Response of Serum LH, FSH and Prolactin to Injection of Synthetic LH–RH into Rat Anterior Pituitary

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Changes in serum LH, FSH and prolactin were measured by radioimmunoassay following injection of synthetic LH-RH into the anterior pituitary and median eminence of rats. The time course of serum levels of LH and FSH after injection of synthetic LH-RH into the pituitary showed a peak at 15 min. The synthetic LH-RH had a more marked releasing effect on LH than on FSH. After injection of 20 ng into the pituitary, the serum LH rose to about 10.7 times the control level but the serum FSH only to about 1.8 times. A significant rise of serum LH over the control level was noted after injection of more than 0.2 ng of synthetic LH-RH, while that of serum FSH was after injection of more than 2 ng. An injection of 2 ng of synthetic LH-RH into the pituitary following 0.1 µg of estradiol benzoate resulted in a fall of serum LH and FSH levels, and a rise of serum prolactin level. An injection of 2 ng of synthetic LH-RH into the median eminence resulted in a tendency towards slight decrease in serum LH and FSH. No significant response of serum prolactin to synthetic LH-RH was noted.

Serum LH, FSH and prolactin; synthetic LH-RH; rat anterior pituitary

The gonad is controlled by the anterior pituitary, which is further regulated by neurohormones of the hypothalamus. As to the relationship between the hypothalamus and the anterior pituitary, Harris (1947), Green and Harris (1947) advanced the hypothesis that special neurohumoral substances produced in the hypothalamic region reach the anterior pituitary via the pituitary portal vessels and regulate the activity of the pituitary. After many studies of extraction and purification of these substances Folkers et al. (1969) and Burgus et al. (1969) for the first time clarified the structure of thyrotropin-releasing hormone (TRH). Matsuo et al. (1971) thereafter clarified the structure of luteinizing hormone-releasing hormone (LH-RH) and synthesized it. LH-RH extracted from porcine hypothalamus is a polypeptide consisting of 10 amino acids. Since synthetic LH-RH became available, dynamic studies on the gonads, anterior pituitary, and hypothalamus became possible, and numerous reports appeared on basic and clinical studies of the mechanism of action of synthetic LH-RH. In order to further clarify the mechanism of action of LH-RH, we injected synthetic LH-RH into the rat.
anterior pituitary and median eminence and studied the dynamic changes of serum LH, FSH and prolactin, making comparison with the effects of its intravenous injection. Other aspects studied were whether or not estrogen affects on the release of LH-RH at the pituitary level and whether the amount of oxygen has any influence.

**MATERIALS AND METHODS**

*Materials.* Female Wistar rats, 90 to 150 days of age and 200–300 g of body weight were used for the present series of experiments. 3 weeks before the experiments, rats received a bilateral oophorectomy. 50 µg of dipropionate estradiol and 25 ng of 17α-hydroxyprogesterone caproate were subcutaneously injected 3 days before the experiment.

*Experimental methods.* Synthetic LH-RH was injected into the anterior pituitary and median eminence, or intravenously. The modified method of Hedge et al. (1966) was employed for injection of the drug solution into the anterior pituitary using a stereotax, an apparatus for the stereotaxic operation on the brain.

The intravenous injection was carried out via the femoral artery. Experiments were conducted under ether anesthesia. Since the circulation through brain is said to decrease under hypoxia, oxygen was simultaneously administered during the experiment. In the present experiment, the control group received an injection of physiological saline. 0.02, 0.2, 2 and 20 ng of synthetic LH-RH were dissolved separately in 0.5 µl of physiological saline and injected into the pituitary. 0.5 µl of the drug solution containing 2 ng synthetic LH-RH was injected to the median eminence. For intravenous injection, 0.2, 2, 20 and 200 ng of synthetic LH-RH were dissolved in 50 µl of physiological saline. Estradiol benzoate suspension used for injection into the anterior pituitary contained 0.1 µg in 0.5 µl of physiological saline. Serum LH, FSH and prolactin were measured by the method of Monroe et al. (1968). As a reference standard, rat LH, FSH, and prolactin Rp-1 supplied from NIAMD were used in double antibody radioimmunoassay. Student’s *t*-test was used for the statistical evaluation of differences of average values.

