The steroid antagonist RU486 given at pro-oestrus induces hypersecretion of follicle-stimulating hormone from oestrus afternoon to early metoestrus in the rat

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Abstract
Administration of the steroid antagonist RU486 to cyclic rats at pro-oestrus blunts the preovulatory surge of LH and suppresses the first and second surges of FSH. In addition, administration of oestradiol to RU486-treated rats reactivates the LH surge the following day. The present study explored the effects of RU486 (4 mg/0.2 ml oil) administered at 0800 h on the day of pro-oestrus, on serum FSH and LH concentrations through oestrus and early metoestrus. RU486 induced a hypersecretion of FSH, which started at 1400 h on the day of oestrus and was maintained until 0800 h on the day of metoestrus. Because the timing and magnitude of this secretion of FSH were similar to those of the periovulatory secretion of FSH during pro-oestrus and early oestrus in intact cyclic rats, we investigated the effects of: 1) LHRH antagonist (LHRHa) injected at either 0900 h or 2000 h on the day of oestrus, 2) oestradiol benzoate injected at 1600 h on the day of pro-oestrus and at 0900 h on the day of oestrus, 3) bovine follicular fluid (bFF) given either at 1100 h or at 2000 h on the day of oestrus, or 4) adrenalectomy (ADX) at 1100 h on the day of oestrus, on serum FSH and LH concentrations at 1800 h on the day of oestrus and at 0200 h on the day of metoestrus in rats injected with RU486 at pro-oestrus. The results showed that 1) both components (late oestrus and early metoestrus) of FSH hypersecretion in RU486-injected rats in pro-oestrus were inhibited by oestradiol benzoate and bFF, 2) the metoestrous component was not affected by LHRHa, whereas the oestrous component was partially reduced, and 3) ADX partially reduced serum FSH concentrations only on the day of metoestrus, possibly because, as the serum concentrations of corticosterone reflected, the antiglucocorticoid activity of 4 mg RU486 lasted only 24 h. The results support the hypothesis that blockade of progesterone actions at pro-oestrus results in the maintenance of the daily neural signal that activates the release of gonadotrophins. Whereas the expression of LH secretion requires high levels of oestradiol, FSH secretion is expressed against a background of low oestradiol levels. The results of this study also indicate that the release of FSH during oestrus and metoestrus in rats injected with RU486 at pro-oestrus is a consequence of the lack of ovarian negative feedback inhibition on the pituitary.

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Introduction
In the cyclic rat, after the preovulatory surges of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the afternoon of pro-oestrus, LH secretion rapidly declines to basal levels, while FSH secretion remains high throughout the night of pro-oestrus and the early part of the day of oestrus (secondary surge of FSH) (1, 2). Although the regulation of the LH-releasing hormone (LHRH)-independent (3–5) secondary FSH surge is not completely understood (6, 7), it is well established that it requires the endocrine effects of the LHRH-dependent preovulatory surge of LH on the ovary (i.e. a decrease in serum inhibin and oestradiol and an increase in serum progesterone) (8–10).

It has been shown that administration of RU486, an antiprogestagen with antiglucocorticoid activity (11), to cyclic rats at pro-oestrus blunts the preovulatory surge of LH and suppresses the primary and secondary surges of FSH (5, 12, 13). In addition, RU486 blocks the effects of inhibin antiserum on the secondary surge of FSH (14).

The blockade of progesterone secretion on pro-oestrus afternoon with pentobarbitone postpones the expression of the LH surge by one day (15, 16). In addition, if the decrease in serum concentration of oestrogen is prevented, antagonism of the action of progesterone by injection of RU486 at pro-oestrus reactivates the LH surge the next day (17). In an initial study, it was found that RU486 injected at pro-oestrus increases serum FSH concentrations at 1830 h on the
day of oestrus (18). It is thus possible that RU486 also reactivates the FSH surges the next day. Therefore, the present experiments were designed to determine serum FSH and LH concentrations during oestrus and metoestrus in rats injected with RU486 at pro-oestrus. As RU486-injected rats had increased FSH serum concentrations during the afternoon of oestrus and early on the day of metoestrus, we aimed to determine whether this FSH secretion depends on LHRH, oestrogen, inhibin or corticosterone.

