Human Investigation Committees.

The National Pituitary Agency supplied human pituitary LH A-3 (LER-1549) which by bioassay contains 2225 IU of LH and 1.2 IU of FSH per mg. For these studies LH A-3 was prepared in 10 ml aliquots of normal saline containing 10 IU/ml with 18% salt-poor human albumin and stored at −20°C until used. Aliquots were checked for bioactivity during and at the end of the present study. Using rat isolated interstitial cells as little as 0.2 mlU/ml LH A-3 in incubation medium consistently increased testosterone production (unpublished data).

LH was determined in duplicate using the methodology reported previously (Nankin et al. 1975). The intra-assay coefficient of variation is less than 6% and the inter-assay variation is 15%. LH concentrations reported herein are in terms of ng of LH-907. In 24 normal men serum LH was 64 ng/ml ± 18 (mean ± sd) (Nankin et al. 1977). For comparison, in the present radioimmunoassay system, 1 mg of LH-907 (60 IU bioassay) is equal to 210.6 IU IRP-2, and is equal to 63 IU (bioassay) of LH A-3 (LER-1549) or bioassay/radioimmunoassay ratio of 1.05 for LH A-3. Data supplied by the National Pituitary Agency indicates that LH A-3 has a bioassay/radioimmunoassay ratio of 1.28 when compared against LER-907. Radioimmunoassay potency differences may be related to the different anti-LH sera used.

Testosterone was determined using previously reported methodology (Nankin et al. 1975) with one minor modification. A new rabbit antitestosterone serum was generated. Using thrice re-crystallized steroid reagents, a mass of dihydrotestosterone equivalent to 14.3 times the mass of testosterone resulted in 50% displacement of column purified [3H]testosterone. Previously, 9.1 times the mass resulted in 50% displacement of testosterone.

In our laboratory testosterone averaged 645 and ranged from 370 to 1140 ng/100 ml in one group of 20 normal men (Nankin et al. 1975) and in 16 other normal men ranged from 301 to 977 ng/100 ml with a mean of 564 (Nankin et al. 1977). The coefficients of variation are 6% for intra-assay variation and 11% for inter-assay reproducibility.

17 OH-progesterone was determined by radioimmunoassay using the methodology reported by Pang et al. (1977) with minor modifications. The same highly specific rabbit antisera to 17 OH-progesterone (prepared by A. H. Surve, Ph.D.) was utilized permitting radioimmunoassay after extraction without the use of chromatography. The intra-assay coefficient of variation is 5.2% while inter-assay variation is 12.3%.

All serum specimens from a given subject were analyzed together for each hormone. Statistical analyses were performed using Student's t-test for paired observations.

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**Table 1.**

<table>
<thead>
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<th>Subject No.</th>
<th>Age (years)</th>
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<th>Total LH A-3 given (IU)</th>
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**Fig. 1.**
LH concentrations in four randomly chosen group members (means ± se)

**Table 2.**

<table>
<thead>
<tr>
<th>Subject No.</th>
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</tr>
<tr>
<td>351</td>
<td>617</td>
</tr>
<tr>
<td>352</td>
<td>464</td>
</tr>
</tbody>
</table>

| mean ± se   | 698 ± 72               | 685 | 749 | 787 | 767 | 804 |
| Elapsed time in min. |
|             | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 | 300 | 330 | 345 | 360 |

b, c, d, e: See Fig. 2 legend.
Results

Mean baseline titers of LH for the 10 normal controls averaged 69 ng/ml ± 27 (SD) and ranged between 36 to 108 ng/ml and were similar to previous studies in other normal volunteers. With 4 of the volunteers (No. 281, No. 335, No. 336 and No. 340), all of the serum specimens from each respective study were analyzed for LH. The means (± SE) are depicted in Fig. 1. LH rose promptly by an average of 5.2-fold with LH administration and declined after the gonadotrophin infusion was discontinued. Limited quantities of serum precluded analyses of all specimens.

