EVALUATION OF THE GONADOTROPHIC RESPONSIVENESS OF THE PITUITARY TO ACUTE AND PROLONGED ADMINISTRATION OF LH/FSH-RELEASING HORMONE (LH-RH) IN NORMAL FEMALES AND MALES

By

Heidi Pinto, Bernardo Leo Wajchenberg, Fabio Bessa Lima, Jayme Goldman, Ana Maria Comaru-Schally, and Andrew V. Schally

ABSTRACT

In normal females, the injection of 25 μg of LH-RH (acute test) induced a greater LH and FSH release from the pituitary in the mid-luteal than in the mid-follicular phase of the menstrual cycle. In normal males, the responsiveness to 25 μg LH-RH was greater than that in females at mid-follicular but not at the luteal stage. The pituitary response to the prolonged LH-RH infusion (0.21 μg/min/8 h) was similar in both phases of the cycle of the females with a decline in serum gonadotrophins after the 4th hour and was paralleled by a significant increase of plasma oestradiol levels. In males the LH, but not the FSH secretion was lower as compared to female subjects, and gonadotrophin levels did not show a fall during the infusion. The acute injection of 25 μg LH-RH at the end of a prolonged infusion induced the same response in the female subjects in both phases of the cycle. In males, the acute test following prolonged infusion produced a similar LH secretion, but a lower FSH response than in females. The comparison of the acute test alone and that preceded by a prolonged LH-RH infusion, demonstrated that, in females, the only sig-
significant differences consisted of a greater LH secretion in the former test in the mid-luteal phase. In males there was greater FSH secretion in the acute isolated test than when this test was given after the prolonged infusion.

Since synthetic LH-RH became available (Schally et al. 1971), gonadotrophin release in response to its stimulus has been studied extensively in animals and in the human beings (Schally et al. 1976, 1978). It has been demonstrated that during prolonged infusion of LH-RH, the pituitary of normal men does not become refractory to LH-RH stimulation (Bremner & Paulsen 1974, 1977; Kley et al. 1974). Moreover, two phases of LH-RH increase have been reported to occur during constant LH-RH administration to the normal adult men (Bremner & Paulsen 1974, 1977; de Lange et al. 1974; de Kretser et al. 1975), to men with idiopathic oligozoospermia (Gennser et al. 1974) and to normal women in the follicular and luteal phases (de Kretser et al. 1976). The biphasic response may be related to the existence of two pools of LH in the human pituitary, one requiring a longer stimulation for the release than the other (Bremner & Paulsen 1974). The effect of sex steroids on the response to LH-RH has also been extensively studied (Gud et al. 1972; Kastin et al. 1972; Nillius & Wide 1972; Schally et al. 1976, 1978; Franchimont 1977; Yen & Tsai 1972; Thomas et al. 1973; Shaw et al. 1974; Jeppsson et al. 1977). In this work we studied the patterns of gonadotrophin release in normal men and women after an acute injection, a prolonged infusion of LH-RH, as well as after the combination of both types of administration in an attempt to shed more light on the dynamics of the response to this hormone.

**Materials and Methods**

*Subjects* – Eight regularly cycling women aged 19–36 years volunteered for this study. An acute injection and a prolonged infusion of synthetic LH-RH were made in the mid-follicular and mid-luteal phases as follows: The acute LH-RH test in the 6th to the 10th day of the cycle (mean ± se: 7.0 ± 1.8 days) and in the 19th to the 24th day (20.6 ± 1.8 days), respectively. Prolonged LH-RH infusion were given in the 8th to the 12th day of the cycle (9.5 ± 1.5 days) and in the 21st to the 26th day (23.1 ± 1.7 days), respectively. The acute test always preceded the chronic one by 2 to 3 days.

Nine healthy adult male volunteers aged 12–26 years were also subjected to the acute test and chronic LH-RH infusion, with an interval of 3 days between them. Informed consent was obtained from all the subjects studied.

