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Follicular and luteal progesterone synergize to maintain 5-day cyclicity in rats

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The length of the ovarian cycle in rat is determined by the duration of progesterone secretion from the corpora lutea (CL) during diestrus. The action of progesterone secretion from the preovulatory follicles on proestrus is also responsible for the cycle length in 4-day cyclic rats. To study whether follicular and luteal progesterone participate in the maintenance of 5-day cyclicity, the effects of the antiprogestagen RU486 (5 mg on proestrus or estrus) on estrous cycle length and on the serum concentrations of LH in 5-day cyclic rats and in 4-day cycle experimentally induced by the dopamine agonist CB154 (1 mg on estrus) were investigated. Furthermore, serum concentrations of progesterone on the day of ensuing ovulation were measured to see whether activation of the CL function after treatment with RU486 had occurred. Both 5-day and CB154-injected rats had a 3-day estrous cycle after RU486 on proestrus, while RU486 on estrus shortened by 1-day the estrous cycle length in 5-day but not in CB154-injected rats. Basal serum concentrations of LH increased and the LH surge decreased after RU486 treatment in both cycle types. Serum concentrations of progesterone rose only in 5-day rats injected with RU486. These results indicate that the actions of both follicular and luteal progesterone synergize in maintaining the length of the estrous cycle in 5-day cyclic rats and that functionally active CL increase progesterone production only under the action of a complete surge of prolactin.

Key words: RU486, Progesterone, Prolactin, Estrous cycle.

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Reproductive cycles in female rats characteristically last 4 or 5 days. The administration of progesterone to 4-day cyclic rats during the time of corpus luteum (CL) activity prolongs the duration of the elevated levels of circulating progesterone during diestrus (17). This results in a 1-day cycle prolongation (15). The blockade of diestrous progesterone actions through the administration of the antiprogestagen RU486 (13, 22) to 5-day cyclic rats results in a 1-day estrous cycle shortening. Additionally, the injection of bromocryptine during estrus to 5-day cyclic rats reduces the duration of progesterone secretion by the CL and the length of the estrous cycle by 1 day (2, 11-13, 24). It is, therefore, widely agreed that 5-day cyclicity is the result of the more prolonged period of progesterone secretion by the CL (2, 5, 10, 11, 13, 26), which retards preovulatory desensitization to negative estrogen feedback (3).

Recently it has been found that, in 4-day cyclic rats, the absence of the action of the luteinizing hormone surge-dependent progesterone secretion on the afternoon of proestrus (21), after treatment with RU486 on proestrus, advances the onset of pituitary desensitization to negative estrogen feedback and enhances pituitary responsiveness to luteinizing hormone (LH)-releasing hormone (LHRH) during diestrus (18). These effects of RU486 on proestrus result in a 3-day estrous cycle (19).

The aim of the present experiments was to study whether follicular and luteal progesterone actions synergize in determining the length of the ovarian cycle in 5-day rats. In addition, in order to evaluate whether RU486 activates the corpus luteum function in 3 and 4-day cyclic rats, as has been reported for 5-day cyclic rats (23), the serum progesterone concentrations were determined on the day of ovulation (day of estrus) in 3-, 4- and 5-day estrous cyclic rats.

Materials and Methods

Animals.– Adult virgin (R x U) hybrids of two Wistar substrains (R-inbred females and U-inbred males) were used. These rats display, almost exclusively, 5-day ovarian cycles (24, 25). They were kept in controlled light (lights on from 03.00 to 17.00 h) and temperature (22 ± 1 °C) conditions, with free access to drinking water and standard food pellets. Rats were housed five per cage and examined daily for vaginal smears. Only rats with at least two typical consecutive 5-day ovarian estrous cycles (26) were used. The day of vaginal metestrus was designated day 1. Under the lighting conditions used, the LH surge began between 2 and 3 hours after the middle of the light period on the day of proestrus, reached its peak around 16.00 h and decreased shortly after the onset of the dark period (8, 9).

Treatments.– Antiprogestagen RU486, 11β-(4-dimethylaminophenyl)-17β-hydroxy-17α-(1-propinyl)-estra-4,9-diene-3-one (Roussel-Uclaf, Romainville, France), was obtained in micronized crystalline form and suspended in olive oil (25 mg/ml).

Dopamine agonist 2-bromo-α-ergocryptine, CB154 mesilate (Sandoz, Basel) was dissolved in 70 % ethanol and administered s.c. at 14.00 h on estrus (1 mg/0.25 ml per rat) to inhibit pituitary prolactin release in the afternoon of estrus.

