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One Day Estrous Cycle Shortening Induced by Antiprogestagen RU486 Administration in Proestrus to 4-Day Cyclic Rats

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Administration of 4 mg of the antiprogestagen RU486 to 4-day cyclic rats in proestrus, which blocks proestrous and diestrous progesterone actions, induced a one day shortening of the ovarian cycle and a reduction of the ovulation rate in the following cycle. These effects were not present when RU486 was administered in estrus or metestrus. RU486 injections either in proestrus or estrus increased the serum levels of LH and 17β-estradiol during metestrus. However, only rats injected with RU486 in proestrus presented a 24 hour advancement of the preovulatory surge of gonadotropins and a lack of the LH-inhibiting effect of exogenous estradiol. These results suggest that, in 4-day cyclic rats, the secretion of progesterone by the corpora lutea during diestrous phase retards the follicular development by lowering the serum concentrations of LH, whereas progesterone secretion by the preovulatory follicles in proestrus regulates the estrous cycle length by antagonizing the desensitization of the pituitary to the estrogen negative feedback on LH secretion.

Key words: 4-day cyclic rats, RU486, Follicular progesterone, Luteal progesterone.

In the 4-day cyclic rat, the preovulatory LH-dependent follicular secretion of progesterone (28) begins to rise early in the afternoon of proestrus, reaches maximum values at 1800 h and then falls to basal levels by early estrus. On the other hand, the LH-independent luteal secretion of progesterone rises in the afternoon of metestrus, reaches peak values by early morning of diestrus and falls to basal levels shortly after (23). The duration of the high serum concentrations of progesterone during diestrous is more prolonged in 5-day cyclic rats (16) and determines the length of the ovarian cycle.

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In 5-day cyclic rats, the reduction of luteal progesterone secretion by administration of bromocriptine (19, 30) or the blockade of luteal progesterone actions with an antiprogesterone serum (13) or with the antiprogestagen RU486 (29), results in a one day shortening of the estrous cycle. However, such treatments do not result in a one day shortening of the ovarian cycle in 4-day cyclic rats (5, 18, 20).

In preliminary observations, we found that a single injection of RU486 in proestrus to 4-day cyclic rats shortens the length of the ovarian cycle by one day. The vaginal smears of this 3-day estrous cycle were estrus, metestrus, proestrus and estrus. In this paper we have studied the physiological characteristics of this RU486-induced 3-day estrous cycle.

Materials and Methods

General.– Female Wistar rats between 180 and 220 g b.w. were used. Rats were housed, five per cage, at 20-22 °C room temperature under controlled conditions of light (lights on 0700 h; and lights off, 1900 h) and food and water *ad libitum.* Vaginal smears were examined daily and only those rats showing at least two consecutive 4-day estrous cycles were used.

Treatments.– Antiprogestagen RU486 (Mifepristone, 11 β-(4-dimethylaminophenyl)-17 β-hydroxy-17 α-(1-propinyl)-estra-4,9-diene-3-one) (Roussel-Uclaf, Romainville, France). This compound has a high affinity for the progesterone receptor (2, 3, 17). In the first experiment RU486 was administered s.c. at a dose of 0.1, 1, 2 or 4 mg/200 µl oil at 0900 h in proestrus or 4 mg/200 µl oil at 0900 h in estrus or metestrus. In the second and third experiments rats were injected s.c. with 4 mg RU486/200 µl oil at 0900 h in proestrus or estrus. Controls were injected with 200 µl oil at 0900 h in proestrus, estrus or metestrus.

Estradiol benzoate (EB) (Sigma) was prepared at a concentration of 60 µg/ml oil and the rats were injected s.c. with 3 µg/100 g body weight at 1300 h in metestrus. Control rats were injected with 100 µl oil.

Experimental design.– The first experiment studied the effects of 0.1, 1, 2 or 4 mg RU486 in proestrus or 4 mg RU486 in estrus or metestrus on both the length of the ovarian cycle and the ovulation rate. Rats were sacrified on the day of vaginal estrus and the number of ova was counted.

In the second experiment the effects of 4 mg RU486 in proestrus or estrus on the serum concentrations of LH and FSH and 17β-estradiol throughout the estrous cycles were determined. Rats were bled by direct jugular venipuncture at 1800 h on each day of the estrous cycles from estrus onwards and the serum stored frozen until the radioimmunoassays of LH, FSH and 17β-estradiol were run.