For the performance of the acute tests, the patients were given 25 μg of LH-RH as a single rapid intravenous injection and blood samples were collected at −5, 0, 10, 25, 30, 40, 50 and 60 min after the injection for the determination of serum LH and FSH. Plasma oestradiol, for the females, and testosterone for the males, were determined by specific radioimmunoassay, in some of the subjects.
In the prolonged test, LH-RH was administered by constant iv infusion, through an indwelling catheter in one arm, at a rate of 0.21 \( \mu \text{g/min} \) for 8 h in 1000 ml saline solution. The prolonged LH-RH infusion test was performed in all male individuals studied and in 7 of the 8 females who had acute tests done. Two baseline blood samples, separated by 15 min were obtained. During the LH-RH infusion, blood was sampled at 60, 120, 180, 240, 260 and 480 min, when the infusion was stopped. In the acute post-prolonged infusion test, at this time, 25 \( \mu \text{g} \) bolus of LH-RH was administered. The blood samples were obtained at the acute test.

Serum concentrations of LH and FSH were measured by radioimmunoassays (Pinto et al. 1975, 1977), against the 2nd IRP hMG as the standard but expressed in terms of LER-907 preparation which has a content of 35 IU FSH and 219 IU LH per mg. Alle values were reported as ng/ml. The standard paired and unpaired Student's \( t \)-tests were utilized in the statistical analysis of the gonadotrophin data. The changes in plasma LH and FSH following LH-RH infusion were analyzed by mean levels prior to LH-RH administration (basal), peak level attained (peak), maximum increment over basal level (\( \Delta \)), and relationship between basal and maximum increment as the percent gonadotrophin response above basal, i.e., the relative response. The calculation of the relative maximum gonadotrophin response (\( \Delta/\text{basal} \times 100 = \% \)) permitted eval-

![Fig. 1.](image)

Serum LH and FSH levels (mean \( \pm \) sn) after the acute administration of 25 \( \mu \text{g} \) LH-RH in 8 normal adult females (at the mid-follicular and mid-luteal phases) of the menstrual cycle and 9 normal males.
Table 1.
LH and FSH responses to LH-RH in normal females and males*

Females

<table>
<thead>
<tr>
<th>Administration</th>
<th>No. of subjects</th>
<th>Basal Value (ng/ml)</th>
<th>Peak Time (min)</th>
<th>Δ (ng/ml)</th>
<th>Δ %</th>
<th>Area (ng/ml x min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH Acute (25 μg)</td>
<td>8</td>
<td>35.1 ± 14.9</td>
<td>120.1 ± 54.9</td>
<td>30.0 ± 7.1</td>
<td>85.0 ± 42.3</td>
<td>245.4 ± 91.3</td>
</tr>
<tr>
<td>LH-RH Prolonged infusion (100 μg LH-RH)</td>
<td>7</td>
<td>40.7 ± 11.7</td>
<td>797.6 ± 217.9</td>
<td>265.7 ± 68.0</td>
<td>756.9 ± 209.0</td>
<td>1881.9 ± 467.0</td>
</tr>
<tr>
<td>Acute (25 μg) after prolonged infusion of 100 μg LH-RH</td>
<td>7</td>
<td>576.7 ± 204.0</td>
<td>764.0 ± 159.0</td>
<td>497.1 ± 7.6</td>
<td>187.3 ± 76.6</td>
<td>43.9 ± 39.1</td>
</tr>
</tbody>
</table>

FSH Acute (25 μg) | 8 | 310.9 ± 63.9 | 562.6 ± 172.7 | 26.3 ± 7.9 | 252.6 ± 171.4 | 85.5 ± 57.2 | 9666 ± 8352 |
| LH-RH Prolonged infusion (100 μg LH-RH) | 7 | 314.0 ± 96.2 | 1139.7 ± 355.7 | 265.7 ± 68.0 | 825.6 ± 315.3 | 274.8 ± 124.1 | 253708 ± 117013 |
| Acute (25 μg) after prolonged infusion of 100 μg LH-RH | 7 | 718.4 ± 185.8 | 1324.6 ± 540.8 | 495.0 ± 8.7 | 605.1 ± 338.7 | 85.3 ± 43.5 | 19557 ± 10251 |