Control and experimental groups.– Five-day cyclic rats and rats with a 4-day cycle, experimentally induced by a single s.c. injection of CB154 at 14.00 h on estrus, were given an s.c. injection of

RU486 (5 mg/0.2 ml) at 10.00 h on proestrus or estrus. Control injections consisted of 0.25 ml ethanol 70 % or 0.2 ml oil.

Blood sample collection.– Except on the day after proestrus where 1 ml blood was taken, less than 0.4 ml blood was obtained by direct jugular venipuncture under light ether anaesthesia at 16.00 h, in each day of the estrous cycle. Blood was kept at 4 °C to clot overnight, and the serum then removed and stored at TM20 °C until assayed for LH and progesterone.

Radioimmunoassays of LH and progesterone.– Serum LH concentrations were measured in duplicate in 25 µl samples using the double antibody RIA method with the RIA kit supplied by NIH (Bethesda, MD), following the microassay method described previously (12). Rat LH-I-9 was labeled with ^{125}I by the chloramine T method (4). LH concentrations were expressed as ng/ml of serum of the reference preparation LH-rat-RP-3. All samples were run in the same assay. The intraassay coefficient of variation was 7 % and the sensitivity of the assay was 7.5 pg/tube.

Serum concentrations of progesterone were measured in duplicate in 100 µl samples using a commercially-obtained kit (Diagnosis Products Corp., Los Angeles, CA). The intraassay coefficient of variation was 6 % and the sensitivity of the assay was 10 pg/tube.

Data evaluation and statistical analysis.– To assess the statistical significance of the effects of the different treatments on the length of the vaginal estrous cycle, the exact probability Fisher's test was used. Values of serum concentrations of hormones 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 significant if $p < 0.01$.

Results

Length of the vaginal estrous cycle (table I).– The length of the cycle in all 5-day cyclic rats injected with CB154 was shortened by 1 day. Although the injection of RU486 either in proestrus or estrus induced the presence of a large number of cornified squamous epithelial cells throughout the vaginal estrous cycle, the length of the ovarian cycle was assessed by the presence of clusters of round nucleated epithelial cells bearing an easily visible nucleus. Approximately 83 % of 5-day and 85 % of 4-day cyclic rats injected with RU486 on proestrus had a vaginal estrous cycle of 3 days' length. Six out of seven of 5-day cyclic rats injected with RU486 in estrus displayed 1-day shortening of the estrous cycle, while RU486 on estrus in 4-day cyclic rats did not induce such a shortening.

Table I. *Effects of RU486 and CB154 on the length of the ovarian reproductive cycle in 5-day cyclic rats.*
Ethanol 70 % (0.25 ml) or CB154 (1 mg) was injected at 14.00 h on estrus. Oil (0.2 ml) or RU486 (5 mg) was injected at 10.00 h on proestrus (P) or estrus (E).

		Number of rats with cycles of		
Treatment	n	3 day	4 day	5 day
Ethanol + Oil	7	0	1	6
Ethanol + RU486 (P)	6	5	1	0
Ethanol + RU486 (E)	7	0	6	1
CB154 + Oil	7	0	7	0
CB154 + RU486 (P)	7	6	1	0
CB154 + RU486 (E)	6	0	6	0

Serum LH concentrations by CB154 and/or RU486 injections.– Administration of RU486 during proestrus or estrus in 5-day as well as in 4-day cyclic rats increased the basal serum LH concentrations in the afternoon of metestrus and, regardless of the duration of the estrous cycle, reduced the serum LH concentration in the afternoon of proestrus. Four-day cyclic rats injected with RU486 on estrus, also recorded increased serum LH concentrations in the afternoon of diestrus (fig. 1).

