FAILURE TO INDUCE DECIDUAL CELL REACTION IN LACTOGENIC HORMONE TREATED AND PSEUDOPREGNANT RATS

By

U. Herlyn, H. F. Geller and Ilse von Berswordt-Wallrabe

ABSTRACT

The decidual cell reaction (DCR) was studied in intact rats under influence of exogenous and/or endogenous (by induction of pseudopregnancy) lactogenic hormone (LGH). No or a very small DCR occurred in rats traumatized on day 6 of the experiment after 5 days of LGH treatment, starting at the time of late vaginal oestrus, or after 5 days of pseudopregnancy, induced by glass rod stimulation during vaginal oestrus. All the rats, killed on day 10, had ovaries with active corpora lutea. The absence of DCR after trauma on day 6 is due to a temporal factor, which prevents the endometrium reacting even in presence of active corpora lutea.

Data on the stimulation of the decidual cell reaction (DCR) by lactogenic hormone (LGH) in normal rats have been presented (v. Berswordt-Wallrabe & Turner 1961 c). Two doses of ovine LGH, i.e. 1 mg and 2 mg/day respectively, stimulated the formation of DCR in about 30% of the tested rat population with a graded reaction as measured by DNA. Failure to obtain a uniform reaction was explained tentatively by the assumption, that LGH is not identical with luteotrophic hormone. In other words: It was assumed, that exogenous LGH did not stimulate corpora lutea to secrete sufficient amounts of progesterone to stimulate DCR after a mechanical trauma. There were, however, no data on corpora lutea and/or endogenous progesterone-production available. The purpose of the present study is to determine, whether the DCR is dependent on active corpora lutea in LGH-treated rats.

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102 rats of the FW 49 strain, fed Altromin Lab Chow, were maintained in an animal room artificially illuminated for 12 h/day at 20–24° C, until regular oestrous cycles were established. Vaginal smears were taken daily until the termination of the experiment. Soon after the rats showed full oestrous smears (vaginal late oestrus), 1 or 2 mg LGH*, diluted in 0.2 ml saline solution, were injected for 5 days (pretrauma period). 12 animals were made pseudopregnant mechanically by stimulation of the cervix uteri with a glass rod during the period of vaginal cornification. Day 1 of pseudopregnancy was the next day, provided that the animals then showed the typical dioestrous smear picture. On day 6, one uterine horn of each animal was traumatized by inserting a needle through the cut tubal end of the uterus as far as the cervix on that side. By withdrawing the needle slantwise, the antimesometrial part of the endometrium was scratched twice throughout its entire length with a sharp pointed needle. The controlateral untraumatized horn served as control. During the posttrauma period, day 6–9, rats received treatment as indicated in Table 1, the first injection being given immediately after the operation. 88 rats were killed by ether, 24 hours after the last injection, on day 10; 14 rats were killed on day 6 instead of being traumatized. Pituitary glands, adrenals, uteri and ovaries were removed. The ovaries were sectioned for histological examination (Geller et al., in preparation). The other organs were transferred to icecold vials, weighed on a Sartorius balance to the nearest 0.01 mg, and put into the deep freeze. The uteri were divided before weighing into control and traumatized horns. After at least 4 days, uteri were thawed and then extracted in ethanol and ether for 6 hours, respectively, placed overnight in a vacuum desiccator and weighed to the nearest 0.01 mg, in order to obtain data for dry fat-free tissue (DFFT).

RESULTS

During the posttrauma period, 5 out of 10 rats started having regular cycles again, following discontinuation of LGH-treatment on day 6. All the other rats, killed on day 6 and day 10, respectively, had luteinised ovaries with active corpora lutea and no distinct follicular development, thus confirming the typical dioestrous picture of the vaginal smears, indicating pseudopregnancy.

Although the wet weights of the traumatized horns were increased by about 30 %, as compared with the control horns, no deciduoma reaction was recorded (Table 1). There was a similar weight increase of about 20 % in DFFT. Those animals, which were killed in oestrus, showed the effect of oestrogen on the uteri: The wet weights of the control horns were significantly elevated, as compared to the dioestrous rats, and weight increases of the traumatized horns remained smaller. However, the rats treated with NIH-LGH had for unknown

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* LGH preparations were kindly supplied by the Endocrinology Study Section U. S. P. H. S., Prolactin ovine (NIH-P-S-3), and Schering A. G., Berlin-West, Charge 115/6 S, with potencies of 15 I. U./mg.
**Table 1.**
Effect of uterine traumatization in lactogenic hormone treated and pseudopregnant rats.

