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Effect of Antiprogestagen RU486 on both Natural and Prolactin-Induced Morphological Luteolysis in Rat

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Adult female cyclic rats were hypophysectomized and their pituitary glands autotransplanted beneath the left kidney capsule (Aptr) on day 1 (metestrus). To induce the luteolytic effect of prolactin (PRL) the rats were injected s. c. with 0.4 mg of 2-bromo-α-ergocryptine (CB154) on cycle days 12, 13 and 14. Other groups of Aptr rats were injected daily with CB154 or ethanol vehicle from day 12 to day 23. To study the role of progesterone (P) on the luteotrophic effect of PRL as well as on the natural and on the PRL-induced luteolysis, rats without CB154 treatment or with long or short CB154 treatment were injected s. c. with 2 mg of the antiprogestagen RU486 or 0.1 ml oil from day 1 to day 23. From each rat the mean weight of the corpus luteum (CL) was noted on day 24. The serum PRL level rose from day 1 to day 12 and fell thereafter by day 15 in CB154-treated rats. The serum level of PRL rose again in short term CB154-treated rats, and remained low in long term CB154-treated rats. No effect of RU486 on serum PRL levels was noted in any group. The serum P level fell rapidly as the treatment with CB154 began after day 12. Neither the rise in serum PRL after day 15 in short term CB154-treated rats nor RU486 treatment affected the P level. Treatment with RU486 did not affect the CL weight on day 24 in ethanol vehicle injected rats. On the contrary, RU486 significantly reduced the CL weight on day 24 in rats with short or long CB154 treatment. The results indicate that while P is not involved in the luteotrophic action of PRL, P seems to play a role in both the natural and the PRL-induced luteolysis in the rat.

Key words: Morphological luteolysis, Progesterone, PRL, RU486.

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In rat, as well as in some of its close relatives, as mouse and hamster, prolactin (PRL) has two effects on the corpus luteum (CL) (15). One is to maintain the

ability of the CL to secrete progesterone, which is referred to as "the luteotropic effect". The other causes rapid regression of the CL which is unable to respond to the luteotrophic action of PRL. This effect is referred to as "the luteolytic effect". The luteolytic effect of PRL, which is a form of structural or morphological luteolysis, is expressed by a marked increase in the rate of regression of the corpus luteum size (10, 11, 19).

Several findings point strongly to an inverse relationship between the CL'ability to produce progesterone and its response to the luteolytic effect of PRL. In the pregnant rat the CL secrete progesterone after day 12, for a period of about 72 h, even if the pituitary and the uterus are removed. In such rats PRL does not have a luteolytic effect until after more than three days of deprivation (10). There is an opposite connection between the secretion of progesterone by the CL and the luteolytic effect of prostaglandins (19, 25). Progesterone treatment tends to prevent the PRL-induced luteolysis (16).

RU486 is a synthetic steroid that possesses great affinity for progesterone receptors with no agonistic activity (2, 3). Thus, RU486 is a powerful tool for exploring the actions of progesterone in animal models. The experiments here described in hypophysectomized autopituitary-transplanted rats are directed towards exploring the possible role of progesterone on the luteotropic effect of PRL, as well as on the natural and on the PRL-induced structural luteolysis.

Materials and Methods

General.— Female Wistar rats were used at a body weight of 200-230 g. They were housed, five per cage, under standard conditions of light (light on from 0700 h to 1900 h) and temperature (20-23 °C). Vaginal smears were examined daily and rats were only used after at least two consecutive 4-day vaginal cycles. The day of vaginal metestrus was assigned as day 1 of the cycle and days were numbered consecutively thereafter.

Hypophysectomy and autopituitary transplant.— Hypophysectomy was done between 0800 and 1100 h on day 1 of diestrus, (day 1 of the cycle) by the parapharyngeal route (17). The removed pituitary was immediately transplanted (Aptr) beneath the left kidney capsule (19). Completeness of hypophysectomy was judged by the condition of the gland at the time of its removal, the absence of remnants in the fossa at autopsy, the absence of LH in serum and the regression in weight of the left adrenal gland (18.5 ± 0.6 mg, n = 43 in Hypox rats vs 34.1 ± 1.3 mg, n = 14 in intact rats). The condition of the transplant was judged by its size, color, and vascularity (1). Only those rats in which hypophysectomy was complete and the graft was functioning, were included in the results.