Materials and methods

Animals

Adult female Wistar rats weighing 180–220 g were used. The rats were housed (five per cage) under controlled conditions of light (lights on from 0500 to 1900 h) and temperature (20–23°C), with free access to tap water, and food available ad libitum. Vaginal smears were examined daily. Only rats exhibiting at least two consecutive 4-day oestrous cycles were used.

Drugs, treatments and surgery

Antiprogestagen RU486 (Mifepriston, 11β-(4-dimethylaminophenyl)-17β-hydroxy-17α-(1-propinyl)-estra-4,9-diene-3-one) was donated by Dr R Deraedt (Roussel-Uclaf, Romainville, France). This compound has high affinity for the progesterone receptor, in addition to antiglucocorticoid activity (11, 19). Rats were injected s.c. with 4 mg/0.2 ml oil. Control injections consisted of 0.2 ml olive oil.

The LHRH antagonist (LHRHa) used was ORG.30276 (Ac-d-p-C1-Phe-d-p-C1-Phe-d-Trp-Ser-Tyr-d-Arg-Leu-Arg-Pro-d-Ala-NH2.CH3.CO OH) (Organon International B.V., Oss, The Netherlands). Immediately before use, the peptide was dissolved in saline to a concentration of 5 mg/ml and rats received s.c. injections of 0.2 ml of this solution. This dosage causes maximal suppression of LH serum levels (20, 21). Controls were injected with 0.2 ml saline.

Oestradiol benzoate (Sigma Chemical Co., St Louis, MO, USA) was dissolved in olive oil to a concentration of 200 µg/ml. Injections (s.c.) consisted of 50 µg/0.25 ml oil. Control injections consisted of 0.25 ml oil.

Bovine follicular fluid (bFF) was used to suppress FSH secretion (22). One pool of follicular fluid, from heifer ovarian follicles measuring between 10 and 15 mm in diameter, was collected in a slaughterhouse (ICCOSA, Córdoba, Spain) and charcoal-extracted according to methods described previously (18). Rats were injected with 0.5 ml bFF i.v. and 0.8 ml bFF i.p. Controls received bovine serum.

Adrenalectomy (ADX) was performed with the rat under ether anaesthesia, at 1100 h on the day of oestrus. Adrenal glands were removed through a subcostal 1 cm incision on both sides. The sham operation consisted of exposing but not removing the adrenals.

Experiments

To determine the action of pro-oestrus afternoon progesterone on oestrus and metoestrus serum FSH and LH concentrations, in the first experiment, groups of rats injected with RU486 or oil at 0800 h on the day of pro-oestrus were decapitated at 2-h intervals starting at 1000 h on the day of oestrus and continuing until 1000 h on the day of metoestrus.

In the second experiment, we studied the effects of RU486 administered at 0800 h on the day of pro-oestrus or of oestrus (or both) on serum FSH and LH concentrations at 1800 h on the day of oestrus and at 0200 h on the day of metoestrus.

In the third experiment, we investigated the effects of: 1) LHRHa either at 0900 h or at 2000 h on the day of oestrus, 2) oestradiol benzoate at 1600 h on the day of pro-oestrus and at 0900 h on the day of oestrus, 3) bFF either at 1100 h or at 2000 h on the day of oestrus or 4) ADX, on FSH and LH serum concentrations at 1800 h on the day of oestrus and at 0200 h on the day of metoestrus in rats injected with RU486 at pro-oestrus. Because ADX partially suppressed serum FSH concentrations at 0200 h on the day of metoestrus in RU486-treated rats, in a final experiment we studied the antiglucocorticoid activity of RU486 by determining the serum concentrations of corticosterone at 1000 h and 1800 h on the day of pro-oestrus, at 0200 h, 1000 h and 1800 h on the day of oestrus and at 1000 h on the day of metoestrus in rats injected with 4 mg RU486 at 0800 h on the day of pro-oestrus. Rats were decapitated and trunk blood was collected to determine serum corticosterone concentrations.

Radioimmunoassay of gonadotrophins

Serum concentrations of LH and FSH were measured in duplicate in 25 µl samples by double-antibody RIA methods using the RIA kits supplied by NIADDK (Baltimore, MD, USA) and following a microassay method described previously (21). Rat LH-I9 and FSH-I8 were labelled with iodine-125 by the Chloramine T method (23). Serum LH and FSH concentrations are expressed as µg/l of the reference preparations LH-rat-RP-3 and FSH-rat-RP-2 respectively. All samples were run in the same assay. The intra-assay coefficients of variation were 8% and 7% for LH and FSH respectively and the sensitivities of the assays were 7.5 and 20 pg/tube for LH and FSH respectively.