The last experiment was carried out to determine the serum concentrations of LH and FSH after the administration of exogenous estradiol in rats injected with RU486 in proestrus or estrus. At 1300 h in metestrus rats were injected with EB or oil and decapitated 24 h after injection. Trunk blood was collected and the serum stored frozen until the radioimmunoassays of LH and FSH were run.

Ovulation.– To assess ovulation, the ampullary region of the fallopian tubes was examined for the presence of eggs between 1000 h and 1100 h of the day of vaginal estrus.

Blood sample collection.– At the times indicated in the second experiment, less than 0.5 ml blood was taken by jugular venipuncture while rats were under light

ether anaesthesia. The blood was allowed to clot, centrifuged at 4 °C, and the serum was stored at –20 °C until assayed by radioimmunoassay for LH, FSH and 17β-estradiol.

Hormone radioimmunoassays.– Serum concentrations of LH and FSH were measured in duplicate in 25 µl samples using doubleantibody RIA methods with the RIA kit supplied by NIH (Bethesda, MD) and following the microassay method described previously (20). Rat LH-I-9 and rat FSH-I-8 were labeled with ^{125}I by the chloramine T method (12). LH and FSH concentrations were expressed as ng/ml of serum of the reference preparation LH-rat-RP-3 and FSH-rat-RP-2, respectively. All samples were run in the same assay. The intraassay coefficients of variation were 7 and 6 % for LH and FSH, respectively. The sensitivities of the assays were 7.5 and 20 pg/tube for LH and FSH, respectively.

Serum concentrations of 17β-estradiol were measured by using a commercially obtained kit (Diagnosis Products Corporation, Los Angeles, CA). The sensitivity of the assay was 1 pg/tube and the intra-assay coefficient of variation was 6.5 %.

Statistics.– Results are given as the mean ± SEM. Data were evaluated for statistically significant differences using one-way analysis of variance (ANOVA) followed by Tukey's Q test. Difference was considered to be statistically significant if $P < 0.05$.

Results

Length of the vaginal estrous cycle and ovulation rate in 4-day cyclic rats injected with RU486 (table I).– The injection of 4 mg RU486 in proestrus induced a one day shortening of the estrous cycle and a reduction in the number of ova shed per ovulating rat in the following cycle. While 1 and 2 mg RU486 in proestrus affected these parameters in a dose dependent manner, 0.5 mg RU486 in proestrus had no effect. The administration of 4 mg RU486 either in estrus or metestrus did not induce estrous cycle shortening or reduction of the number of ova. Interest-

Table I. *Number of rats with one day estrous cycle shortening and ovulation rate in 4-day cyclic rats injected with RU486.*
RU486 (RU): 0.5, 1, 2 or 4 mg/200 µl oil (s.c.) at 0900 h in proestrus or 4 mg/200 µl oil (s.c.) at 0900 h in estrus or metestrus. Controls (oil): 200 µl oil at 0900 h in proestrus, estrus or metestrus (the three groups are represented together since no significant differences were found among then).

Treatment on cycle days			Proportions of rats with one day estrous cycle shortening	Number of ova per ovulating rat
Proestrus	Estrus	Metestrus		
Oil	Oil	Oil	0/18	13.8 ± 0.8
RU (0.5 mg)			0/8	13.6 ± 1.1
RU (1 mg)			5/8	12.7 ± 1.0
RU (2 mg)			6/8	9.4 ± 1.2[a]
RU (4 mg)			9/9	8.1 ± 1.0[b]
	RU (4 mg)		0/7	13.1 ± 1.3*
		RU (4 mg)	0/7	14.4 ± 0.6

a = $p < 0.05$, b = $p < 0.01$ vs. oil-treated rats (ANOVA) and Tukey's Q test.
* = 55 ± 10 % of the ova lacked cumulus.

