PITUITARY LACTOGENIC HORMONE RELEASE DURING ONSET OF PSEUDOPREGNANCY IN INTACT RATS

By


ABSTRACT

The release of lactogenic hormone (LGH) was studied at the onset of pseudopregnancy in 90 rats. The LGH-content of the pituitary glands was determined by the pigeon crop sac assay. Glass rod stimulation of the cervix during vaginal oestrus was followed by a significant decrease in the LGH-content of the pituitary glands, thus demonstrating a correlation between LGH-release and beginning pseudopregnancy. Anaesthesia by Nembutal almost prevented this effect of cervical stimulation.

It is generally accepted, that during pseudopregnancy the corpora lutea of the cycle persist because of the influence of endogenous lactogenic hormone (LGH) (Mayer 1951; Klein 1954). This theory is supported by reproducible observations, e.g. administration of exogenous LGH to rats normal with cycles, which causes pseudopregnancy (Herlyn et al. 1964), or the persistence of the corpora lutea under the influence of secretion(s) of autotransplanted pituitary grafts (Everett 1956). Wolthuis & De Jongh (1963) gave additional evidence that the autotransplanted pituitary grafts secrete increased amounts of LGH, as measured by LGH-determination in immature hypophysectomized female rats. On the other hand, small amounts of oestradiol also result in persistent corpora lutea in intact rats (Nelson 1951). According to Desclin (1949) oestrogen has a synergistic effect on the luteotrophic activity of prolactin, as dis-

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Thus LGH plays a key role in pseudopregnancy. However, so far as we are aware, there are no data about pituitary LGH at the onset of pseudopregnancy. The purpose of this study was to determine whether the LGH is directly involved in the onset of pseudopregnancy – in other words, an attempt was made to collect data about LGH-«secretion» under the influence of cervical stimulation by means of a glass rod during vaginal oestrus. This procedure was successful in our experiments in inducing pseudopregnancies (Herlyn et al. 1964; v. Berswordt-Wallrabe et al. 1964).

As a criterion for the »secretion« of LGH we decided to determine the LGH-content of the pituitary glands of rats before and 30 min following glass rod stimulation, a model, which was introduced by Grosvenor & Turner (1958 a, b) for the determination of pituitary concentration of LGH under the influence of suckling litters.

**MATERIAL AND METHODS**

*Rats:* A total of 90 rats of the FW 49 strain were housed and fed under identical conditions, as previously described (Herlyn et al. 1964). Vaginal smears were taken daily until the termination of the experiment.

When 30 of the rats showed full oestrous smears, they were killed with ether. Pituitary glands were quickly removed, weighed on a Sartorius balance to the nearest 0.01 mg and stored in the deep freeze until assayed for the LGH content.

Thirty rats, showing full oestrus in the vaginal smears, were stimulated with a glass rod at the cervix uteri, as if inducing pseudopregnancy. Thirty rats were injected with Nembutal, 45 mg/kg i.p. When completely anaesthetized, the stimulus described above was applied. Thirty min after the stimulus, the animals were sacrificed and the pituitary glands dealt with as described.

**Assay procedure of the pigeon crop sac test:** 251 adult common pigeons of both sexes weighing 221–484 g were obtained and housed in a room artificially illuminated during the normal daylight hours. They were fed mixed grain and aqua fort. ad libitum.

The test was carried out according to Grosvenor & Turner (1958 a). 13 to 16 pigeons were used for each assay. The ovine LGH preparation was dissolved in sterile NaCl 0.9% solution. The centre of the crop sacs over which the feathers had been removed, was marked by a spot of indigocarmine 4 mg/ml. 1 ml of the solution was then injected intradermally daily for 4 days.

24 hours after the last injection the pigeons were killed by decapitation; the entire crop sac was removed and divided in two halves. Each half was then spread by 2 investigators over a light source, and the area of response was matched by using

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* The LGH-preparation was kindly supplied by Schering AG, West-Berlin, Prolaktin Charge 115/116 S with potencies of 15 IU/mg.
### Table 1.

Pigeon crop sac assay, log dose response curve.