* mean ± sd.

ulation of the gonadotrophin response to the LH-RH independent of factors influencing the basal level. The areas under the response curves in the 60 min (acute test), 480 min (prolonged test) and 480–540 min (acute post-prolonged test) of gonadotrophin concentrations above basal were used as an index for comparing relative quantitative changes of gonadotrophin secretion. The units used to express this function of gonadotrophin secretion were ng/ml⁻¹.
### Females

#### Luteal phase

<table>
<thead>
<tr>
<th>Administration</th>
<th>No. of subjects</th>
<th>Basal Value (ng/ml)</th>
<th>Peak Time (min)</th>
<th>△ Value (ng/ml)</th>
<th>△ %</th>
<th>Area (ng/ml x min⁻¹)</th>
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<tr>
<td>LH Acute (25 µg)</td>
<td>8</td>
<td>34.0 ± 13.6</td>
<td>25.6</td>
<td>391.0</td>
<td>1114.5</td>
<td>15825 ± 10528</td>
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<tr>
<td>LH-RH Prolonged infusion (100 µg LH-RH)</td>
<td>7</td>
<td>29.0 ± 5.3</td>
<td>240.0</td>
<td>652.6</td>
<td>2241.1</td>
<td>211748 ± 86073</td>
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<tr>
<td>Acute (25 µg) after prolonged infusion of 100 µg LH-RH</td>
<td>7</td>
<td>424.1 ± 154.6</td>
<td>429.9</td>
<td>71.4</td>
<td>6751</td>
<td>130.6 ± 3660</td>
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<tr>
<td>FSH Acute (25 µg)</td>
<td>8</td>
<td>334.5 ± 47.9</td>
<td>35.0</td>
<td>425.5</td>
<td>130.6</td>
<td>17573.4 ± 4868.7</td>
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<tr>
<td>LH-RH Prolonged infusion (100 µg LH-RH)</td>
<td>7</td>
<td>288.1 ± 67.1</td>
<td>274.0</td>
<td>785.3</td>
<td>273.4</td>
<td>258222 ± 122333</td>
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<tr>
<td>Acute (25 µg) after prolonged infusion of 100 µg LH-RH</td>
<td>7</td>
<td>595.9 ± 227.3</td>
<td>496.4</td>
<td>476.4</td>
<td>85.6</td>
<td>15204 ± 5748</td>
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### RESULTS

**Acute LH-RH tests**

The results are shown in Fig. 1 and Table 1. The LH and FSH levels increased progressively after the injection of 25 µg LH-RH, attaining the mean peak in 15 and 30 min blood sample for LH and FSH, respectively, at the mid-follicular phase. During the luteal phase, the gonadotrophin responsiveness was greater than in the follicular phase, although basal values for both gonado-
Table 1 (cont.).
LH and FSH responses to LH-RH in normal females and males*

