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Unilateral Ovariectomy, Flutamide Treatment and HCG Reverse the Anovulatory Action of Antiprogesterone RU486 in Rat

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Administration of antiprogesterone RU486 (4 mg/day) from estrus through proestrus to cyclic rats blocked ovulation. Moreover, RU486 increased basal serum concentrations of LH, PRL, testosterone and estradiol, while it decreased basal serum concentration of FSH. Both unilateral ovariectomy and antiandrogen flutamide treatment, as well as an ovulatory injection of HCG in the proestrus afternoon partially reversed, the ovulatory blockade of RU486. These results indicate that both the decreased FSH concentration and the increased testosterone concentration, as well as the reduced ovulatory LH release are responsible for the anovulatory effects of RU486.

Key words: Antiprogesterone, Ovulation, LH, FSH, PRL, Testosterone, Estradiol, Estrous cycle.

Administration of RU486, an antiprogestagen with high affinity for the progesterone receptors (4), to cyclic rats from the day after ovulation to the day before the ensuing expected ovulation, produces an ovulatory impairment, reduces the amount of the preovulatory luteinizing hormone (LH) released (21, 25, 32) and increases the rate of follicular atresia (21).

While reduction in the ovulatory LH released justifies the ovulatory deficit, the mechanism of RU486-induced follicular atresia and its role in the RU486-induced ovulation failure are not known. Besides these effects on ovulatory events, RU486 increases both serum LH: follicle-stimulating hormone (FSH) and serum testosterone: estradiol ratios during the period of follicular growth (26). Moreover, administration of RU486 to cyclic female

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rats increased serum prolactin (PRL) levels (34, 35).

Since normal follicular development in the rat is supported by basal levels of LH, FSH and estradiol, and since testosterone and PRL are favourable to follicular atresia (13), the aim of these experiments was to evaluate the involvement of the decreased basal FSH and the increased testosterone and PRL serum levels in the RU486-induced ovulation failure.

Materials and Methods

Animals. — Experiments were performed on Wistar adult female rats (body weight between 200 and 230 g). They were housed under standard conditions of light (lights-on: 07.00 h; and lights-off: 19.00 h) and temperature (20-22 °C), with food (Sanders rat chow) and tap water available *ad libitum*. Rats were examined daily for vaginal smears. Only those rats showing at least two consecutive 4-day estrous cycles were used.

Compounds and application. — Antiprogesterone RU486 (Roussel-Uclaf, Romainville, France) was dissolved at a concentration of 20 mg/ml in olive oil. Rats were given 0.2 ml (s.c.) a day starting on the day of estrus until the day of proestrus. Control rats received 0.2. ml of oil. The dose of RU486 used derived from a dose-response effect of this compound on ovulation rate in the rat (25).

Flutamide (Schering-Plough, Madrid, Spain) was dissolved at a concentration of 10 mg/ml in ethanol 70 %. Rats were given 0.2 ml (s.c.) on metestrus, diestrus and proestrus. This dose completely blocks the action of testosterone (6).

Bromocryptine (Sigma) was dissolved at a concentration of 4 mg/ml in ethanol 70 %. Rats were injected (s.c.) 1 mg/0.25 ml on metestrus, diestrus and proestrus. This dose is sufficient to block the secretion of PRL (23).

Human chorionic gonadotrophin (HCG) (Pregnyl, Organon, Oss, The Netherlands) was given (10 IU, s.c.) at 17.00 h in proestrus. Prior to the HCG injection, a sc injection of sodium pentobarbital (35 mg/kg BW) was given at 15.00 h to block the endogenous LH surge. Oil-treated rats were injected with either sodium pentobarbital or with sodium pentobarbital and HCG to check the effectiveness of both compounds (32). The ovulatory response was measured the following day.

As a tool to increase the FSH levels (1, 2, 36), unilateral ovariectomy was performed under light ether anesthesia at 09.00 h on metestrus.

Experiments. — Experiment 1 was designed to check the effects of RU486 on the basal concentrations of LH, FSH, PRL, progesterone, testosterone and oestradiol. Rats injected with oil vehicle or with RU486 were killed by decapitation at 09.00 h on the day of proestrus. Blood samples were allowed to clot at 4 °C. Serum samples were stored at -20 °C until assayed.

In experiment 2, rats injected with oil or RU486 were adscribed to the following experimental groups: unilateral ovariectomy, bromocryptine or flutamide treatments and exogenous ovulatory dose of HCG. These groups were formed to study the involvement of the decreased concentration of FSH, the increased concentration of both PRL and testosterone, and the decreased ovulatory LH release in the proestrus afternoon (21, 25, 32) on RU486-induced ovulation blockade.