All of the mean testosterone results are given in Table 2. Mean baseline titers of testosterone based on four specimens per subject ranged from 464 to 1139 ng/100 ml in the study population. Pre-treatment testosterone averaged 698 ng/100 ml ± 72 (mean ± SE) for this group. Thirty minutes of LH infusion resulted in no significant change (mean 685 ng/100 ml) and there was a non-significant increase by 60 min (to 749 ± 64). By 90 min testosterone rose to 787 ± 67 which was significantly greater than baseline values (P < 0.03). During the remainder of the LH infusions mean ± SE testosterone titers were: 767 ± 60 at 120 min (P < 0.04); 804 ± 69 at 150 min (P < 0.001); and 764 ± 66 at 180 min (P = N.S.). With termination of the LH infusion testosterone titers fell to 709 ± 65 at 210 min (P = N.S. compared to 180 min) and to 650 ± 47 at 240 min (P < 0.01, compared to 180 min). Thereafter there were no significant changes of testosterone although the titers progressively rose at 330, 345, and 360 min. The mean responses are depicted in Fig. 2. Subjects No. 281 and No. 344 who had the highest baseline titers also demonstrated the smallest responses to gonadotrophin administration. In fact, the mean testosterone concentrations during LH infusions were lower than baselines for these two individuals (No. 281 baseline 1139, infusion 1129 ng/100 ml; No. 344 baseline 1036, infusion 929 ng/100 ml). For the other 8 subjects mean testosterone titers were higher during the 3 h gonadotrophin infusion than respective baseline titers.

17 OH-progesterone levels were determined in 9 of the subjects (Table 3). Baseline concentrations were 142 ng/100 ml ± 16 (mean ± SE). With LH stimulation this testosterone precursor gradually rose to 143 ± 18 at 30 min; to 151 ± 15 at 60 min, to 170 ± 13 at 90 min, and to 178 ± 18 at 120 min (P < 0.05 compared to baseline). At 150 and 180 min the concentrations declined slightly. By 240

Testosterone and 17 OH-progesterone concentrations (mean ± SE). Statistical comparisons a, b, c, and d are made with respective basal concentrations, whereas e and f are comparisons with respective 180 min steroid concentrations – a: P < 0.05; b: P < 0.04; c: P < 0.03; d: P < 0.001; e: P < 0.01; f: P < 0.005.
min 17 OH-progesterone had fallen to 134 ± 12 (P < 0.005 compared to 180 min). Although titers gradually rose there were no significant changes during the remainder of the study. The mean responses are depicted in Fig. 2.

Discussion

The current study was performed utilizing purified human pituitary LH, which although 5% more potent biologically than indicated by the current radioimmunoassay, can be closely approximated by this radioimmunoassay. With the use of 2 IU/kg (bioassay) one-third given as a 1 min iv push and the rest by 3 h infusion causing a 5.2-fold rise of LH (by RIA), it was found that a group of 10 normal men demonstrates testosterone elevations averaging 15% (at 150 min) or less, and 17 OH-progesterone mean elevations of 25% (at 120 min) or less. Mean levels of both steroids show non-significant declines during the final hour of LH infusions. With termination of the 180 min gonadotrophin infusions concentrations of both steroids fell significantly at 240 min. Although both steroids rose gradually between 300 and 360 min these changes were not significant.

Similar patterns to testosterone and 17 OH-progesterone were reported after hCG administration. Saez & Forest (1979) report that testosterone initially peaks within 2 h after 6000 IU hCG given im, and peaks between 2 to 4 h after 6000 IU hCG given iv. Forest et al. (1979) noted peak rises of 17 OH-progesterone to occur within 2 h of 6000 IU of hCG given iv or im. Testosterone plateaued at lower concentrations for 24 h and 17 OH-progesterone decreased for 8 h and then both steroids have secondary rises. The possible causes for early peaks, nadirs and secondary peaks are reviewed in those two reports and in the next paragraph. The present study was performed with LH which has a much shorter half life than hCG and ended at 360 min – so we do not know if a statistically significant secondary rise occurred for testosterone or for 17 OH-progesterone.

