Pattern of prolactin secretion and pituitary responsiveness to LRH in pseudopregnant rats maintained in constant light

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Abstract. Secretion of LH in response to an LRH infusion (104 ng/h during 21 h), secretion of progesterone (P) and 20α-dihydroprogesterone (DHP), as well as the 24 h rhythm of prolactin (Prl) secretion were investigated in rats rendered persistently oestrous by exposure to constant light and in which subsequently pseudopregnancy (PSP) had been induced by cervical stimulation after induction of ovulation by hCG. Similar persistently oestrous rats, but otherwise untreated, served as controls.

LRH was infused through an intra-jugular cannula and blood samples for assay of LH were taken via an intracarotid cannula. LH responses were judged on the basis of the mean maximal height (MH; ng LH ml plasma) and the rate of decrease or half life (t½) of the plasma LH concentrations after 2 h of LRH infusion. For assay of P and DHP blood samples were taken from the orbital plexus. In another series of animals blood, for assay of Prl, was sampled every 2 h for 24 h from a cannula with the tip in the right atrium.

It was observed that in persistently oestrous rats LH-responses were high (MH = 3326 ± 305) and steep (t½ = 65 ± 10 min). On the day after the night of ovulation (day 0 of PSP) and on days 1, 5 and 9 of PSP the responses were lower (MH between 1315 and 1041) and more blunted (t½ varied between 236 and 142 min), whilst on day 12 of PSP they were again higher (MH = 4416 ± 575) and steeper (t½ = 101 ± 12 min). P and DHP concentrations were low in persistently oestrous rats and high during days 5 and 8 of PSP. On day 12 of PSP P concentrations were low again, while the DHP concentrations had increased further.

In persistently oestrous rats the plasma Prl levels were low with incidental elevations (up to 141 ng Prl/ml plasma). During days 8–9 of PSP Prl was secreted at irregular intervals (1 to 3 peaks/24 h) with peak levels of up to 400 ng Prl/ml plasma. On days 11–12 of PSP, Prl peaks were lacking almost completely: only a few minor elevations (up to 41 ng Prl/ml plasma) were measured. It is concluded that during days 0 to 10 of pseudopregnancy under constant light the endocrine state of the animals (in terms of LRH-responsiveness and P and DHP secretion) differs significantly from that during the previous and the following days. As similar changes are observed in PSP rats maintained under standard lighting conditions, it is apparent that the lighting conditions do not influence these changes. The pattern of Prl secretion during PSP, on the other hand is affected by the lighting conditions.

In the female rat the pattern of prolactin (Prl) secretion and the responsiveness of the pituitary gland to luteinizing hormone-releasing hormone (LRH) are both influenced by the endocrine state of the animal. In cyclic rats, for instance, a Prl surge is generated every 4 and 5 days on the afternoon of pro-oestrous (Butcher et al. 1974; Döhler & Wuttke 1974; Smith et al. 1975), while in pseudopregnant rats the hormone is secreted as 2 daily surges, one diurnal and the other nocturnal (Freeman & Neill 1972; Freeman et al. 1974). Pituitary responsiveness to LRH, on the other hand, changes continuously during the oestrous cycle (Cooper et al. 1973, 1974; Aiyer et al. 1974; Gordon & Reichlin 1974; Blake 1978; Schuiling et al. 1980), but remains unchanged during most of pseudopregnancy (PSP; Koiter et al. 1979). At the end of PSP the pituitary gland does not only become more responsive to LRH (Koiter et al. 1979), but the state of the neuroendocrine mecha-

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nism which controls Prl secretion seems to change also: on pro-oestrous only the diurnal surge is seen (Freeman et al. 1974; Smith & Neill 1976b).

The pattern of Prl secretion is also influenced by the lighting conditions. In rats rendered persistently oestrous by constant light, Prl is continuously secreted at a low rate (Bethea & Neill 1979; Yogev & Terkel 1980), but when such rats are made PSP the hormone is secreted at a frequency of up to 4 surges per 24 h (Bethea & Neill 1979; Pieper & Gala 1979).