<table>
<thead>
<tr>
<th>Administration</th>
<th>No. of subjects</th>
<th>Basal Value</th>
<th>Peak Value</th>
<th>Δ Time</th>
<th>Δ %</th>
<th>Area (ng/ml × min⁻¹)</th>
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<tbody>
<tr>
<td><strong>LH</strong></td>
<td></td>
<td>(ng/ml)</td>
<td>(ng/ml)</td>
<td>(min)</td>
<td>(ng/ml)</td>
<td></td>
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<tr>
<td>Acute (25 μg)</td>
<td>9</td>
<td>28.67 ± 9.87</td>
<td>261.89 ± 159.52</td>
<td>22.22 ± 6.18</td>
<td>233.22 ± 158.21</td>
<td>892.00 ± 541.63</td>
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<tr>
<td>LH-RH Prolonged infusion (100 μg LH-RH)</td>
<td>9</td>
<td>24.1 ± 11.1</td>
<td>330.0 ± 84.8</td>
<td>452.1 ± 267.9</td>
<td>404.9 ± 261.5</td>
<td>1883.0 ± 1231.8</td>
</tr>
<tr>
<td>Acute (25 μg)</td>
<td>9</td>
<td>205.5 ± 61.1</td>
<td>682.7 ± 220.2</td>
<td>498.1 ± 5.3</td>
<td>477.2 ± 166.3</td>
<td>232.5 ± 44.6</td>
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<td>Prolonged infusion of</td>
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<tr>
<td>FSH</td>
<td></td>
<td>(ng/ml)</td>
<td>(ng/ml)</td>
<td>(min)</td>
<td>(ng/ml)</td>
<td></td>
</tr>
<tr>
<td>Acute (25 μg)</td>
<td>9</td>
<td>344.0 ± 84.0</td>
<td>905.0 ± 378.5</td>
<td>21.0 ± 7.0</td>
<td>560.7 ± 380.4</td>
<td>171.1 ± 130.8</td>
</tr>
<tr>
<td>LH-RH Prolonged infusion (100 μg LH-RH)</td>
<td>9</td>
<td>374.3 ± 150.9</td>
<td>865.5 ± 183.9</td>
<td>346.6 ± 40.0</td>
<td>429.0 ± 206.7</td>
<td>154.6 ± 90.8</td>
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<tr>
<td>Acute (25 μg)</td>
<td>9</td>
<td>905.1 ± 240.8</td>
<td>1135.7 ± 250.5</td>
<td>497.7 ± 8.3</td>
<td>230.6 ± 140.9</td>
<td>27.5 ± 14.5</td>
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<tr>
<td>Prolonged infusion of</td>
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</table>

Peak values were attained at 30 min and 25 min for LH and FSH, respectively. The comparison of the response has shown that the peak values, maximum increments, relative maximum response and total secretory area for both LH and FSH were significantly greater during the luteal than at the follicular phase.

No significant differences existed in mean basal levels of serum LH and FSH when males were compared with females either at the follicular or luteal phase.
phase. The mean peak LH value after the LH-RH injection, was attained in the males at 25 min. The mean peak value, maximum increment, relative maximum response and secretory area, regarding LH response, were significantly greater than those obtained in normal females at the mid-follicular phase, but not in the mid-luteal phase.

When the same comparisons were made for FSH, males presented significantly higher mean peak values (and also at an earlier time than females) and mean maximum increment over basal values at mid-follicular phase. No significant differences were noticed in all parameters studied, between males and females at the luteal phase.

**Prolonged infusion of LH-RH**

The results shown in Figs. 2 and 3 and Table 1 indicate that in general the gonadotrophin response patterns to prolonged LH-RH infusion in females were similar regardless of the phase in the cycle. Both gonadotrophins reached peak levels within 4 h, except for LH at the luteal phase where a plateau was attained at 180 min. After 4 h, there was a decline of the gonadotrophins up to the time when the 25 µg bolus of LH-RH was given iv. All other response

![Graph showing LH levels during LH-RH infusion](image_url)
Serum FSH levels (mean ± sd) during 8-h continuous infusion of LH-RH (0.21 μg/min) followed by a 25 μg pulse in 7 normal adult females (at the mid-follicular and mid-luteal phases of the menstrual cycle) and 8 normal males.

Figure 3.

Parameters for LH and FSH were significantly different during the LH-RH infusion between the follicular and the luteal phase.

When men were compared to the females in the follicular phase, basal LH levels, peak values and maximum increment above basal and the secretory area, were significantly lower, but not the relative maximum responses.

The comparison between males and females at the mid-luteal phase revealed significant differences only in the total LH secretory area, which was lower in the males. When FSH response was evaluated in males (Fig. 3), a different pattern was observed in relation to females, i.e., progressive increase in FSH levels up to the end of infusion. Basal FSH levels, peak values and the total secretory area were the same, but maximum increments over basal and max-
imum relative response were significantly lower in males in relation to females either at the follicular or luteal phase.

**Acute LH-RH test following prolonged LH-RH infusion**

As shown in Figs. 2 and 3 and Table 1, the LH and FSH levels attained mean peak value in 20 min and 10 min, respectively at the mid-follicular and in 15 min sample for both gonadotrophins at the mid-luteal phase. All parameters of LH and FSH secretion were similar in both phases of the menstrual cycle.