Treatments.— To antagonize the progesterone action, antiprogesterone RU486 (Roussel-Uclaf, Romainville, France) was used. It was dissolved in oil at a concentration of 20 mg/ml. Treatment consisted in daily s.c. injections of 0.1 ml of this solution from day 1 throughout the experiment. Control groups were injected with 0.1 ml oil.

2-Bromo-α-ergocryptine, CB154 mesilate (Sandoz, Basel, Switzerland), used to depress prolactin secretion, was dissolved immediately before use in 70 % ethanol at a concentration of 2 mg/ml, each injection comprising 0.4 mg and being administered sc.

Experimental design.— Each rat was hypophysectomized and had its pituitary gland transplanted (Aptr) beneath its left kidney capsule on day 1 of the estrous cycle. In such rats the pituitary autotransplant secretes PRL indefinitely at a fairly constant rate and the corpora lutea se-

cretes progesterone for several months (12, 18). In these rats the luteolytic effect of prolactin was induced by being injected with CB154 on days 12, 13 and 14 and ethanol vehicle from day 15 to day 23 after Aptr. Other two groups of cyclic rats were Aptr and injected with CB154 or ethanol vehicle from day 12 to day 23, and served as controls of the luteolytic effect of PRL. The role of progesterone on the luteotrophic or luteolytic effect of PRL was tested by injecting RU486 or oil vehicle.

Blood samples collection.— Blood samples of 1 ml each were obtained by jugular venipuncture from rats under light ether anesthesia on cycle days 1, 5, 9, 12, 15, 18, 21 and 24 after the surgery. Blood was allowed to clot, centrifuged, and the serum stored at –20 °C until assayed for LH, prolactin and progesterone.

Hormone radioimmunoassay.— Serum concentrations of LH and PRL were measured in duplicate in 25-ml serum samples by double antibody radioimmunoassays (20) and by kits supplied by NIH (Bethesda, MD, USA). Rat LH-I-6, PRL-I-5 were labeled with 125-I by the chloramine T method, LH and PRL values being expressed as ng/ml of the reference preparation LH-rat-RP-2 and PRL-rat-RP3. The sensitivities of the assays were 7.5 and 10 pg/tube, respectively. Serum progesterone was determined in duplicate with antiserum GDN337 from G.D. Niswender (Colorado State University, Fort Collins, CO) as described (17). Values are expressed as ng/ml. All samples were assayed in the same specific assay and the intra-assay coefficients of variations were 9, 8 and 10 % for LH, PRL and progesterone, respectively.

Autopsy and corpus luteum weight.— The rats were killed on day 24 after the last blood sample was taken. The left adrenal glands were removed, cleaned and weighed. Also, from each pair of ovaries, all corpus luteum (n = 11-15) was removed, cleaned and weighed as a group. The mean corpus luteum weight for each group of rats was calculated from each rat's mean weight as described (19, 25).

Statistics.— Results of corpus luteum weight given in the text are expressed as means ± SEM. Differences between groups were compared by analysis of variance (ANOVA) followed by Duncan's multiple range test. Values of $P < 0.05$ were considered significant. No special statistical treatment was needed for the changes in serum LH, PRL and progesterone levels, since these were self evident.

Results

Serum concentrations of LH, PRL and progesterone.— The serum concentrations of LH were combined, since no significant differences were observed between groups at any time of the experiment (fig. 1). Overall, the serum concentration of LH fell after autopituitary transplantation and remained low throughout the experiment.

The serum concentrations of PRL and progesterone rose from day 1 to day 12 (fig. 1). Treatment with CB154 on days 12, 13 and 14 reduced the serum prolactin level by day 15. After cessation of the treatment serum PRL level rose again and reached the value of ethanol vehicle-treated rats by day 24. On the contrary, the continued CB154 treatment maintained low the serum PRL level until day 24. The serum progesterone level fell rapidly as the CB154 treatment began and remained low until day 24 regardless of the serum level of PRL. The serum concentrations of PRL and progesterone remained high until day 24 in ethanol vehicle-treated rats. Neither PRL nor progesterone levels were affected by RU486 in any group. Because of that, they are represented together.