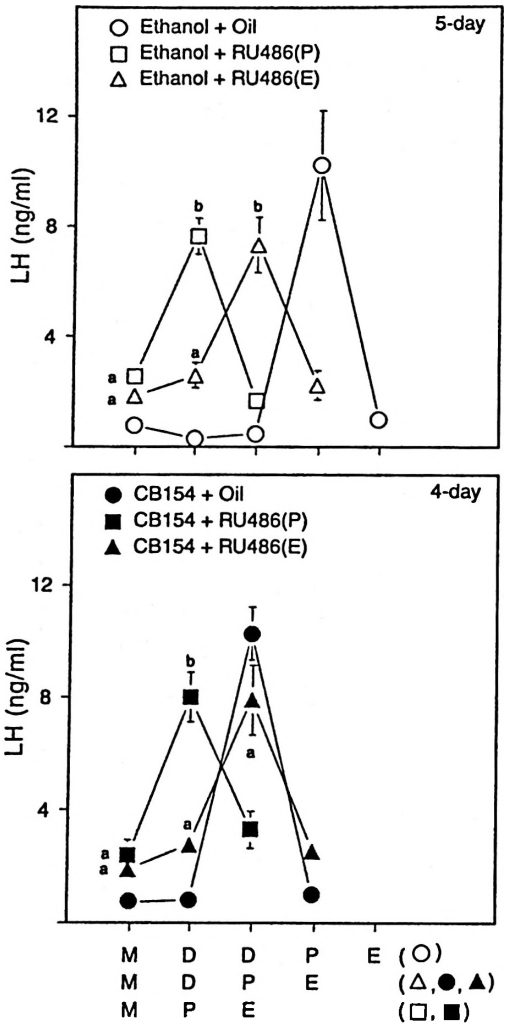

Fig. 1. *LH serum levels at 16.00 h on each day of the estrous cycle of 5-day cyclic and of CB154-induced 4-day cyclic rats injected with RU486.*
Rats were injected (s.c.) with RU486 (5 mg/0.2 ml oil) at 10.00 h on proestrus (P) or estrus (E). CB154 (1 mg/0.25 ml ethanol 70 %) was injected (s.c.) at 14.00 h on estrus. M = metestrus and D = diestrus. Data are mean ± SEM for 5-7 rats. (a) $p < 0.05$ and (b) $p < 0.01$ vs. oil-treated rats (ANOVA and Tukey's Q test).

Effect of CB154 and/or RU486 on serum progesterone concentrations (fig. 2).– After 16.00 h estrus serum concentrations of progesterone were low in both 5-

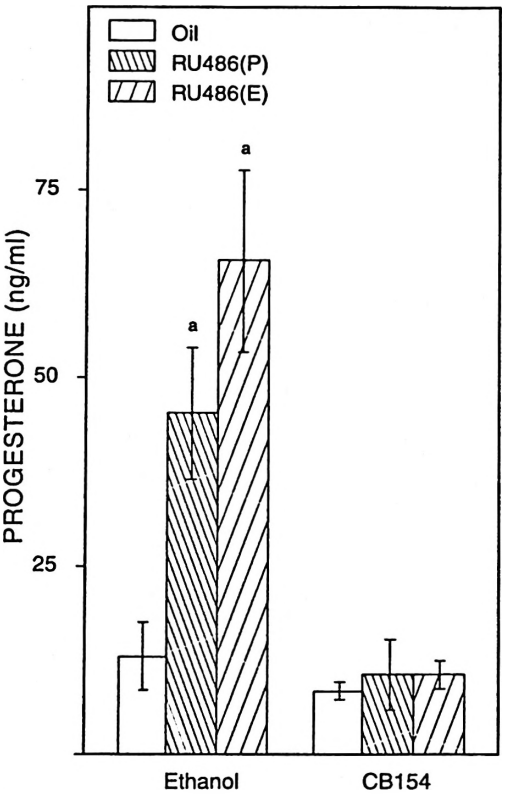

Fig. 2. *Progesterone serum levels at 16.00 h on the day of estrus in 5-day cyclic and of CB154-induced 4-day cyclic rats injected with RU486.*
Legend as in fig. 1. (a) $p < 0.01$ vs. oil-treated rats.

and 4-day cyclic rats. The injection of RU486 during proestrus or estrus in 5-day cyclic rats resulted in a significant increase in the serum concentrations of progesterone in the afternoon of the day after proestrus. On the other hand, injections of RU486 either on proestrus or estrus did not affect the serum concentrations of progesterone in CB154 injected rats.

Discussion

The main finding of these experiments is that the blockade of proestrous afternoon progesterone actions through the administration of the antiprogestagen RU486 during proestrus resulted in a 1- and 2-day advancement of LH surge and shortening of the ovarian cycle in CB154-induced 4-day and in 5-day cyclic rats, respectively. These effects of RU486 resulted in estrous cycles of 3-days in both types of rats. While in 5-day cyclic rats the estrous cycle shortening effect of RU486 either on proestrus or estrus was associated with activation of the corpus luteum, the 1 day shortening effect of RU486 on proestrus in CB154-induced 4-day cyclic rats was not.