Radioimmunoassay of corticosterone

Serum corticosterone concentrations were measured using commercially obtained kits (ICN, Biomedicals, Costa Mesa, CA, USA). Assay sensitivity was 2.5 ng/tube and the intra-assay coefficient of variation was 4%.
Data evaluation and statistical analysis

Results are expressed as means ± S.E.M. Data were analysed by one-way analysis of variance using Duncan’s multiple range test. A difference was considered to be statistically significant at $P < 0.05$.

Results

Serum FSH and LH concentrations during oestrus and metoestrus in rats injected with RU486 at pro-oestrus

Figure 1 summarizes these findings. Rats injected with RU486 at pro-oestrus displayed hypersecretion of FSH on the afternoon and evening of the day of oestrus and during the early hours of the day of metoestrus. FSH secretion began at 1400 h on the day of oestrus, attained plateau levels between 1600 h on the day of oestrus and 0400 h on the day of metoestrus, peaking at 1800 h on the day of oestrus, and returning to baseline levels at 0800 h on the day of metoestrus. Baseline LH concentrations during oestrus and early metoestrus were increased by administration of RU486 at pro-oestrus.

Effects of RU486 at pro-oestrus or oestrus on serum FSH and LH concentrations at 1800 h on the day of oestrus and at 0200 h on the day of metoestrus

Serum FSH concentration increased significantly in rats injected with RU486 at pro-oestrus or at pro-oestrus and oestrus. In addition, injection of RU486 on the day of oestrus alone had no effect on serum FSH concentrations either at 1800 h on the day of oestrus or at 0200 h on the day of metoestrus. Serum LH concentrations were increased, except at 1800 h on the day of oestrus in rats injected with RU486 on that day. These results are presented in Table 1.

Effects of LHRHa, estradiol benzoate, bFF or ADX on serum FSH and LH concentrations at 1800 h on the day of oestrus and at 0200 h on the day of metoestrus in rats injected with RU486 at pro-oestrus

Because no differences in serum FSH and LH concentrations were found among RU486-treated rats injected with the different vehicles or sham-adrenalectomized, they are represented together as a single group in Table 2, which summarizes these results. Administration of LHRHa at 0900 h on the day of oestrus partially suppressed serum FSH concentrations at 1800 h on that day. In contrast, injection of LHRHa either at 0900 h or at 2000 h on the day of oestrus did not affect serum FSH concentrations at 0200 h on the day of metoestrus. Injections of oestradiol benzoate reduced serum FSH concentrations at 1800 h on the day of oestrus, and at 0200 h on the day of metoestrus. Whereas injections of bFF at 1100 h on the day of

Table 1 Serum FSH and LH concentrations at 1800 h on the day of oestrus and at 0200 h on the day of metoestrus in rats injected s.c. with RU486 (4 mg/0.2 ml oil) at 0800 h on the days of pro-oestrus or oestrus. Values are means ± S.E.M. (number of rats).

<table>
<thead>
<tr>
<th>Treatment on cycle day</th>
<th>FSH (μg/l)</th>
<th>LH (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oestrus</td>
<td>1800 h oestrus</td>
</tr>
<tr>
<td>Oil</td>
<td>Oil</td>
<td>3.5 ± 0.4 (7)</td>
</tr>
<tr>
<td>RU486 Oil</td>
<td>Oil</td>
<td>9.1 ± 0.8 (8)**</td>
</tr>
<tr>
<td>Oil</td>
<td>RU486</td>
<td>3.1 ± 0.3 (9)</td>
</tr>
<tr>
<td>RU486 RU486</td>
<td>RU486</td>
<td>9.7 ± 0.5 (8)**</td>
</tr>
</tbody>
</table>