Ovulation. — To assess ovulation, the fallopian tubes were exposed and the ampullary region examined for the presence of eggs as previously described (24).

Hormone assays. — Concentrations of LH, FSH and PRL were measured without replicate in 25 µl serum samples by

double-antibody radioimmunoassay assays (25) and kits supplied by NIH (Bethesda, MD, USA). Rat LH-I-6, FSH-I-6 and PRL-I-5 were labeled with ^{125}I by the chloramine T method. LH, FSH and PRL values are expressed as ng/ml of the reference preparations LH-rat-RP-2, FSH-rat-RP-2 and PRL-rat-RP-3 respectively.

Serum levels of testosterone and estradiol 17β were determined using a commercially obtained kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Serum concentrations of progesterone were determined with G.D. Niswender's antiserum GDN337 was obtained as previously described (23).

All samples were assayed in the same specific assay and the intraassay coefficients of variation were: 9 %, 6 %, 9 %, 9 %, 7 % and 8 % for LH, FSH, PRL, progesterone, testosterone and oestradiol, respectively.

Statistical analysis. — Results are expressed as mean ± SEM. Means were compared using the Student's t test or Fisher's exact probability test where appropriate. A value of $P < 0.05$ was considered significant.

Table I. *Serum concentrations of LH, FSH, PRL, progesterone, testosterone and estradiol at proestrus (09.00 h) in rats injected with RU486 (mean ± SEM).*

RU486 treatment consisted in one sc injection a day (4 mg/0.2 ml oil) from estrus through proestrus. Number of rats per group, 8.

	Oil	RU486
LH (ng/ml)	1.5±0.5	4.8±1.5**
FSH (ng/ml)	4.5±0.3	3.2±0.3*
PRL (ng/ml)	5.0±1.5	16.7±7.0**
Progesterone (ng/ml)	9.1±1.6	7.0±2.1
Testosterone (ng/ml)	0.5±0.2	5.5±1.0***
Estradiol (pg/ml)	71.0±6.5	135.5±14.5**

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with oil injected rats (Student's t test).

Results

Serum concentrations of gonadotropins and steroids at proestrus in cyclic rats treated with RU486. — Table I shows serum concentrations of gonadotropins (LH, FSH, PRL) and steroid hormones (progesterone, testosterone and estradiol) in cyclic rats at 09.00 h on proestrus and the effect of RU486. The antiprogesterone RU486 increased significantly serum concentrations of LH, PRL, testosterone and estradiol, while it significantly decreased FSH serum concentration. No effects of RU486 on progesterone serum concentrations were noted.

Effect of bromocryptine, unilateral ovariectomy, flutamide and HCG on ovu-

Table II. *Effects of bromocryptine (BCR), unilateral ovariectomy (ULO), flutamide (FLT) and HCG on ovulation rate in 4-day cyclic rats treated with antiprogesterone RU486.*

Treatments: RU486 (see legend of table I). BCR: 1 mg/0.25 ml ethanol 70 % (s.c.) on metestrus, diestrus and proestrus. FLT: 2 mg/0.2 ml ethanol 70 % (s.c.) on metestrus, diestrus and proestrus. HCG: 10 IU (s.c.) at 17.00 h in proestrus. ULO was performed at 09.00 h on metestrus. PBT (sodium pentobarbital): 35 mg/k BW (s.c.) at 15.00 h in proestrus.

Groups	Proportion of rats ovulating	Number of ova ovulating rat
Oil	9/9	13.8 ± 1.0
RU486	0/9	—
Oil + BCR	7/7	14.1 ± 0.6
RU486 ± BCR	0/7	—
Oil + ULO	7/7	14.9 ± 1.0
RU486 + ULO	3/9*	9.8 ± 0.5**
Oil + FLT	5/5	13.2 ± 0.4
RU486 + FLT	4/8*	7.4 ± 2.8**
Oil + PBT	0/8	—
Oil + PBT + HCG	8/8	11.7 ± 1.7
RU486 + PBT + HCG	2/8*	3.5 ± 0.4**

* $p < 0.01$ compared with its control (Fisher's exact probability test).
** $p < 0.01$ compared with its control (Student's t test).

lation rate in RU486-treated cyclic rats. — Treatment with RU486 caused an ovulation blockade. While bromocryptine treatment did not reverse the effects of RU486 on ovulation, unilateral ovariectomy, flutamide treatment and HCG restored partially, both the number of ova per ovulating rat and the proportion of ovulating rats: 33 %, 50 % and 25 %, respectively (table II).