Moreover, the results of these experiments have confirmed that administration of bromocryptine on the afternoon of estrus in 5-day cyclic rats reduces the length of the ovarian cycle by 1 day (2, 13, 24), as the suppressive effect of bromocryptine on PRL release on the afternoon of estrus (7, 14) eliminates the antiluteolytic and luteotrophic actions of PRL (11). This results in a duration reduction of the of progesterone secretion by the corpus luteum to a level similar to that exhibited in 4-day cyclic rats. Additionally, these experiments have replicated previous findings concerning the neutralization effects of progesterone actions during diestrus on the length of the estrous cycle in 4- (12, 18, 20) and 5-day cyclic rats (13, 22).

In the cyclic rat, the pattern of progesterone secretion shows two increases. The first, of follicular origin, takes place in the afternoon of proestrus and depends on the preovulatory surge of LH (21). The second, of luteal origin, occurs during the diestrous phase and is independent from the pituitary (16). Because of the absence of the inhibitory and stimulatory effects of progesterone on basal LH release and on LH surge (12), respectively, in all groups of rats treated with RU486 and regardless of the length of the cycle, the serum concentration of LH during the diestrous phase increased and the preovulatory surge of LH in the afternoon of proestrus decreased.

As previously described for spontaneous 4-day cyclic rats (19), the blockade of progesterone actions on proestrous afternoon by administration of RU486 on proestrous morning reduced the length of the cycle by 1 day in experimentally-induced 4-day cyclic rats and by 2 days in 5-day cyclic rats. This would appear to mean that the LH surge-dependent progesterone secretion on proestrous afternoon has a role in determining the length of the cycle in all cycle types (5- and spontaneous and experimental 4-day cycles). The physiological mechanism of this ovary-mediated effect of proestrous afternoon progesterone secretion is at present unknown, although ovarian non-steroidal substances seem to be involved (18).

On proestrous afternoon, together with the LH surge there is also a PRL surge (16). This secretion of PRL acts luteolytically on the CL from the previous estrous cycle, which has lost the capacity to secrete progesterone (1). However, PRL acts luteotrophically when the CL is functionally active in terms of progesterone production (6). When, as in the present experiments, LH

surge is advanced, the PRL surge is also advanced (22). The 24 or 48 h PRL surge advancement coincides, in 5-day cyclic rats,with a CL still producing progesterone (5, 10, 26), which resulted in raised progesterone serum concentrations on the ovulation day.

The 1-day estrous cycle shortening due to administration of RU486 on proestrus in CB154-induced 4-day cyclic rats was not followed by activation of the CL, despite the temporal coincidence of a CL still producing progesterone (13) and the PRL surge. It may be that CB154 injection, besides reducing the duration of the corpus luteum progesterone secretion (11), also affected the magnitude of the PRL surge on proestrus. This would be possible since the interval between the administration of 1 mg CB154 (14.00 h on estrus) and the expected PRL surge (16.00 h on proestrus) in rats with a 3-day estrous cycle is 50 hours and administration of 1 mg CB154 at 09.00 h on estrus slightly reduces the proestrous surge of PRL some 78 h later (13).

In summary, the results of these experiments show that the actions of proestrous afternoon progesterone of follicular origin synergizes with those of diestrous progesterone of luteal origin in determining the length of the ovarian cycle in 5-day cyclic rats. Furthermore, the results indicate that activation of CL in rats treated with RU486 is due to the luteotrophic effect of the advanced prolactin surge.

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J. E. SÁNCHEZ-CRIADO, A. RUIZ, M. TÉBAR y J. A. M. MATTHEIJ. *Sinergismo de las acciones de la progesterona folicular y lútea en el mantenimiento de la duración del ciclo estral en la rata de 5 días.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (4), 223-230, 1995.

La duración de la secreción de progesterona procedente del cuerpo lúteo (CL) durante la fase de diestro determina que la longitud del ciclo ovárico en la rata sea de 4 ó de 5 días. A su vez, en las ratas con ciclos de 4 días, la ausencia de la acción de la progesterona de origen folicular en la tarde de proestro acorta la duración del ciclo ovárico a 3 días. Para estudiar si también las acciones de la progesterona lútea y folicular sinergizan en el mantenimiento de la longitud del ciclo de 5 días, se estudian la longitud del ciclo estral y las concentraciones séricas de LH tras la administración del antiprogestágeno RU486 (5 mg) en proestro o en estro en ratas con ciclos de 5 días. Estos mismos parámetros se analizan en ratas de 5 días, con un ciclo de 4 días inducido experimentalmente por inyección del agonista dopaminérgico CB154 (1 mg) en estro. Además, se estudian las concentraciones séricas de progesterona en el día de la siguiente ovulación. La inyección de RU486 en proestro acorta a 3 días la longitud del ciclo, tanto en las ratas de 5 días como en las de 4 inyectadas con CB154, mientras que la administración de RU486 en estro sólo acorta la duración del ciclo a 4 días en las ratas de 5 días. El tratamiento con RU486 aumenta las concentraciones séricas basales de LH, reduce la magnitud de la liberación preovulatoria de LH con independencia de la longitud del ciclo e incrementa las concentraciones séricas de progesterona sólo en las ratas no inyectadas con CB154. Estos resultados indican que tanto la progesterona folicular como la lútea sinergizan en el mantenimiento de la duración del ciclo ovárico en las ratas de 5 días, y que la producción de progesterona por el CL se incrementa en las ratas tratadas con RU486 por la acción luteotrópica de la prolactina.

