Comparison of in-vitro bioactivity and immunoreactivity of serum LH in normal cyclic and hypogonadal women treated with low doses of LH-RH

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Summary. The in-vitro test of rat interstitial cell testosterone secretion and a radioimmunoassay (RIA) were used to explore the nature of the LH released in women in response to LH-RH. The ratio of in-vitro bioactivity to immunoreactivity (B:I) calculated for serum samples collected from hypogonadal women using the standard LER-907 for comparison was 13.25 ± 0.56. This mean ratio was significantly elevated ($P < 0.02$) above that found for normal cyclic women, 9.48 ± 0.49. After one i.v. injection of 10 µg LH-RH, 5 hypogonadal and 6 luteal-phase women showed an initial significant drop in the B:I ratio ($P < 0.05$ and $P < 0.02$ respectively) followed by a steady significant rise ($P < 0.03$; $P < 0.01$ respectively). This drop in the B:I ratio as immunoreactive LH rose and the rise in the B:I ratio as immunoreactive LH fell may be the result of the release of two or more kinds of LH, one or more with a slower in-vivo clearance rate and an increased B:I ratio.

Introduction

The use of in-vitro bioassays for human serum luteinizing hormone (LH) has recently increased (Dufau, Beitins, McArthur & Catt, 1976; Graesslin et al., 1976; Romani, Robertson & Diczfalussy, 1977) due to disparities found between LH quantifying techniques using immunoreactivity and those utilizing receptor activation. Serum LH levels measured by in-vitro bioassay are frequently higher than those determined by radio-immunoassay. These differences in the biological and immunological potency of serum LH are more marked in men and in post-menopausal women, and in normal late-follicular and luteal-phase women after subcutaneous injection of 100 µg LH-RH than in normal cyclic women with no LH-RH stimulation (Dufau et al., 1976). Changes in the ratio of biologically active:immunologically active LH in the blood could be associated with physiological differences in the rate or nature of LH secretion. Because structural alterations in the LH molecule may affect the biological and antigenic properties of this molecule differently and may arise too rapidly to be observed by infrequent sampling, we have used rapid sampling times, and an in-vitro bioassay and a radioimmunoassay for LH to explore the nature of the LH released in response to low doses of LH-RH.

0022-4251/82/030045-07$02.00/0
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Materials and Methods

Twenty-one regularly cyclic women with cycle lengths of 27–30 days and 12 hypogonadal women with ovarian deficiency of diverse aetiology (post-menopause, ovariectomy, gonadal dysgenesis, and premature ovarian failure) volunteered for this study. Gonadotrophin and ovarian steroid concentrations were determined in appropriate daily blood samples. These and the menstrual calendar were used to identify the stage of the cycle. The LH release in response to pulses of LH-RH (2 i.v. injections of 10 μg LH-RH 2 h apart) or a constant LH-RH infusion (0.2 μg/min infused over a 4-h interval using a calibrated Harvard pump) was assessed by in-vitro bioassay and radioimmunoassay. Blood samples were obtained at 15-min intervals for 30 min before LH-RH administration and then throughout the remainder of the study, according to a standard technique (Yen, VandenBerg, Rebar & Ehara, 1972).

The bioactivity of plasma LH was determined by the rat interstitial cell testosterone assay as described by Dufau et al. (1976) and the immunoreactive LH was measured by a double-antibody radioimmunoassay (RIA) (Yen, Llera, Pearson & Littell, 1968). The standard used in the bioassay was the pituitary standard 69/104 which is an ampouled version of LER-907 derived from the same source (Bangham et al., 1973). By bioassay and RIA, 2.05 ng 69/104 = 1 ng LER-907. The bioassay and RIA results were compared in terms of the LER-907 standard. The interassay coefficient of variation for the RIA ranged from 14.7% at 70% bound to 9.9% at 20% bound. The interassay coefficient of variation for the bioassay was 12.0%.