To our knowledge data concerning the LRH-responsiveness and the pattern of Prl secretion during the later phases of PSP in animals maintained in constant light, have not been published so far. In this report we present data on these two phenomena.

Materials and Methods

Female 10 weeks old Wistar rats, bred at the Department of Pharmacology of Leiden University, were placed in permanently illuminated animal quarters. Three months later vaginal smears were taken daily, and the animals were used for experiments when they exhibited at least 7 consecutive oestrous vaginal smears. At that time they weighed about 200 g. Persistently oestrous rats were rendered pseudopregnant by iv administration of an ovulatory dose of hCG (Pregnyl®; Organon Ltd. 50 IU/100 g b.w.) followed by electrical stimulation of the cervix uterus at 17.00 h the same day and at 15.00 h the next day (de Greef & Zeilmaker 1974). The first day following the last cervical stimulation is denoted day 1 of pseudopregnancy (PSP). During pseudopregnancy vaginal smears were taken daily. On the day after the hCG injection an oestrous smear was found while during the subsequent 12 days only dioestrous smears were seen. Furthermore in 10 animals plasma progesterone (P) and 20 α-dihydroprogesterone (DHP) concentrations were measured on the day before hCG treatment as well as on the days 0, 1, 5, 8 and 12 of pseudopregnancy. (Experiment 1).

Prolonged stimulation of LH secretion by the pituitary gland was effected by constant rate infusion of LRH (104 ng/h during 21 h) via a cannula inserted into the right jugular vein as described by Schuiling & Gnodde (1976). The infusions were started at 12.00 h. During LRH infusion the animals were anaesthetized with sodium phenobarbitone in order to damp the hypothalamic LRH secretion. For this purpose rats first received an ip injection of 80 mg/kg b.w. at 11.00 h and then another one of 30 mg/kg b.w. at 15.00 h.

During LRH infusions blood samples (300 μl) for assay of LH were taken from a cannula inserted into right carotid artery. During insertion of these cannulas (be-
Plasma progesterone (black bars) and 20α-dihydroprogesterone (open bars) concentrations (mean ± SEM) in rats in permanent oestrus (PO) and in persistently oestrous rats, rendered pseudopregnant, on days 0, 1, 5, 8 and 12 of pseudopregnancy (PSP)

provided $\beta \neq \alpha$, the course of the plasma LH concentrations directly reflects the course of the LH secretion rate, so that the constant $\beta$ is the characteristic constant of the process which causes the LH secretion rate to decrease.

With the help of a computer programme developed at the Groningen Dept. of Pharmacology the present experimental data were fitted to a multi-exponential model by means of the least squares method.

A one-exponential model with a plateau (equation 1) described the experimental data satisfactorily as compared (F-test) with models with two exponentials (with or without a plateau). Estimates for the constant $\beta$ were thus obtained.

Since the use of the parameter half-life or $t_{1/2}$ instead of the $\beta$ (with $t_{1/2} = \ln 2 / |\beta|$) may be more illustrative, the $t_{1/2}$'s were tabulated in Table 1. Differences between $t_{1/2}$'s were assessed by the Mann-Whitney-U-test. Differences between means were assessed by the unpaired, two-tailed Student's $t$-test. The level of significance was chosen at 0.05.

**Table 1.**
Maximal height (MH; ng LH/ml plasma; mean ± SEM) and the half-life characterizing the rate of decrease of the LH secretion after $t = 2$ h ($t_{1/2}$; min; mean ± SEM) of LH-secretory responses induced by continuous infusion of LRH at the rate of 104 ng/h.