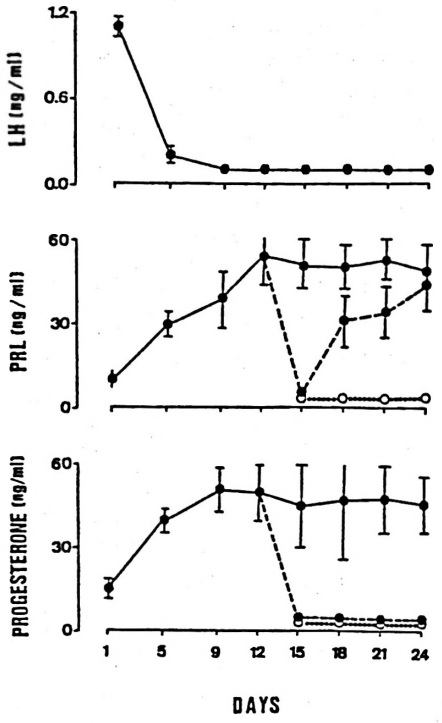

Fig. 1. *Serum concentrations of LH (upper panel), PRL (middle panel) and progesterone (lower panel) on days 1, 5, 9, 12, 15, 18, 21 and 24 in day 1 autopituitary transplanted (Aptr) rats.*
One group of rats (—) was injected sc with 0.4 mg CB154 from day 12 to 23 and other group from day 12 to 14 (....). A third group of rats was injected with ethanol 70 % vehicle from day 12 to day 23 (---). Half of the rats in each of the three groups were injected sc daily (from day 1 to day 23) with 0.1 ml olive oil and the other half with 2 mg RU486. Groups consisted of 6-8 rats. Since the serum concentrations of hormones were similar in all three groups regardless of whether they were injected with oil or RU486, they are represented together.

Corpus luteum weight .— There was no significant difference in the mean weight of the corpus luteum between oil- or RU486- treated rats injected with ethanol vehicle on day 24 of the cycle (table I). On day 24, the corpus luteum decreased in weight in rats injected with CB154 from day 12 to day 14 but not in those injected from day 12 to day 23. Treatment with RU486 resulted in a significant fall in the mean corpus luteum weight in rats with short or long CB154 treatment.

Table I. *Effect of antiprogestagen RU486 on the weight of the corpus luteum (mg) on day 24 in rats bearing their pituitaries as autotrasplant.*
Values are mean SEM (n = 6-8 rats). All rats were hypophysectomized and had their pituitaries autotransplanted on day 1 (metestrus) of an estrous cycle. RU486 treatment consisted in sc injections of 0.25 ml. of ethanol 70% from day 12 to day 23. Bromocryptine (0.5 mg) was injected (sc) once a day from day 12 to day 23 or from day 12 to day 15. $^{a}p < 0.05$ and $^{b}p < 0.01$ vs ethanol, $^{c}p < 0.05$ vs oil.

Treatments	Oil	RU486
Ethanol (12-23)	1.53 ± 1.10	1.46 ± 0.07
Bromocryptine (12-23)	1.41 ± 0.09	1.22 ± 0.04^{ac}
Bromocryptine (12-15)	0.90 ± 0.07^{b}	0.62 ± 0.02^{bc}

Discussion

In the intact rat, RU486 treatment increases LH and PRL secretion (20), while RU486 had no effect on the secretion of these hormones by the transplanted pituitary. This finding shows the effect of RU486 to be exerted at the hypothalamic level. In rats bearing a pituitary graft, treatment with RU486 altered neither the CL weight on day 24 nor the PRL or the progesterone secretion from day 1 to day 24, indicating that PRL is sufficient to stimulate the secretion of progesterone from the CL (15) even in the absence of the progesterone action. Similarly, RU486 did not affect the functional luteolysis induced by PRL withdrawal. These observations reinforce the critical role of PRL in the maintenance of progesterone secretion in the rat (25), and also indicate that the luteolytic effect of PRL, as well as the spontaneous morphological luteolysis, are enhanced in rats treated with RU486. Since RU486 has strong antiprogesterone

activity (2, 3) and it did not alter the secretion of PRL by the grafted pituitary (fig. 1), the structural regression of the corpus luteum in the rat could have been blunted through the blockade of progesterone action.