**$P < 0.01$ compared with rats injected with oil (one-way ANOVA followed by Duncan’s multiple range test).
Table 2 Serum FSH and LH concentrations at 1800 h on the day of oestrus and at 0200 h on the day of metoestrus in rats injected s.c. with RU486 (4 mg/0.2 ml oil) at 0800 h on the day of pro-oestrus. Effects of LHRH antagonist (LHRHa), oestradiol benzoate (OB), bovine follicular fluid (bFF) or adrenalectomy (ADX). Values are means ± S.E.M. (number of rats).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FSH (μg/l)</th>
<th>LH (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1800 h oestrus</td>
<td>0200 h metoestrus</td>
</tr>
<tr>
<td>RU486 + Vehicles + Sham-ADX</td>
<td>9.6 ± 0.3 (25)</td>
<td>7.4 ± 0.7 (24)</td>
</tr>
<tr>
<td>RU486 + LHRHa</td>
<td>6.8 ± 0.3 (8)**</td>
<td>6.3 ± 0.7 (8)</td>
</tr>
<tr>
<td>RU486 + LHRHa2</td>
<td>7.2 ± 0.5 (10)</td>
<td>0.1 ± 0.1 (10)**</td>
</tr>
<tr>
<td>RU486 + OB</td>
<td>2.4 ± 0.4 (9)**</td>
<td>1.9 ± 0.1 (10)**</td>
</tr>
<tr>
<td>RU486 + bFF1</td>
<td>1.4 ± 0.2 (10)**</td>
<td>8.0 ± 0.2 (8)</td>
</tr>
<tr>
<td>RU486 + bFF2</td>
<td>2.9 ± 0.3 (10)**</td>
<td>4.2 ± 0.2 (8)**</td>
</tr>
<tr>
<td>RU486 + ADX</td>
<td>9.8 ± 0.5 (8)</td>
<td>4.2 ± 0.2 (8)**</td>
</tr>
</tbody>
</table>

LHRHa, 1 mg/0.2 ml saline (s.c.) at 0900 h on the day of oestrus; LHRHa2, 1 mg/0.2 ml saline (s.c.) at 2000 h on the day of oestrus; OB, 50 μg/0.25 ml oil (s.c.) at 1600 h on the day of pro-oestrus and at 0900 h on the day of oestrus; bFF1, 0.5 ml (i.v.) and 0.8 ml (i.p.) at 1100 h on the day of oestrus; bFF2, 0.5 ml (i.v.) and 0.8 ml (i.p.) at 2000 h on the day of oestrus; ADX, at 1100 h on the day of oestrus. Vehicles, 0.2 ml saline, 0.2 ml oil and 1.3 ml bovine serum. *P < 0.05, **P < 0.01 compared with RU486 + Vehicles + Sham-ADX (one-way ANOVA followed by Duncan’s multiple range test).

Serum corticosterone concentrations during pro-oestrus, oestrus and early metoestrus in rats injected with RU486 at pro-oestrus

Control rats had characteristic increases in serum corticosterone in the afternoon of the days of pro-oestrus and oestrus. Administration of RU486 at 0800 h on the day of pro-oestrus significantly increased serum corticosterone concentrations from 1000 h on the day of pro-oestrus to 1000 h on the day of oestrus. However, at 1800 h on the day of oestrus or at 1000 h on the day of metoestrus, there were no significant differences in serum corticosterone concentrations between rats injected with oil or with RU486 (Fig. 2).

Discussion

These studies show that administration of RU486 to cyclic rats at pro-oestrus induces a hypersecretion of FSH during oestrus and metoestrus. This finding is of interest, as administration of RU486 in the cyclic rat decreases: a) baseline serum FSH levels during the dioestrous phase (12, 21), b) the increase in serum FSH concentration after unilateral ovariectomy (24), c) the primary surge of FSH on pro-oestrus afternoon (5, 13) and d) the secondary surge of FSH early on the day of oestrus (12–14, 25, 26). The results might also be valuable in providing new in vivo evidence for the differential control of LH and FSH secretion.