<table>
<thead>
<tr>
<th>Endocrine state (days)</th>
<th>n</th>
<th>MH</th>
<th>$t_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistently oestrous rats</td>
<td>6</td>
<td>3326 ± 305$^a$</td>
<td>65 ± 10$^a$</td>
</tr>
<tr>
<td>Pseudopregnant rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>1227 ± 56$^b$</td>
<td>208 ± 21$^b$</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>1315 ± 132$^c$</td>
<td>236 ± 44$^c$</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>1059 ± 180$^d$</td>
<td>142 ± 16$^d$</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>1041 ± 89$^e$</td>
<td>152 ± 18$^e$</td>
</tr>
<tr>
<td>12</td>
<td>7</td>
<td>4416 ± 575$^f$</td>
<td>101 ± 12$^f$</td>
</tr>
</tbody>
</table>

$P(a vs b,c,d,e) < 0.001$  \  $P(a vs b,c,d,e) < 0.01$

$P(f vs b,c) < 0.01$  \  $P(a vs f) < 0.05$

$P(f vs d,e) < 0.001$  \  $P(f vs b,c,d,e) < 0.05$
Results

Plasma progesterone and 20α-dihydroprogesterone concentrations (Fig. 1)
Upon induction of PSP, both the progesterone (P) and 20α-dihydroprogesterone (DHP) concentrations in the plasma increase. P concentrations are highest on days 5 and 8 of PSP and low again on day 12. DHP concentrations are constantly high throughout PSP and have risen further on day 12.

LH-responses to LRH infusion (Fig. 2 and Table 1)
Concomitantly with the induction of PSP, the state of responsiveness of the pituitary gland to LRH changes: in persistently oestrous rats both the MH and the constant of the process which causes the LH secretion rate to decrease, β, were significantly larger (and hence the t½ shorter) than during days 0, 1, 5 and 9 of PSP. During these days of PSP the LH-responses were essentially the same. With regard to both parameters, however, they differed from responses induced on the 12th day of PSP. On this latter day the responses were higher and steeper again. The t½'s, but not the MH's of the responses of persistently oestrous and day-12 PSP rats differed significantly.

Prolactin secretion patterns
A. Persistently oestrous rats (Fig. 3).
In persistently oestrous rats concentrations were relatively low, although incidental elevations of up to 141 ng Prl/ml plasma occurred irregularly throughout the day.

![Graphs](image-url)
B. Pseudopregnant rats

Days 8–9. (Fig. 4-A) During days 8–9 of PSP the pattern of Prl secretion showed irregular fluctuations. Plasma Prl concentrations of up to 400 ng/ml were measured. One-three peaks occurred every 24 h, but a systematic periodicity seems to be lacking.

Days 11–12. (Fig. 4-B) During days 11–12 of PSP plasma Prl concentrations were relatively low. The pattern of Prl secretion resembled that of persistently oestrous rats, although plasma Prl levels higher than 41 ng/ml were not observed. The incidental elevations occurred at irregular intervals.

Discussion

The values of the plasma progesterone and 20α-dihydroprogesterone concentrations during the days after hCG injection and cervical stimulation indicate that the rats had become PSP and that on the 12th day of PSP physiological luteolysis had occurred indicating that the life-span of the ‘activated’ corpora lutea in rats maintained in constant light is similar (about 10 days) to that of such corpora lutea in rats maintained under normal lighting conditions (cf. Hashimoto et al. 1968; Bartosik & Szarowski 1973).

In a previous study (Koiter et al. 1981) it was shown that the LRH-responsiveness of the pituitary gland of persistently oestrous rats changes after administration of an ovulatory dose of hCG. Twenty-four h after such a treatment the constant β but not the maximal height (MH) of LH-responses induced by infusion of LRH, is decreased (and the t½ increased), and as it was furthermore shown that the constant β (or the t½) does not change when the blood oestrogen level is manipulated, it was concluded that changes in this constant are associated with the formation of corpora lutea. The present experiments show that the LH-responses also change when pseudopregnancy is induced in persistently oestrous rats (Table 1): both the MH and the constant β decreased (and hence the t½ has increased). The decrease of the MH may be due to the LH release as effected by the stimulation (cf. Brown-Grant et al. 1973; Davidson et al. 1973), and/or to changes in the endocrine environment (such as the secretion of Prl). The newly acquired state of LRH-responsiveness is maintained for at least the first 10 days of PSP. On day 12 of PSP the responsiveness appeared to have changed once more: both the MH and the decay constant β of the LRH-induced LH-responses had increased again (and hence the t½ has decreased).