Present results suggest that men who have baseline titers of testosterone at the upper limits of the normal range have blunted testosterone responses but normal 17 OH-progesterone elevations to the acute infusion of LH. Most probably, these men have just experienced spontaneous testosterone elevations. The failure of the testes to then respond to administered gonadotrophin may be related to changes within the Leydig cell. These same me-
Mechanisms may be involved in the early peaking of these two steroids. Among the mechanisms which could be involved are that LH can cause desensitization of LH receptors and biosynthetic blocks (Dufau et al. 1978). Those investigators also found small doses of hCG block 17–20 lyase, while larger doses of gonadotrophin block pregnenolone formation. It is possible that the two men with blunted testosterone rises and normal 17 OH-progesterone increases demonstrate 17–20 lyase blocks. It has been speculated that a buildup of oestradiol, 20α-dihydroprogesterone or 20α-dihydropregnenolone could block testosterone biosynthesis. Using in vitro techniques, these steroids have been shown to inhibit production of testosterone (Yanaihara et al. 1972; Fan et al. 1974). These blocks may be either relative or temporary as several Leydig cell stimulation protocols utilizing large doses of hCG have demonstrated consistent and substantial elevations of testosterone in normal men within 30 min and lasting for at least 360 min (Maurer et al. 1973), or by 12 and 24 h after the start of a 4 h infusion (Vivanco et al. 1973), and lasting up to 72 h after a single im injection (Smals et al. 1979). These results may be related to the longer half-life of hCG (when compared to LH) and the doses of hCG administered. This topic was reviewed by Mahoudeau et al. (1975).

Kjeld et al. (1976) administered hLH alone to two men, and hLH with hFSH on four occasions beginning after 12.00 h. Twenty-five per cent of the gonadotrophin was given as a priming dose and the remainder was given as a 4 h infusion. During the infusion circulating LH rose more than 18 times basal as measured by radioimmunoassay. They noted that testosterone responses were sluggish and reached maximum concentrations 7–8 h after finishing the infusion. Testosterone was increased from 17% to 68% over levels determined on respective basal days. Based on hourly blood samples, those investigators did not note the statistically significant testosterone elevations during LH infusion that are presently reported. The reason for this difference is not clear. One protocol variation should be noted. We began LH infusions at 09.00 h while Kjeld et al. (1976) started gonadotrophin administration between 12.00 and 14.00 h. We have preliminary data suggesting that in men Leydig cell responsiveness is different in the morning than it is in the afternoon.

Under normal circumstances what increases Leydig cell production of testosterone? An experimental protocol, with partial LH suppression has shown that LH pulses also cause pulsatile increases of testosterone (Loriaux et al. 1977). This may be the mechanism for nocturnal pulsatile testosterone rises in pubertal boys where low daytime LH titers are followed by nocturnal increases in gonadotrophin. However, in adult males under normal circumstances it was either not possible to find LH elevations followed by testosterone pulses (Wieland et al. 1973; Murray & Corker 1973; Alford et al. 1973b; DeLacerda et al. 1973), or else testosterone elevations occurred after one-third of LH rises (Naftolin et al. 1973; Judd et al. 1973). Therefore, in normal men most pulsatile rises of LH are not promptly followed by elevations of androgen. LH is released by the pituitary tonically with superimposed periodic elevations (Nankin & Troen 1975), and tonic LH stimulation as demonstrated herein appears to cause enhanced Leydig cell function. Tonic hCG stimulation also increases testosterone production by Leydig cells.

In conclusion, a 3 h LH infusion causes significant increases in circulating testosterone and 17 OH-progesterone in a group of 10 normal men. The two men with the highest baseline mean concentrations of testosterone demonstrated the smallest responses of that steroid, but in both 17 OH-progesterone titers adequately rose. This could represent a biosynthetic block. Both steroids peak before the LH infusion ends. Leydig cell 'autoregulation' and tonic gonadotrophin stimulation appear to have major influences on testosterone production.

Acknowledgments

These studies were supported by the Veterans Administration and NIA-NIH Grant No. 1 RO1 AG 01217-01 REB. The National Pituitary Agency kindly supplied LH A-3 (LER-1549) and some of the reagents used for gonadotrophin radioimmunoassay. Antiserum to 17 OH-progesterone was a generous gift from Dr. A. H. Surve. Appreciation is expressed to Mrs S-W Ke, Mrs I Grim, Mr J Marchwinski, Mr J Kilgore and Mr J Lane for technical assistance and to Mrs A Martin for secretarial assistance.

References


Received on October 16th, 1979.