The in-vitro bioassay was a 3 point bioassay, run in duplicate. Slopes of control sera were found to be parallel to LER-907, 69/104, as well as to preparations of PMSG and hCG. The 69/104 standard and the control sera proved parallel by RIA to LER-907. The immunoassay showed less than 1% cross-reactivity with the alpha subunit and total cross-reactivity with the beta subunit. FSH did not cross-react.

The LH used for i.v. injection in one experiment with a luteal-phase woman was human pituitary LH (Lot no. A-2, 2000 i.u./vial by the ventral prostate test). This human pituitary LH was from the National Pituitary Agency.

Statistical analyses were done by using Student’s paired t tests, group t tests, linear regression analysis, and analysis of variance. On all graphical displays, standard error bars are shown.

Results

Initially, baseline serum LH concentrations were determined by both assays for all 33 women. The mean B:I ratio for hypogonadal women was significantly elevated above that of luteal-phase women (P < 0.02) although the ranges were similar in the two groups. When LH release in luteal-phase women in response to 2 injections of LH-RH was measured by bioassay and RIA, the profiles differed significantly (Text-fig. 1a) as shown by the changes in the B:I ratio. As LH rose (total rise by bioassay = 468.5% and by RIA = 824.8%) in response to the first LH-RH injection, the B:I ratio fell (P < 0.02 when each of the three baseline samples was independently compared with the B:I ratio of the sample 15 min after the first LH-RH injection). As LH values fell (46.0% by bioassay and 69.0% by RIA), the B:I ratio began to rise; the ratio peaked at the point where immunoreactive LH was back to baseline levels (P < 0.01 when the B:I ratio for the sample 15 min after the first LH-RH injection was compared with that after 105 min). With the second LH-RH injection, LH again rose (total rise = 159.5% by bioassay, 442.9% by RIA) and B:I ratio dramatically fell (P < 0.02 when the B:I ratio for the sample immediately before the second LH-RH injection was compared with that for the sample 15 min after the second LH-RH injection). Serum LH levels then fell (total fall = 38.3% by bioassay and 67.0% by RIA) and the B:I ratio rose (P < 0.02 for comparison of samples 15 min and 105 min after the second LH-RH injection).
Text-fig. 1. The results on serum LH values (mean ± s.e.m.) of (a, b) two injections of 10 μg LH-RH 2 h apart into (a) 6 normal luteal-phase women, and (b) 5 hypogonadal women and (c) of infusion of LH-RH at 0.2 μg/min in 4 h into 5 periovulatory women. The number of samples is indicated if not equal to that of the whole group.
The half-lives of LH disappearance (not correcting for basal LH release from the subject) were calculated by linear regression analysis for LH falling in the circulation after the 2 LH-RH injections: the value was 110.7 min for LH measured by bioassay and 68.8 min for LH measured by RIA (P < 0.0003). The rises in the B:I ratio starting 30 min after each LH-RH injection, when LH was rapidly falling, were linear (P < 0.004 after the first LH-RH injection, P < 0.0005 after the second LH-RH injection) and were not significantly different.

The results for 5 hypogonadal volunteers are shown in Text-fig. 1(b). While LH rose in response to the first LH-RH injection (total rise = 189.9% by bioassay and 321.8% by RIA), the B:I ratio fell significantly (P < 0.05 when the mean B:I ratio from the 3 baseline samples was compared with that of samples collected 15, 30 and 45 min after the injection). Then, while the LH fell (total fall = 28.8% by bioassay and 39.2% by RIA), the B:I rose (P < 0.03, for the samples collected 15, 30 and 45 min after the first LH-RH injection compared with samples collected 75, 90 and 105 min after the first LH-RH injection). Although LH concentrations rose (both assay methods) after the second LH-RH injection, the B:I ratio followed no consistent pattern.

Five periovulatory women (4 early luteal and 1 late follicular) volunteered for a third study which involved continuous infusion of LH-RH (Text-fig. 1c). There was a large increase in LH values (total rise = 1922.1% by bioassay and 2187.7% by RIA) and the B:I ratio dropped significantly (P < 0.006 for samples collected 15, 30 and 45 min after the start of the infusion compared with samples collected at 120, 135 and 150 min). During the rest of the infusion the LH values and the B:I ratios stabilized.