<table>
<thead>
<tr>
<th>Daily injected LHG - amount</th>
<th>No. of crop sac sides</th>
<th>Diameter of response (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg</td>
<td>IU</td>
<td></td>
</tr>
<tr>
<td>0.35</td>
<td>0.00525</td>
<td>15</td>
</tr>
<tr>
<td>0.70</td>
<td>0.0105</td>
<td>14</td>
</tr>
<tr>
<td>1.40</td>
<td>0.021</td>
<td>29</td>
</tr>
<tr>
<td>2.80</td>
<td>0.042</td>
<td>14</td>
</tr>
<tr>
<td>5.60</td>
<td>0.084</td>
<td>25</td>
</tr>
<tr>
<td>11.20</td>
<td>0.168</td>
<td>14</td>
</tr>
</tbody>
</table>

* standard error ($s_R = \frac{s}{\sqrt{n}}$)

### Fig. 1.

Pigeon crop sac test, regression line.

Gauges consisting of a very thin circle of metal, connected to a handle. 19 gauges were used, ranging in diameter from 5 to 50 mm. Most of the proliferation areas are almost circular and can be measured accurately by this method. A response area of 10 mm is the lowest diameter, which can be measured accurately.
The log dose response curve was calculated according to Bliss (1952), resulting in a 6 point assay (Table 1), with 111 birds a regression line with $y = 0.950 + 1.221 x$. $\lambda = 0.352$ (Fig. 1).

Five pituitary glands were pooled for each assay. These were crushed in an agate mortar and suspended in 10 ml of NaCl 0.9%. As in the log dose response curve assay, 0.1 ml of this solution was injected intradermally for 4 days, over one of the crop sides of 13 to 16 pigeons. A saline solution of standard LGH was similarly injected above the opposite crop sac side in some of the assays.

RESULTS

The LGH content was highest in the pituitary glands of the rats, which received no treatment and were killed during vaginal oestrus. In contrast, the LGH content of the glass rod stimulated rats was significantly smaller, 40 098 IU as against 8715 IU. Those rats, stimulated while completely anaesthetized by Nembutal, had a hypophyseal LGH content, which was only slightly reduced, 40 098 IU as against 30 645 IU, at the borderline of significance (Table 2).

Table 2.
LGH content in pituitary glands.

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>No. of Assays</th>
<th>No. of pituitary glands per assay</th>
<th>Average weight of pituitary glands</th>
<th>LGH content of 30 pituitary glands (IU)</th>
<th>Average LGH contents of 5 pituitary glands (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats in oestrus</td>
<td>6</td>
<td>5</td>
<td>10.06</td>
<td>40.980</td>
<td>6.825 ± 0.675*</td>
</tr>
<tr>
<td>Rats stimulated in oestrus</td>
<td>6</td>
<td>5</td>
<td>10.47</td>
<td>30.645</td>
<td>5.100 ± 0.270</td>
</tr>
<tr>
<td>under Nembutal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rats stimulated in oestrus</td>
<td>6</td>
<td>5</td>
<td>10.30</td>
<td>8.715</td>
<td>1.455 ± 0.255*</td>
</tr>
</tbody>
</table>

Student's t-test:

\[ \begin{align*}
   & 2 - 3 & \{ P < .001 \} \\
   & 1 - 3 & \{ P < .01 \} \\
   & 1 - 2 & .025 < P < .05
\end{align*} \]

* standard error \( s_{\overline{x}} = \frac{s}{\sqrt{n}} \)

DISCUSSION

The data presented provide the first report, giving quantitative evidence of LGH depletion of pituitary glands under the influence of mechanical stimula-
tion (by glass rod) of the cervix uteri during vaginal oestrus. Since it was shown, that LGH discharge in response to nursing stimuli is blocked when the mother rats are anaesthetized by Nembutal (Grosvenor & Turner 1958 a, b), it is not surprising, that glass rod stimulated rats, as shown in this report responded by a greatly reduced LGH release from the pituitary gland when under Nembutal anaesthesia.

Although there are no data as yet, which indicate increased LGH content in the serum after cervical stimulation, these short term experiments nevertheless indicate, that LGH probably plays an important role in the onset of pseudopregnancy. Factors, which are involved in the maintenance of pseudopregnancy with particular regard to LGH synthesis and/or secretion, are still unknown, although the findings of Rothchild (1962) and Wolthuis (1963) indicate, that the LGH production is enhanced by a positive feedback.

REFERENCES


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