Discussion

Administration of RU486 from metestrus to proestrus in the cyclic rat blocks ovulation (25, 32, 34) and blunts the proestrus afternoon LH surge (25, 32). Moreover, it increases the rate of follicular atresia (25) with temporarily related increases in both LH: FSH and testosterone: estradiol ratios (26).

The results of the present experiments replicate previous data concerning the anovulatory effect of RU486 in the cyclic rat (25, 32). Furthermore, the results show that rats injected with RU486 increased basal concentrations of LH and PRL while decreasing that of FSH.

During the rat estrous cycle the secretion of LH, FSH and PRL from late estrus to early proestrus is low because of the negative feedback action provided by ovarian steroids (estradiol and progesterone) and non-steroid (inhibin) hormones (29). The increased basal levels of LH in response to RU486 may be due to the negative effect of this compound on the ability of progesterone to potentiate estrogen negative feedback on gonadotropin secretion (8). On the other hand, PRL levels are increased because of the stimulating effects of estradiol not counteracted by progesterone on PRL secretion (7, 14). The decrease in FSH serum concentration is the result of an increase in ovarian inhibin secretion (26).

Moreover, out of the three steroids measured, only progesterone serum concentration is not affected by RU486. This has been previously reported in 4-day cyclic rats (25, 27), and it is a reflection of the pituitary-independent corpus luteum-progesterone secretion during dioestrous in the cyclic rat (23). On the contrary, oestradiol, and especially testosterone serum concentrations increased in RU486-treated rats as a response of the ovary to the increased LH/FSH ratio (10).

In the cyclic rat, follicles entering the growing ovulatory pool at the beginning of the estrous cycle (17) are stimulated by the gonadotropins (FSH and LH) to move into more advanced stages of development during the diestrous and early proestrous phases (13). Moreover, the physiological rising levels of estradiol during diestrus facilitate follicular development (12).

Sustained increases in serum LH activity stimulates the growth of follicles to the preovulatory stage (22) and estradiol has mitogenic activity on follicular granulosa cells (13). The antiandrogen flutamide and unilateral ovariectomy partially restored, the effects of RU486 on ovulation. This is consistent with the well known atretogenic effects of both androgens (18) and abnormal low levels of FSH (11, 19, 20). Hyperprolactinemia, by interfering FSH effects on follicular function (9, 31) induces atresia of preovulatory follicles and bromocryptine treatment delays the occurrence of atresia (33). The results of these experiments show that bromocryptine treatment, which is able to reduce PRL levels (23), did not modify the effects of RU486 on ovulation which indicate that the moderate increase in PRL levels in RU486-treated rats was not sufficient to alter follicular development.

As it has been suggested (21, 25, 32), the attenuated LH surge in the proestrus afternoon in rats treated with RU486 is responsible, although only in part, for the effects of RU486 on ovulation. This is clear since the injection of an ovulatory dose of HCG increases both the number

of ovulating rats and the number of ova per ovulating rat.

Beside the application of RU486 as contragestagen when administered during the early pregnancy (3), the antiprogesterone RU486 administered to women during the follicular phase disrupts normal follicular development (15) and lowers serum estradiol concentration (30), attenuates (28) or blocks (5) spontaneous midcycle LH surge and inhibits ovulation (16). Because of these effects, it has been speculated that RU486 could be used in the design of an estrogen-free oral contraceptive (16). The mechanism of RU486-induced follicular growth failure and ovulation blockade in women is not known. From the present experiments in the rat, it can be suggested that either androgens as well as FSH are involved in the antiprogesterone RU486-induced follicular atresia.

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Resumen

La administración del antiprogestágeno RU486 (4 mg/día) desde el día de estro hasta el de proestro induce un fallo de la ovulación, y aumento de las concentraciones basales de LH, PRL, testosterona y estradiol en suero. Por el contrario, disminuye la concentración basal de FSH en suero. Tanto la ovariectomía unilateral, como la antagonización de los receptores de andrógenos con flutamida, y la inyección ovulatoria de HCG en la tarde de proestro, revierten, parcialmente, el bloqueo de la ovulación inducido por el RU486. Estos resultados indican que tanto la disminución de FSH como el aumento de testosterona en suero durante la fase de desarrollo folicular, y la disminución de la secreción ovulatoria de LH en las ratas inyectadas con RU486 son los responsables del efecto anovulatorio encontrado.

Key words: Antiprogesterona, Ovulación, LH, FSH, PRL, Testosterona, Estradiol.

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