A preliminary experiment involving injection of a luteal-phase woman with 200 i.u. of a purified human pituitary extract (B:I ratio of 2.1) was done. As the LH concentration rose after injection, the B:I ratio dropped, and remained constant for the rest of the study although LH values fell (Text-fig. 2).

Discussion

The range in the B:I ratio for circulating LH in normal premenopausal and hypogonadal women (Table 1) indicates that separate structural properties of the molecule are being recognized by the LH receptor involved in cell activation and the antibody employed for radioimmunoassay (RIA). Although the ratios in Table 1 are higher than those reported by Dufau et al. (1976), this is primarily attributable to the standard used to compare the bioassay and immunoassay results (LER-907 in this study, the urinary standard HMG 2nd IRP in the study by Dufau et al., 1976). The standard 69/104 derived from LER-907 contains immunoreactive LH which can be separated from bioactive LH by isoelectric focussing (Robertson, Froya & Diczfalussy, 1978) and hence gives a higher value for RIA measurements. The baseline B:I ratios reported here are consistent with those reported by Romani et al. (1977), who used pituitary standards similar to ours for comparison between assays.

Table 1. The bioassay to immunoassay ratio (B:I) for LH measurements in women

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of subjects</th>
<th>No. of samples</th>
<th>B:I</th>
<th>Range</th>
<th>Mean ± s.e.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early follicular</td>
<td>1</td>
<td>9</td>
<td>5.52–8.13</td>
<td>6.80 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>Late follicular</td>
<td>2</td>
<td>11</td>
<td>4.22–16.10</td>
<td>8.59 ± 1.02</td>
<td></td>
</tr>
<tr>
<td>Surge</td>
<td>2</td>
<td>14</td>
<td>5.48–10.44</td>
<td>6.87 ± 0.32</td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td>16</td>
<td>79</td>
<td>2.58–26.27</td>
<td>10.65 ± 0.66</td>
<td></td>
</tr>
<tr>
<td>All phases</td>
<td>21</td>
<td>113</td>
<td>2.58–26.27</td>
<td>9.68 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>Hypogonadal</td>
<td>12</td>
<td>109</td>
<td>6.13–28.85</td>
<td>13.25 ± 0.56</td>
<td></td>
</tr>
</tbody>
</table>
Consistent with the data reported by Dufau et al (1976), the B:I ratio for LH in the sera of hypogonadal women was significantly elevated above the ratio in normal cyclic women. The rise in the B:I after LH-RH administration in luteal-phase women shown by Dufau et al. (1976) was also confirmed. However, after immunoactive LH values began to fall we observed a consistent drop in the B:I ratio preceding this rise. While LH was rising most rapidly, the B:I ratio was at its lowest point; then, as LH concentrations were falling in the circulation, the B:I ratio rose. This was found to be true for low-dose LH-RH administration experiments (2 injections 2 h apart and 4 h LH-RH infusion) in luteal-phase women and for the first 10 µg LH-RH injection in hypogonadal women. These data differ from those of Beitins, Dufau, O'Loughlin, Catt & McArthur (1977) and Beitins et al. (1980) who used a similar infusion protocol but found no changes in the B:I ratio with LH release in men and women.

The B:I ratios for plasma LH during the period of the LH surge have been reported to be significantly lower than those throughout the remainder of the cycle (Robertson, Puri, Lindberg & Diczfalusy, 1979). Since LH rises most rapidly during the LH surge, the lower B:I ratio found for plasma samples during the surge is consistent with the decreased B:I ratios observed as LH was rapidly rising in the circulation in the present study. Ioelectric focussing has shown that bioactive LH in mid-cycle plasma is physicochemically different from that in post-menopausal plasma (Robertson, Van Damme & Diczfalusy, 1977), indicating that different forms of LH may be responsible for changes in the B:I ratio.