Serum FSH levels during oestrus and metoestrus in rats injected with RU486 at pro-oestrus increased from 1400 h on the day of oestrus until 0800 h on the day of metoestrus, with a plateau phase lasting 12 h and peak values synchronized with the light–darkness cycle. This pattern of FSH secretion resembled that normally found during pro-oestrus afternoon and early on the day of oestrus in intact rats (1, 2, 5). In addition, administration of RU486 at pro-oestrus increased baseline LH levels throughout the experiment, while administration on the day of oestrus did not affect serum FSH concentrations on oestrus afternoon and metoestrus morning, in rats injected with either oil or RU486 at
pro-oestrus. These further findings are consistent with the concept that the blockade of the actions of progesterone on pro-oestrus afternoon, in addition to increasing basal LH secretion, reactivates the neural signal for the gonadotrophin surges in cyclic rats 24 h later. Increased baseline levels of serum LH in rats injected with RU486 are the consequence of a lack of progesterone inhibitory action on LH secretion, and an increased pituitary sensitivity to LHRH (21).

Administration of pentobarbitone at pro-oestrus, which blocks the increase in serum progesterone and prevents the decrease in serum levels of oestradiol (27, 28), delays the expression of the LH surge by one day (16, 29). Furthermore, blockade of the action of progesterone on pro-oestrus afternoon by RU486 does not elicit release of the LH surge on the day of oestrus unless exogenous oestradiol is administered (17, present results). This indicates that the absence of pro-oestrus progesterone secretion or action allows the activation, 24 h later, of the neural signal controlling the gonadotrophin surge (15) expressing an LH or an FSH surge, depending on the preceding high or low background of oestradiol (30) respectively.

Administration of RU486 at pro-oestrus does not prevent either ovulation or the decrease in serum oestradiol and inhibin on pro-oestrus evening (5, 12, 13). This partial absence of the ovarian negative feedback at pituitary level might cause the increased serum FSH levels triggered by the reactivated central signal in the absence of pro-oestrus afternoon progesterone activity. Serum FSH levels remain increased until FSH-stimulated follicles secrete enough inhibin and oestradiol which, in turn, suppress FSH secretion (10, 31, 32). The oestrous component of the FSH hypersecretion in RU486-treated rats was inhibited by bFF, but not affected by ADX, as occurs in the preovulatory surge of FSH on pro-oestrus afternoon (33–35). The metoestrous component showed a similar endocrine dependency to that of the secondary surge of FSH during early oestrus (25, 27, 34, 36). Therefore, FSH secretion during oestrus and metoestrus in rats injected with RU486 in pro-oestrus cannot be considered a delayed periovulatory secretion. Increased levels of oestradiol on the evening of dioestrus and the morning of pro-oestrus are obligatory for preovulatory gonadotrophin surges, as they are both attributed to a similar neuroendocrine mechanism resulting from increased serum oestrogen levels: increased LHRH secretion and enhanced pituitary responsivity to LHRH (reviewed in 7). However, oestradiol benzoate completely inhibited, and LHRHα only slightly suppressed, FSH secretion on the day of oestrus in RU486-treated rats. Thus it would appear that the mechanism underlying the oestrous component of FSH hypersecretion in pro-oestrus RU486-injected rats is analogous to the mechanism responsible for ovariectomy-induced FSH hypersecretion (36).

The finding that ADX on the morning of oestrus in rats injected with RU486 at pro-oestrus partially decreased FSH serum concentrations at 0200 h on the day of metoestrus prompted us to determine the serum corticosterone levels during pro-oestrus, oestrus and metoestrus in RU486-treated rats in order to study the antiglucocorticoid activity of RU486. Serum corticosterone concentrations in rats injected with RU486 in pro-oestrus were increased for only about 24 h after treatment. This hitherto unreported finding offers an explanation for the effect of ADX on the day of oestrus in decreasing serum FSH concentrations at 0200 h on the day of metoestrus. In rats injected with RU486 at pro-oestrus, and supports the proposed stimulatory role of the adrenal glands on the secondary surge of FSH (25, 37).

Although RU486 might exert agonist-like effects through activation of the progesterone receptor (38), in most systems RU486 does not display any agonistic action (19). This fact, together with the results of the present study, allow us to conclude that the absence of progesterone activity on pro-oestrus afternoon permits the release of gonadotrophin surges 24 h later. Prolonged maintenance of increased circulating levels of oestradiol through pro-oestrus and oestrus is essential to expression of the LH surge, while a background of low serum oestradiol concentrations facilitates FSH release. Our findings indicate that the release of FSH during oestrus afternoon and evening and early metoestrus in rats injected with RU486 at pro-oestrus is a consequence of the release of the pituitary from ovarian negative feedback inhibition in the presence of the central nervous system signal.

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