Regarding the males, basal LH values, at the end of the prolonged infusion, were significantly lower in comparison to the females and the mean LH and FSH peaks were attained in the 15 min blood samples (Figs. 2 and 3). Only the maximum increment and relative maximum response were different (higher) in males than in the females. Regarding the FSH levels, although the basal values were higher in males than in the females, the maximum increment, the relative maximum response and secretory area were significantly lower.

**Comparison between the acute LH-RH test preceded or not by prolonged LH-RH infusion**

As would be expected, both basal values and peak LH levels in response to 25 \( \mu \text{g} \) LH-RH were significantly greater after the prolonged infusion of this hormone in the mid-follicular phase \((P < 0.05)\). The LH peak was also attained in a shorter time after the prolonged infusion than during the rapid injection (Figs. 1 and 2). The relative maximum response, but not the secretory area, was significantly greater after the acute test alone than when this was preceded by infusion \((P < 0.05)\).

When the same type comparison was made for serum FSH in the follicular phase, it showed significantly greater basal values \((P < 0.05)\) and the post-infusion peak \((P < 0.05)\) at an earlier time in the acute test following prolonged infusion than in the case of the acute administration alone. The relative increment was similar in both tests but the mean secretory area was somewhat greater after the acute test following prolonged infusion.

The comparison between the acute administration of LH-RH preceded or not by the prolonged on in the luteal phase showed that basal LH and FSH values both were significantly greater and the time to attain the peaks was shorter after prolonged LH-RH infusion. The relative maximum LH and FSH increments, but not the maximum increments, were significantly greater \((P < 0.05)\) after the isolated acute LH-RH injection.

In males, the comparison between the acute LH-RH test and the same test preceded by prolonged infusion, demonstrated that mean basal LH values, the peak LH level and the maximum increment were significantly greater after
the prolonged infusion ($P < 0.05$). However, the relative maximum increment
was significantly greater after isolated acute injection ($P < 0.05$). The same
comparisons made for FSH levels demonstrated higher mean basal level after
prolonged infusion, but the maximum increment, relative maximum response
and secretory area were significantly greater ($P < 0.05$) in the acute test alone.

In men, neither the acute, rapid LH-RH test nor the prolonged infusion in¬
duced significant changes in plasma testosterone during the time of blood col¬
lection (basal = 612 ± 96 ng/100 ml, 60 min value = 562 ± 100 ng/ml and basal
= 642 ± 64 and 705 ± 73 ng/100 ml after 480 min, respectively). No significant
changes were noticed in the oestradiol ($Oe_2$) levels in plasma after the acute
LH-RH test either at the mid-follicular or mid-luteal phase in normal females.
The basal plasma $Oe_2$ levels were significantly higher ($P < 0.05$) in the luteal
$(168.1 ± 53.9$ pg/ml) than in the follicular phase $(86.7 ± 35.8$ pg/ml). The
levels increased significantly at 240 and 480 min of the LH-RH infusion, att¬
taining the values of $112.6 ± 34.0$ and $161.1 ± 28.8$ at the follicular phase
and $211.5 ± 59.4$ and $254.6 ± 55.0$ pg/ml at the luteal phase respectively
(Table 2).

**DISCUSSION**

The studies reported here confirm and extend previous investigations on the
responsiveness to LH-RH in human beings (Kastin et al. 1972; Nillius & Wide
1972; Yen & Tsai 1972; Thomas et al. 1973; Shaw et al. 1974; Schally et al.
1972, 1976; Gual et al. 1972; Wollensen et al. 1976; Bremner & Paulsen 1974;

<table>
<thead>
<tr>
<th>Table 2.</th>
<th>Oestradiol levels during prolonged LH-RH infusion in females.</th>
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<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of Subjects</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>Follicular</td>
<td>7</td>
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<tr>
<td>phase</td>
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<td>Luteal</td>
<td>4</td>
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<tr>
<td>phase</td>
<td></td>
</tr>
</tbody>
</table>