<table>
<thead>
<tr>
<th>Group Nr</th>
<th>LGH treatment: mg/d</th>
<th>No. of rats</th>
<th>Body weight (g)</th>
<th>Adrenals mg/100 g BW</th>
<th>Weight of uterine horns/100 g BW (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretrauma Posttrauma</td>
<td></td>
<td></td>
<td></td>
<td>Wet weights</td>
</tr>
<tr>
<td></td>
<td>Period</td>
<td>d 1-5</td>
<td>d 6-9</td>
<td>d 1</td>
<td>d 10</td>
</tr>
<tr>
<td>1</td>
<td>NIH 1</td>
<td>20</td>
<td>190 ± 5</td>
<td>212 ± 6</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>2</td>
<td>1 Schering 1</td>
<td>30</td>
<td>192 ± 3</td>
<td>214 ± 4</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>3</td>
<td>2 Schering 2</td>
<td>16</td>
<td>193 ± 2</td>
<td>221 ± 2</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>4 a</td>
<td>1 Schering None</td>
<td>5*</td>
<td>187 ± 10</td>
<td>210 ± 6</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>4 b</td>
<td>1 Schering None</td>
<td>5</td>
<td>186 ± 8</td>
<td>210 ± 9</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>5</td>
<td>1 Schering —</td>
<td>14**</td>
<td>192 ± 3</td>
<td>211 ± 3</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>6</td>
<td>None None</td>
<td>12***</td>
<td>200 ± 9</td>
<td>233 ± 4</td>
<td>31 ± 2</td>
</tr>
</tbody>
</table>

* Cycles in posttrauma period, killed in oestrus or LE.
** killed on day 6.
*** pseudopregnant (glass rod stimulation).
pretrauma period d 1-5.
posttrauma period d 6-9.

BW = Body weight
d = day
DFFT = Dry fat-free tissue
LGH = Lactogenic hormone
NIH = National Institute of Health

Student's t-Test

\[
\begin{align*}
2 \text{)-4)} & : P < 0.001 \\
3 \text{)-4)} & : 0.05 < P < 0.1 \\
6 \text{)-4)} & : 0.2 < P < 0.3
\end{align*}
\]
reasons somewhat elevated uterine wet weights, as compared with all the other dioestrous rats. The wet weights of the adrenal glands were within the normal range, thus excluding the possibility of a severe stress condition. This is in agreement with a normal body weight increase during the experimental period.

**DISCUSSION**

The uniform absence of any DCR was a very unexpected result: All the animals, glass rod stimulated as well as LGH-treated, had active corpora lutea as judged by histological examination. Furthermore, all the animals showed typical vaginal dioestrous during the experiment. These criteria indicate the presence of progesterone in the circulation. It is generally accepted that progesterone, secreted by corpora lutea and/or adrenals, stimulates DCR in intact and/or hypophysectomized pseudopregnant rats (Astwood 1939; Sydnor 1945; Lyons et al. 1953). Hence Astwood (1939) and Rothchild & Meyer (1942), suggested the use of DCR for the assay of progesterone. As little as 0.1 mg progesterone/day (Astwood 1939) or 0.25 mg progesterone/day (Velardo & Hisaw 1951) gave DCR in pseudopregnant rats, ovariectomized at the time of the uterine trauma. Even in the absence of the pituitary gland, after an appropriate progesterone-treatment, DCR could be elicited in ovariectomized rats (Rothchild et al. 1940).

The uterine weights did not indicate any oestrogens in the circulation. Therefore, the inhibiting effects of oestradiol on DCR shortly before trauma (Rothchild & Meyer 1942) or during the posttrauma period (Velardo & Hisaw 1951; Hisaw & Velardo 1954) can be excluded.

Under continuing treatment with LGH, as shown in this study, which was performed with the aim of prolonging the uterine sensitivity by endogenous progesterone, trauma at day 6 completely failed to induce DCR. These results are in agreement with the findings of De Feo (1963), obtained in glass rod stimulated rats: This author was unable to produce »maximal« sensitivity to trauma on day 5 with LGH and progesterone, respectively. In contrast, rats ovariectomized during oestrous, and then treated with sensitivity-preserving amounts of 1.5 mg progesterone/day, consistently showed DCR when traumatized on day 6, provided that a combination of progesterone, oestradiol and thyroxine was given during the posttrauma period (v. Berswordt-Wallrabe & Turner 1961 a, b).

The first explanation at hand for the negative results of this study was the assumption, that complete absence of DCR might have been due mainly to a suboptimal progesterone-production of corpora with advancing pseudopregnancy. This speculation, however, is ruled out by the findings in the LGH-treated rats of this study: It was assumed, that continued treatment with LGH
would give an adequate stimulus for the persisting corpora to produce progesterone in amounts, sufficient for DCR. Since DCR was not induced at all while vaginal dioestrous and active corpora indicated the presence of endogenous progesterone, it must be taken into consideration, that a time factor is involved in DCR. This factor, which is independent of progesterone, seems to render the endometrium incapable, responding with DCR after trauma on day 6.

Temporal aspects of DCR thus become of particular interest: Yochim & De Feo (1962) reported remarkable DCR in glass rod stimulated rats; trauma was applied on day 4 of pseudopregnancy. Astwood (1939) found endometrial trauma to be most effective in normal, pseudopregnant rats, when performed on day 4. Optimal conditions for implantation in the rat endometrium were found around day 4 after ovulation (Stein-Werblowsky 1962). In agreement with this, De Feo (1963) described maximal uterine sensitivity during day 4 after oestrus in glass rod stimulated rats. As early as 24 hours later, i.e. by day 5, most of the sensitivity of the uterus was definitely lost.

In order to find out, if maximal sensitivity on day 4, as described by De Feo (1963) for pseudopregnant rats, was also true for LH-treated rats, this temporal aspect of DCR has been studied and is presented in a separate paper (v. Berswordt-Wallrabe et al., in prep.).

REFERENCES


De Feo V. J.: Endocrinology 72 (1963) 305.