Two possible explanations for the biphasic initial drop followed by a rise in the B:I ratio after a low-dose LH-RH injection are: (1) a metabolic change in the LH molecule after release from the pituitary, which renders it more bioactive and/or less immunoreactive, or (2) release of

![Image](image-url)

**Text-fig. 2.** The effects on serum LH values of injection of 200 i.u. purified human pituitary LH into one luteal-phase woman.
2 or more kinds of LH, one or more with a slower in-vivo clearance rate and an increased B:I ratio. It is also possible that differences in the B:I ratio are related to the beta subunit LH reacting with the RIA system but being devoid of biological activity. LH subunits are released with LH in response to LH-RH administration (Hagen & McNeilly, 1975; Rosemberg & Bulat, 1979). However, the plasma levels of subunits reported by Hagen & McNeilly (1975) are too low to account for all the decrease in the B:I ratio when LH values are rapidly rising; additional factors must therefore be responsible for at least part of this effect. We have not tested the possibility that the beta subunit alone is responsible for changes in the B:I ratio with LH-RH stimulated LH release.

Changes in the B:I ratio are more likely to occur during the process of gonadotrophin secretion, rather than through alterations in the molecular structure of the circulating hormone (Sharpe, Shahmanesh, Ellwood, Hartog & Brown, 1975; Liu, Ax & Jackson, 1979; Mukhopadhyay, Leidenberger & Lichtenberg, 1979). LH released from rat pituitary glands during in-vitro stimulation with high concentrations of LH-RH has a higher B:I ratio than pituitary LH or LH secreted in response to low doses of LH-RH (Sharpe et al., 1975). Similar results have been found with rat pituitary cultures, showing that the B:I ratio for LH released in response to LH-RH differs from a form of rat pituitary LH which has a lower B:I ratio and is of larger molecular weight (Liu et al., 1979; Mukhopadhyay et al., 1979). This pituitary LH form has been proposed as a possible prohormone for LH (Liu et al., 1979).

Although there is some evidence that metabolic changes in LH occur after its secretion in man (Leidenberger et al., 1976) and rat (Campbell, Nansel, Meinzer, Aiyer & Bogdanove, 1978), we suggest that the changes in the B:I ratio after LH-RH injection are the result of a secretory phenomenon. When highly purified human pituitary LH with a low B:I ratio was injected into a luteal-phase woman the B:I ratio fell as serum LH was rising after the injection (Text-fig. 2) but did not rise as LH concentrations were reduced.

The changes in the B:I ratio after LH-RH stimulation of LH release presented here may be consistent with the “two pool theory” proposed by Hoff, Lasley, Wang & Yen (1977). The initial drop in the B:I ratio observed as LH levels rose in the circulation may be due to secretion of an acute pool form of LH with a low B:I ratio. As LH is activated from the reserve to the acute pool LH, through LH-RH stimulation, this newly activated LH with an elevated B:I ratio may be released and cause an increase in the B:I ratio as LH is disappearing from the circulation. This would be consistent with the addition of carbohydrate which has been shown to be a late step in rat pituitary LH synthesis and which can be stimulated by LH-RH (Liu & Jackson, 1978). An LH molecule with an increased number of sialic acid residues may therefore have a greater influence on receptor binding and target cell activation than on immunoreactivity. Since the changes in the B:I ratio occur in hypogonadal and normal cyclic women after LH-RH administration, and there is such a wide range in the baseline B:I ratio independent of the serum concentrations of steroid hormones, we suggest that the endogenous or exogenous LH-RH concentration or potency may be very important in the determination of what species of LH is released at any particular time. It may also be concluded that, because of the large differences between B:I ratios for LH in baseline and LH-RH-stimulated samples, the RIA does not consistently provide a good estimate of bioactive LH in any particular serum sample.

We are grateful to Dr Jean Rivier (Salk Institute) for the supply of synthetic LH-RH and to J. Aurand for her excellent technical assistance. We thank Dr A. Lein and Dr K. Benirschke for their helpful advice. The LH reference preparation was provided by the National Institute of Biological Standards and Control, London.

References


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Received 9 June 1981