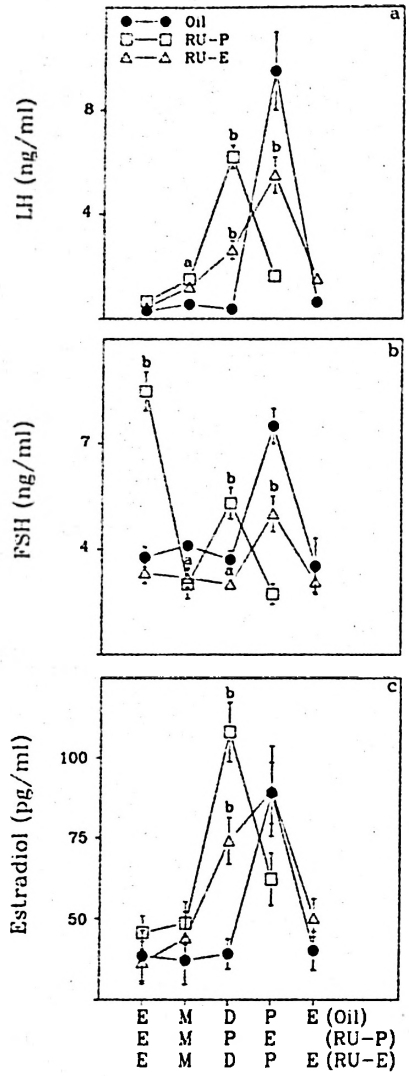

Fig. 1. *Serum concentrations of LH, FSH and 7β-estradiol at 1800 h throughout the estrous cycles in rats injected with RU486.*

Rats injected s.c. at 0900 h with 200 µl oil in proestrus or estrus (Oil; both groups are represented together since no significant differences were found between them) or with 4 mg RU486/200 ml oil in proestrus (RU-P) or estrus (RU-E). Rats injected with RU486 in proestrus displayed a one day estrous cycle shortening and vaginal smears were estrus (E), metestrus (M), proestrus (P) and estrus. D = diestrus. Data are mean ± SEM of 8-10 rats. (a) $P < 0.05$ and (b) $P < 0.01$ vs oil-injected rats (ANOVA and Tukey's Q test).

ingly, 55 ± 10 % of the ova found in the rats injected with 4 mg RU486 in estrus lacked cumulus.

Serum concentrations of LH, FSH and 17β-estradiol throughout the ovarian cycle in rats injected with RU486 (fig. 1).– The injection of RU486 in either proestrus or estrus increased the serum concentrations of LH in metestrous evening and reduced the primary surge of LH. Rats treated with RU486 in proestrus had a one day advancement of the preovulatory surge of LH while those injected with RU486 in estrus showed a significant increase in the serum concentration of LH in diestrous evening and the preovulatory surge of LH was present on the following day (fig. 1a). Both basal concentrations of FSH and the preovulatory surge of FSH were decreased by the injection of RU486 in proestrus or estrus. Rats treated with RU486 in proestrus presented a one day advancement of the preovulatory FSH surge while those injected with RU486 in estrus showed a preovulatory surge of FSH at the same time than controls (fig. 1b). Furthermore, the injection of RU486 in proestrus induced a significant increase in the serum concentrations of FSH in estrous afternoon (fig. 1b). The serum concentrations of 17β-estradiol paralleled those of LH but no significant differences were noted in metestrus among groups (fig. 1c).

Serum concentrations of LH and FSH after the injection of estradiol benzoate to rats injected with RU486 (table II).– Both in controls and in rats treated with RU486 in estrus, the administration of EB in metestrus significantly reduced the serum concentrations of LH 24 hours after the injection. This LH-suppressing effect of estrogen was absent in rats injected with RU486 in proestrus. No differences were

Table II. *Serum concentrations of LH and FSH (ng/ml) 24 h after the injection of estradiol benzoate EB) to rats treated with RU486 in proestrus or estrus.*
RU486: 4 mg/200 µl (s.c.) at 0900 h in proestrus or estrus. Controls (Oil): 200 µl oil at 0900 h in proestrus or estrus (both groups are represented together since no significant differences were found between them). EB: 6 µg/100 µl oil (s.c.) at 1300 h in metestrus.

	Control		RU486 in proestrus		RU486 in estrus	
	Oil	EB	Oil	EB	Oil	EB
LH	0.4 ± 0.06	0.14 ± 0.03[b]	1.57 ± 0.13	1.57 ± 0.2	0.95 ± 0.1	0.72 ± 0.07[a]
FSH	3.72 ± 0.41	3.7 ± 0.32	3.19 ± 0.31	3.1 ± 0.34	3.79 ± 0.22	4.2 ± 0.35

a = $p < 0.05$ compared with its control (ANOVA) and Tukey's Q test.
b = $p < 0.01$ compared with its control (ANOVA) and Tukey's Q test.

found in the serum concentrations of FSH after the EB injection in any group.

Discussion

The results of the present experiments showed an inverse relationship between the amount of RU486 injected in proestrus and the number of ova per ovulating rat in the following cycle and also a lack of the effect of RU486 on the ovulation rate when injected in estrus or metestrus. It is well established that the secondary release of FSH at early estrus initiates recruitment of growing follicles in estrous afternoon, that these follicles are ovulated at the following estrus (11) and that the administration of the antiprogestagen RU486 in proestrus blocks the secondary surge of FSH (14, 21, 22) by antagonizing proestrous corticosterone and, probably, progesterone actions (25, 26). There is a significant correlation between the release of FSH in estrus and the recruitment of preantral follicles (10), as well as between the doses of RU486 in proestrus and the degree of suppression of the secondary surge of FSH (14, 22). All these findings and data indicate that the effect of RU486 in proestrus on the ovulation rate in the ensuing cycle is a consequence of a reduction of the secondary FSH surge-induced follicular recruitment in estrous afternoon.