**RESULTS**

*Effect of oxygen on serum LH, FSH and prolactin levels*

After injection of 2 ng synthetic LH-RH into the anterior pituitary, the serum prolactin level became significantly lower in the group without oxygen administration than in the group with oxygen administration (*p*<0.01). The serum LH and FSH in the former group were also lower by 31.1 and 12.4% in the average values, respectively, though the differences were not significant (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Serum LH (ng/ml)</th>
<th>Serum FSH (ng/ml)</th>
<th>Serum prolactin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With oxygen</td>
<td>540.8±49.5</td>
<td>463.0±21.2</td>
<td>186.4±17.2</td>
</tr>
<tr>
<td>Without oxygen</td>
<td>372.1±48.2</td>
<td>405.8±39.0</td>
<td>57.5±3.7</td>
</tr>
</tbody>
</table>

Mean values±s.e.
Time courses of the LH and FSH levels

Time courses of changes of the LH and FSH levels following injection of 2 ng of synthetic LH-RH into the anterior pituitary revealed that the LH and FSH levels reached their maxima 15 min after injection, the values of which were approximately 4.3 and 1.6 times the control values, respectively, thereafter both of them tended to decrease gradually until 30 min after injection (Table 2). Consequently, all the following experiments were performed under oxygen administration and measurements of serum LH, FSH and prolactin were carried out on samples obtained 15 min after injection.

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>Serum LH (ng/ml)</th>
<th>Serum FSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>155.7±46.2</td>
<td>298.2±30.0</td>
</tr>
<tr>
<td>5</td>
<td>348.6±40.6</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>398.5±67.5</td>
<td>398.7±18.9</td>
</tr>
<tr>
<td>15</td>
<td>540.8±49.5</td>
<td>463.0±31.2</td>
</tr>
<tr>
<td>30</td>
<td>267.2±63.5</td>
<td>346.1±44.7</td>
</tr>
</tbody>
</table>

Mean values±s.e.

Dose of LH-RH and LH value

The measurement of serum LH value following injection of synthetic LH-RH into the pituitary revealed that a slight but insignificant increase was caused at a dose of 0.02 ng, a significant increase of about 10.7 times the control value at 20 ng ($p<0.001$).

Following intravenous injection of synthetic LH-RH, the serum LH level rose. A value approximately 1.5 times the control was measured after administration of 0.2 ng, but this was not statistically significant. At a dose of 2 ng, the serum LH rose to about 1.9 times the control level ($0.05<p<0.1$). At 20 and 200 ng, the values rose to about 2.8 ($p<0.01$) and 10.9 ($p<0.001$) times, respectively (Fig. 1).

Dose of LH-RH and FSH value

After injection of 0.2 ng of synthetic LH-RH into the pituitary, the serum FSH rose above the control level but this was not statistically significant. Significant increases of serum FSH were noted after injection of 2 ng ($p<0.01$) and 20 ng ($p<0.001$), to about 1.8 times the control level at the latter dose.

An intravenous injection of 20 ng of synthetic LH-RH caused a significant increase of serum FSH above the control level ($p<0.01$), while injections of 0.2 and 2 ng tended to give higher values than in controls but the differences were not significant. An injection of 200 ng resulted in an increase of serum FSH to approximately 1.7 times the control level (Fig. 2).
Fig. 1. Values of rat serum LH after intravenous and intrapituitary injections of various doses of synthetic LH-RH. ■, intravenous injection; □, intrapituitary injection.

Fig. 2. Values of rat serum FSH after intravenous and intrapituitary injections of various doses of synthetic LH-RH. ■, intravenous injection; □, intrapituitary injection.
Dose of LH-RH and prolactin value

The serum prolactin value showed no significant change from the control level either in the group received an injection of the synthetic LH-RH into the pituitary or in the group received an intravenous injection. No definite relationship was noted among groups given different doses of synthetic LH-RH (Fig. 3).

Effects of estradiol benzoate on LH, FSH and prolactin values

Twenty min after injection of 0.1 μg of estradiol benzoate into the anterior pituitary, the injection of 2 ng of synthetic LH-RH caused a fall of serum LH by 46.7% as compared with the level attained without injection of estradiol benzoate. This fall was statistically significant (p<0.02). In the group pretreated with estradiol benzoate, the serum FSH level after LH-RH injection was lower by 19.8% than the corresponding level in the group without estradiol benzoate (0.05<p<0.1). In the former group, an injection of 2 ng LH-RH into the pituitary resulted in a significant increase of serum prolactin, as compared with the level attained without estradiol benzoate (p<0.01) (Table 3).

Responses of LH and FSH to the injection of LH-RH into the median eminence

When 2 ng of the synthetic LH-RH was injected into the median eminence, the serum LH and FSH rose significantly from each control level (p<0.05), but the
TABLE 3. Values of rat serum LH, FSH and prolactin 15 min after injection of synthetic LH-RH into the anterior pituitary (AP) and the median eminence (ME)

<table>
<thead>
<tr>
<th>Materials injected</th>
<th>Site</th>
<th>Serum LH (ng/ml)</th>
<th>Serum FSH (ng/ml)</th>
<th>Serum prolactin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH-RH 2 ng</td>
<td>AP</td>
<td>540.8±49.5</td>
<td>463.0±21.2</td>
<td>186.4±17.2</td>
</tr>
<tr>
<td>Estrogen +</td>
<td>AP</td>
<td>288.5±31.5</td>
<td>371.5±50.9</td>
<td>301.8±38.8</td>
</tr>
<tr>
<td>LH-RH 2 ng</td>
<td>ME</td>
<td>289.0±60.9</td>
<td>411.4±18.5</td>
<td>106.8±16.6</td>
</tr>
</tbody>
</table>

Mean values±s.e.

increases were not as great as those in the group received an injection into the anterior pituitary (Table 3).