In the absence of the luteotropic support of PRL, the corpus luteum of the rat continues producing progesterone, which is rapidly converted to its less active metabolite 20α-hydroxyprogesterone, a conversion that is inhibited by PRL (8) and stimulated by prostaglandin $F_{2\alpha}$ (23). In addition to progesterone, the CL also produces prostaglandins, which, in the absence of progesterone secretion, induce structural luteolysis (15). In fact, indomethacin (a prostaglandin synthesis inhibitor) treatment prevents the structural luteolysis in rats subjected to the conditions that induce the luteolytic effect of PRL (19). Moreover, intra-ovarian bursa implants of progesterone interfere with the luteolytic action of PRL (16). These data, together with the findings of the present experiments, support the concept of a critical role of intraluteal progesterone action on the structural luteolysis (15).

Little is known about the physiological events that lead to structural luteolysis. The morphological appearance of both luteal regression (9, 26), and ovarian follicular atresia (7, 24) includes apoptosis. Apoptosis is a physiological programmed cell death (22) associated to a Ca^{2+}-dependent endonuclease (26). Prostaglandin $F_{2\alpha}$ induces apoptosis (9, 21) as well as luteolysis through an acute increase in intracellular Ca^{2+} (6), and indomethacin treatment prevents structural luteolysis (19, 25) and inhibits Ca^{2+}-transport (4).

Treatment with progesterone blunts the luteolytic effect of PRL in the rat (16), inhibits luteal prostaglandins synthesis or action in all mammalian species (15), and suppresses apoptosis in progesterone dependent-tissues (13). On the other hand, RU486 treatment induces apoptosis in the uterine epithelium in the ovariectomized rabbit (13) and, in our study, it accelerated the structural luteolysis. Finally, apoptosis occurs in the CL after progesterone secretion has ceased (9). In 1965 and 1981 ROTHCHILD proposed that the CL'ability to make progesterone protects the CL from luteolysis (14, 15). The results of the experiments reported in this paper, together with the direct effect of progesterone on cellular proliferation (5), support such a view.

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J. E. SÁNCHEZ-CRIADO, A. RUIZ y M. TÉBAR. *Efectos del antiprogestágeno RU486 sobre la luteolisis morfológica natural y la inducida por prolactina en la rata.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (2), 97-102, 1994.

Se utilizan ratas cíclicas hipofisectomizadas y autotrasplantadas (HA) en el día de metestro (día 1). En este modelo, la PRL secretada por la hipófisis estimula la secreción de progesterona por el cuerpo lúteo (CL), sin que se secreten otras hormonas hipofisarias. El tratamiento con el antiprogestágeno RU486 (2 mg/0,2 ml de aceite) durante 23 días no modifica la secreción de PRL, ni la de progesterona, ni altera el peso del CL en relación a las inyectadas con aceite solo. Otro grupo de ratas HA se inyecta con bromocriptina (0,4 mg) en los días 12, 13 y 14 y con el vehículo etanol desde el día 15 hasta el 23, para inducir el efecto luteolítico de la PRL (grupo experimental). Como control del efecto luteolítico de la PRL se utilizan ratas HA inyectadas con bromocriptina desde el día 12 hasta el 23. La antagonización

de los receptores de la progesterona con RU486 no modifica los niveles de PRL o de progesterona. Por el contrario, el peso de los CL en el día 24 es significativamente menor (luteolisis estructural), tanto en el grupo experimental como en el grupo control. Los resultados indican que si bien la progesterona no participa en los efectos luteotróficos de la PRL, retrasa la luteolisis natural y la inducida por PRL.

Palabras clave: Luteolisis morfológica, Progesterona, PRL, RU486.

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