In the cyclic rat, estradiol and progesterone secreted during diestrous (15, 31), but not during estrus (9), affect negatively the basal secretion of LH and FSH (4, 24). Thus, the finding that RU486 administration either in proestrous or estrous morning increased the serum levels of LH in metestrus, together with the well known pharmacological characteristics of RU486 (2, 3, 17), clearly demonstrates that the actions of progesterone during diestrous were blocked by the administration of 4 mg RU486 either in proestrus or estrus.

The more prolonged secretion of luteal progesterone during diestrus in 5-day cyclic rats (6, 13, 16, 19) retards follicle maturation and, by antagonizing the desensitizing effect of estrogen (7), delays the forthcoming ovulation. In contrast, our results have shown that, in 4-day cyclic rats, the blockade of follicular and luteal progesterone actions by injecting RU486 in proestrus induced a one day estrous cycle shortening which was associated with an absence of the negative estrogen feedback on LH secretion, while rats injected with RU486 in estrus had normal estrous cycle length and the injection of estradiol benzoate lowered LH secretion. The effect of the increased LH

serum concentrations on the follicular maturation was demonstrated in rats injected with RU486 in estrus by the presence of 55 % of the ova lacking cumulus. However, this effect could not be observed in rats injected with RU486 in proestrus because the preovulatory release of LH was advanced 24 hours. This suggests that the absence of progesterone actions during diestrous dissociates follicular maturation and the preovulatory pituitary desensitization to the negative feedback action of estrogen. In agreement with ADVIS *et al.* (1), our results showed an absence of negative feedback of estrogen on FSH secretion during diestrous.

The finding that the serum concentrations of FSH in estrous afternoon were significantly increased in rats treated with RU486 in proestrus confirms results from our laboratory (27). This hypersecretion of FSH in estrus seems to be related to the absence of the LH-dependent progesterone rise in proestrus like that of LH in pentobarbital-treated rats (8). In contrast to the hypersecretion of LH in estrus, the hypersecretion of FSH in rats lacking proestrous afternoon progesterone actions would need low plasma levels of inhibin and estradiol (27).

In summary, the results of these experiments indicate that, in 4-day cyclic rats, the preovulatory desensitization to the negative estrogen feedback is antagonized by the preovulatory LH-dependent progesterone secretion in proestrus, while the LH-independent progesterone secretion during metestrus and early diestrus retards follicle maturation via lowering tonic LH secretion. The mechanism by which proestrous afternoon progesterone secretion antagonizes the preovulatory desensitization to the negative estrogen feedback in 4-day cyclic rats is not known. The long interval (48-72 hours) between the proestrous secretion of progesterone and the desensitization to the negative feedback of estrogen supports the concept of an indirect effect on ovarian substances different from progesterone (steroids or peptides).

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El bloqueo de las acciones de la secreción de progesterona en proestro y en estro mediante la administración de 4 mg del antiprogestágeno RU486 en proestro a ratas con ciclos de 4 dias, induce acortamiento de un día del ciclo ovárico así como reducción de la tasa de ovulación en el ciclo siguiente. Estos efectos no se presentan cuando el RU486 se administra en estro o en metestro. El RU486, tanto en proestro como en estro, incrementa los niveles séricos de LH y estradiol 17β durante el metestro. Sin embargo, sólo las ratas inyectadas con RU486 en proestro presentan un avance de 24 h de la liberación preovulatoria de gonadotrofinas y pérdida del efecto inhibidor del estradiol exógeno sobre la secreción de LH. Estos resultados sugieren que, en las ratas con ciclos de 4 días, la secreción de progesterona por los cuerpos lúteos durante la fase de diestro retarda el desarrollo folicular disminuyendo las concentraciones séricas de LH, mientras que la secreción de progesterona por los folículos preovulatorios en proestro regula la longitud del ciclo ovárico antagonizando la desensibilización de la hipófisis a la acción de feedback negativo que ejercen los estrógenos sobre la secreción de LH.

Key words: Ratas cíclicas de 4 días, RU486, Progesterona folicular, Progesterona lútea.

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