Palabras clave: RU486, Progesterona, Prolactina, Ciclo estral.

References

1. Billeter, E. and Flückiger, E. (1980): *Experientia*, 27, 464-465.

2. Boehm, N., Plas-Roser, S. and Aron, Cl. (1984): *Acta Endocrinol.*, 106, 188-192
3. Döcke, F., Rohde, W., Chaoui, R., Stürzebecher, J. and Dörner, G. (1987): *J. Endocrinol.*, 114, 409-414.
4. Greenwood, F. C., Hunter, W. M. Glover, J. S. (1963): *Biochem. J.* 89, 114-123.
5. Kaneko, S., Sato, N., Sato, K. and Hashimoto, I. (1986): *Biol. Reprod.*, 34, 488-494.
6. Krey, L. C. and Everett, J. W. (1973): *Endocrinology*, 93, 377-384.
7. López, F. J., Domínguez, J. R., Sánchez-Criado, J. E. and Negro-Vilar, A. (1989): *Endocrinology*, 124, 536-542.
8. Mattheij, J. A. M., Swarts, J. J. M., de Grooth, R. and Kusters, I. (1992): *Exper. Clin. Endocrinol. (Life Sci. Adv.)*, 11, 85-91.
9. Mattheij, J. A. M., Swarts, J. J. M., van het Hof, K. H., Verburg, J. and Visser, J. (1992): *Reprod. Fertil.*, 95, 775-782.
10. Nequin, L. G., Alvarez, J. and Schwartz, N. B. (1979): *Biol.Reprod.*, 20, 659-670.
11. Sánchez-Criado, J. E., van der Schoot, P. and Uilenbroek, J. Th. J. (1988): *J. Endocrinol.*, 117, 455-460.
12. Sánchez-Criado, J. E., Bellido, C., Galiot, F., López, F. J. and Gaytán, F. (1990): *Biol. Reprod.*, 42, 877-886.
13. Sánchez-Criado, J. E., Uilenbroek, J. Th. J. and Karels, B. (1992): *J. Endocrinol.*, 132, 115-122.
14. Sánchez-Criado, J. E., López, F. J. and Aguilar, E. (1986): *Endocrinology*, 119, 1083-1088.
15. Sánchez-Criado, J. E. and van der Schoot, P. (1989): *Rev. esp. Fisiol.*, 45, 235-238.
16. Smith, M. S., Freeman, M. E. and Neill, J. D. (1975): *Endocrinology*, 96, 219-226.
17. Taya, K., Terranova, P. F. and Greenwald, G. S. (1981): *Endocrinology*, 108, 2324-2330.
18. Tebar, M., Ruiz, A., Bellido, C. and Sánchez-Criado, J. E., (1996): *Biol. Reprod.*, 54, 1266-1270.
19. Tébar, M., Ruiz, A., Gaytán, F. and Sánchez-Criado, J. E. (1995): *Biol. Reprod.*, 53, 1183-1189.
20. Tébar, M., Ruiz, A. and Sánchez-Criado, J. E., (1995): *Rev. esp. Fisiol.*, 51, 85-92.
21. Uchida, K., Kadowaki, M. and Miyake, T. (1969): *Endocrinol. Japon.*, 16, 227-237.
22. Uilenbroek, J. Th. J. (1991): *Endocrinology*, 129, 423-429.
23. van der Schoot, P., Bakker, G. H. and Klijn, J. G. M. (1987): *Endocrinology*, 121, 1375-1382.
24. van der Schoot, P. and Uilenbroek, J. Th. J. (1983): *Endocrinology*, 97, 83-89.
25. van der Schoot, P. (1980): *J. Endocrinol.*, 86, 451-457.
26. van der Schoot, P. and de Greef, W. J. (1976): *J. Endocrinol.*, 70, 61-68.