* mean ± sd.
** $P < 0.05$
The responses to the acute administration of 25 µg LH-RH were greater at the mid-luteal than at the mid-follicular phase in normal females studied, possibly due to the interaction of the gonadal steroids, particularly oestradiol, with the LH-RH at the pituitary level (Gnaal et al. 1972; Schally et al. 1972; Lasley et al. 1975; Nillius & Wide 1972; Yen & Tsai 1972). In effect, the greater responsiveness of both gonadotrophins at the luteal phase rather than during the follicular phase is probably due to the augmenting effect of the progesterone levels on the pituitary sensitivity to LH-RH in the presence of higher oestradiol levels (Lasley et al. 1975; Nillius & Wide 1978). Greater pituitary responsiveness to LH-RH at the luteal phase could also be due to the decreased endogenous LH-RH secretion, accounted for by the negative feedback system involving ovarian steroids, with the resultant greater sensitivity to the exogenous LH-RH. In this study we have used a dose of 25 µg LH-RH, much smaller than the usually employed dose of 100 µg, considered by Kastin et al. (1972) and Wollensen et al. (1976) as the minimum effective dose inducing a response for both LH and FSH. Using this dose we have shown that pituitary response to LH-RH is greater for LH in relation to FSH considering only the relative maximum response, the parameter usually described in the literature. Gonzales-Barcena et al. (1973) and Medeiros-Neto et al. (1973) were the first to show a biphasic LH response and an improved response, respectively, after a prolonged infusion of LH-RH, but did not speculate as to the possible mechanism. Vilchez-Martinez et al. (1976) demonstrated, in rats, that the pre-treatment with actinomycin D failed to affect the rise of serum LH and FSH levels after a quick iv injection of LH-RH or 1 h after the start of a 4-h LH-RH infusion, but significantly suppressed the response at 2, 3 and 4 h. These data are also compatible with the existence, in the pituitary gland, of two functional pools of LH and probably of FSH, one that is preformed and readily releasable, and another consisting possibly of a newly synthesized gonadotrophin which requires prolonged stimulation with LH-RH to be released.

The behaviour of normal men to the acute LH-RH administration was similar regarding both gonadotrophins to that of women at the mid-luteal phase, but with the mean FSH values greater and mean LH levels lower, probably due to the effect of endogenous androgens on the pituitary (Franchimont 1977). After infusion of LH-RH for 8 h, in contrast to the acute administration, there was no significant difference in responses of both gonadotrophins by all the criteria analyzed, despite the modulating effect of the ovarian steroids which is different in the two phases of the menstrual cycle. During the infusion we missed the early releasable gonadotrophin peak (Bremner & Paulsen 1974, 1977), since no blood samples were drawn in the 1st hour. The progressive decline of the LH and FSH levels after the 4th and 6th hour of the infusion is suggestive of decrease in pituitary sensitivity to LH-RH previously described
by Jewelewicz et al. (1977). However, the concept of refractoriness or decreased sensitivity at the end of the 8-h infusion cannot be readily accepted, since there was an evident response when an acute injection of 25 μg of LH-RH was given. The most probable explanation for this phenomenon is negative feedback on the pituitary by ovarian steroids, primarily oestradiol, attained during the prolonged gonadal stimulation, which can be overcome by the acute administration of LH-RH.

In males, the LH response to the 8-h infusion of LH-RH showed the same pattern as that demonstrated by the female subjects, i.e., a decrease in the mean values at the 6th and 8th hour, but no significant increases in testosterone levels were noticed. In contrast to the females, the FSH levels gradually increased, as described by Kley et al. (1974) and by Bremner & Paulsen (1977). From the data presented, no explanation was apparent for the dissociation between LH and FSH response in normal males.

Regarding the acute administration of LH-RH after a prolonged infusion, there were no significant differences between the results obtained in the two stages of the menstrual cycle for FSH and LH. This suggests that, after the prolonged infusion, the acute release of gonadotrophin with a 25 μg dose of LH-RH is maximal or constant independently of the phase of the cycle.

ACKNOWLEDGMENTS

The work reported here was supported by grants from Hochst do Brazil and Ayerst Lab. do Brazil.

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Received on November 7th, 1978.