**DISCUSSION**

According to the experimental results of Gordon et al. (1972) on dynamic changes of TSH following injection of synthetic TRH, a peak appeared at 5 min after intravenous injection but somewhat later after injection into the pituitary. In our present study, a peak was noted 15 min after injection of synthetic LH-RH. This time course is slightly slower than that reported by Spies and Niswender (1973) after intravenous injection. Such a delay might be explained by the fact that only a small portion of the injected LH-RH did act on gonadotropin-secreting cells and that the remaining large part was injected into the interstitial tissue. Ondo et al. (1973) injected synthetic LH-RH into the hypophyseal portal vessel over a period of 30 min, and demonstrated the peak of LH 30 min after the end of injection.

A definite release of LH and FSH was noted after injection of the synthetic LH-RH. Injection of the synthetic LH-RH into the anterior pituitary at higher doses than 0.2 ng caused a significant rise of serum LH, while at higher doses than 2 ng it also caused a significant rise of serum FSH. At the dose of 20 ng serum LH rose to about 10 times the control level and serum FSH about 1.8 times, indicating that a response of FSH release was considerably lower than that of LH release. However, Arimura et al. (1972), who studied similar responses of LH and FSH to a continuous administration of synthetic LH-RH over a period of 4 hr, noted that such a long-term administration of LH-RH caused a more marked release of FSH.

In our experiments, an injection of only 0.2 ng of synthetic LH-RH caused a significant rise in the serum LH. Furthermore the response was greater than that seen after the intravenous injection, confirming that the synthetic LH-RH acts directly on the pituitary. When the synthetic LH-RH is injected into the median eminence it will probably act directly on the nerve tissue rather than spread to the pituitary. The levels of both LH and FSH in serum significantly increased after injection into the median eminence, but the responses were considerably less prominent than those after direct injection into the pituitary, and approximately similar to the responses to an intravenous injection of the same
dose. This result is consistent with that noted by Gordon et al. (1972) who injected TRH into the median eminence.

The intravenous injection of 200 ng of LH-RH resulted in a rise of serum LH up to 1364.1 ng/ml. This is a similar level obtained after injection of 20 ng of LH-RH into the pituitary. That the serum LH level obtained by the injection of 2 ng of LH-RH into the pituitary was higher than that obtained after intravenous injection of 20 ng of LH-RH, indicates that more than 10 times as much LH-RH is necessary by an intravenous route as compared with a direct injection into the anterior pituitary.

Since the serum prolactin values sometimes decreased and sometimes increased in response to the administration of hypothalamic extract, the presence of PIH and PRH has been suggested. Although the influence of synthetic LH-RH on the prolactin release is an interesting question, no significant change in prolactin was found in the present experiment. Debeljuk et al. (1972) reported similar findings.

Bowers et al. (1971) demonstrated a marked rise of blood prolactin along with serum TSH in response to synthetic TRH, suggesting the action of TRH as PRH. Vale et al. (1973) also reported an increase of prolactin in response to TRH in vitro. In man, therefore, TRH might be PRH itself.

In our present study, an injection of estradiol benzoate into the anterior pituitary prior to injection of 2 ng synthetic LH-RH resulted in a fall of serum LH by about 46% (a significant difference). Serum FSH also fell by about 19% (0.05 <p<0.1). Direct action of estrogen on the pituitary in terms of negative feedback was demonstrated by Bogdanove (1963) in an experiment of pituitary implantation. Recently, Schally et al. (1972) reported the in vitro inhibition by estrogen of the activity of LH-RH in releasing LH and FSH. Negro-Vilar et al. (1973) reported the inhibition of LH release by LH-RH in estrogen treated rats in vitro.

In view of the present experiment with direct injection of estrogen into the pituitary, a direct action of estrogen was suggested on anterior pituitary cells to release prolactin. Kanematsu and Sawyer (1963) suggested the same based on their observation in which the synthesis and storage of prolactin in the pituitary increased following an implantation of estradiol benzoate into the hypothalamus. Chen et al. (1970) demonstrated a release of a large amount of prolactin following injection of estrogen from the transplanted pituitary of rat. Despite the difference in methods used, these results are in good agreement with ours.

In our present experiment, oxygen deficiency was shown to influence on the serum levels of LH, FSH and especially prolactin values following injection of the synthetic LH-RH into the pituitary.

Acknowledgment

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References