As the pituitary responsiveness to LRH of PSP rats maintained under normal lighting conditions develops similarly (Koiter et al. 1979), it appears that this development is not under the control of the lighting conditions. Koiter et al. (1979) showed that on the basis of this development the period of
PSP under normal lighting conditions can be divided into three phases. Such a division accords with the state of activity of the corpora lutea. Thus, phase 1, comprising only day 1 of PSP, corresponds with metoestrus of the ovulatory cycle, and phase 2, comprising days 2 to 10 of PSP, corresponds with the period of 'activated' corpora lutea. Phase 3 begins at the onset of luteolysis and lasts until ovulation (Hashimoto et al. 1968; Bartosik & Szarowski 1973; Smith et al. 1975; Welschen et al. 1975).

In the present PSP rats the first phase is not characterized by a very small LH-response: the MH but not the total amount of LH released during the 21 h-lasting LRH-infusion (as determined by computing the area under the curve representing the plasma LH concentrations) has decreased (cf. Koiter et al. 1979). The explanation for this may be that while at the beginning of PSP under normal lighting conditions the LH content of the pituitary is appreciably decreased as a result of the release during the pre-ovulatory LH-surge, pituitary LH content of the present persistently oestrous rats is only slightly affected since only a
small amount of LH is released following cervical stimulation and hCG treatment (own observations).

In persistently oestrous rats plasma Prl concentrations are maintained at a relatively low level, although irregular elevations occur (cf. Bethea & Neill 1979; Yogev & Terkel 1980). Cervical stimulation causes high Prl peaks in persistently oestrous rats (Brown-Grant et al. 1973; Bethea & Neill 1979; Pieper & Gala 1979), and peaks seem to continue until phase 3 of PSP: Bethea & Neill (1979) observed Prl peaks at a 6–8 h periodicity during days 3–4. An present observations also reveal an irregular pattern of Prl secretion during days 8–9. At the transition to the third phase of PSP, however, the pattern of Prl secretion changes and peaks are almost completely absent. Thus, changes in the pattern of Prl secretion coincide with changes in the state of activity of the CI and this suggests that the CI are part of the neuroendocrine mechanism which controls Prl release. Similarly, CI are involved in the development of the LRH-responsiveness of the pituitary: once luteolysis has commenced the development of pituitary responsiveness to LRH proceeds probably under the influence of oestrogen secreted by the developing follicles (e.g. Aiyer & Fink 1974; Legan & Karsch 1975; Schuling et al. 1979, 1980). The disappearance of Prl-surges at the end of PSP under normal lighting conditions seems also to be associated with the decline of luteal activity (de Greef & Zeilmaker 1978). Yet the diurnal and nocturnal surges seem to be regulated differently. The diurnal surge is generated by progesterone (de Greef & Zeilmaker 1979) and accentuated by oestrogen, whilst the nocturnal surge is mainly under the control of progesterone (Freeman & Sterman 1978). In constant light, however, not only the pattern of Prl-peaks differs from that under light-dark conditions, but the concepts ‘diurnal’ and ‘nocturnal’ loose their meaning as well. Furthermore, it has been shown that under alternating lighting conditions the time of appearance of Prl-surges following cervical stimulation is related to the time of day rather than to the time of stimulation (Smith & Neill 1976a). The question of the hormonal control of the present Prl-surges, therefore, needs further investigation.

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