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The Luteotrophic Effects of Estradiol and Prolactin in the Absence of LH in the Hysterectomized, Pseudopregnant Rat[1] (41419)

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Abstract. The ability of estradiol and prolactin to substitute for LH as luteotrophins was investigated in Day 5 (Day 1 = ovulation)-hysterectomized pseudopregnant (PSP) rats. All animals received either a sc injection of oil, 1 μg estradiol (E-1), or 150 μg prolactin (PRL) on Day 9 in combination with either a normal horse serum (NHS) or LH antiserum (LHAS) injection. The ability of oil, E-1, or PRL to maintain luteal function was assessed by monitoring serum progesterone levels through Day 12 and their direct effects on the ovary monitored by measuring luteal and extra-luteal ovarian tissue concentrations of estradiol and progesterone on Day 12. NHS treatment in combination with either oil, E-1, or PRL maintained elevated serum progesterone levels through Day 12 of PSP. LHAS/oil-treated rats underwent luteolysis while E-1 and PRL effectively maintained luteal function in LHAS-treated animals. PRL raised luteal and extraluteal ovarian estradiol concentrations compared to oil/NHS-treated controls and LHAS/oil-treated rats. E-1 induced an intraluteal rise in estradiol levels in LHAS-treated rats. While luteal progesterone concentrations fell following oil/LHAS treatment, E-1 and PRL effectively counteracted this LHAS-induced fall. The results of these studies indicate that (i) E-1 and PRL can effectively replace LH as luteotrophins during PSP in the rat and (ii) that PRL and E-1 effectively maintain luteal levels of estradiol and progesterone following LH deprivation. It is suggested that E-1 and PRL may exert their luteotrophic actions by either an E-1-induced increase in PRL which, in turn, acts on the luteal cells to increase intraluteal estradiol concentrations, or by a direct effect of E on the corpus luteum to bypass the requirement for LH.

The rat corpus luteum (CL) depends upon prolactin (PRL) to maintain, and LH to modulate, progesterone secretion during pseudopregnancy (PSP) (1–3). While PRL is an essential luteotrophin throughout PSP (4), LH becomes essential only after Day 9 (Day 1 = ovulation) (1–3, 5). In the absence of either hormone at this time, luteolysis occurs. However, recent reports in the intact or hypophysectomized-hysterectomized pregnant rat (6–9) and in the hysterectomized-PSP (2, 3) rat indicate that experimental administration of either PRL or estradiol can prevent luteolysis in the absence of LH. While very large doses of estrogen were required for luteal maintenance in the pregnant rat model, very low doses were able to maintain progesterone secretion from the PSP rat CL. The luteotrophic effect of estrogen in the pregnant rat has been explained by the increased intraluteal levels of estradiol which occurs following steroid treatment (8) and the ability of estrogen to substitute for LH (10, 11). However, the mechanism through which PRL and the low dosages of estradiol substitute for LH in the PSP rat remain to be elucidated. This study was designed to clarify the systemic and intraovarian alterations in estradiol and progesterone concentrations which occur as a result of exogenous estradiol and PRL treatment in the hysterectomized-PSP rat.

Materials and Methods. *Animals.* Adult, female Sprague–Dawley rats weighing 200 to 300 g were used in this study. All animals were maintained in separate cages under a controlled photoperiod of 14 hr light/day (lights on 0600 hr). Food and water were available *ad libitum*.

Pseudopregnancy was induced by cervical stimulation with a glass rod on the afternoon of proestrus and morning of estrus. The last day of vaginal cornification was denoted Day 1 of PSP.

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Surgery and tissue collection. Hysterectomy was performed in ether-anesthetized rats following midventral laporotomy. The uterine connections with the oviduct and cervix were ligated and severed. The incision site was surgically closed and the rats allowed to recover in a holding box.

Ovarian tissue (i.e., luteal and extraluteal) was collected at the termination of the experiment. Both ovaries were rapidly removed, placed on dry ice, and cleaned. Using a dissecting microscope, luteal tissue was isolated and dissected free of the remaining ovarian (i.e., extraluteal) tissue. The corpra lutea (CL) of both ovaries were pooled, weighed to the nearest 0.1 mg, and stored at −70° unitl assayed. The extraluteal ovarian tissue of both ovaries was treated in an identical manner.

Hormone treatment and serum collection. Estradiol was administered in sesame oil vehicle (0.2 ml) sc as a single, 1-μg (E-1) dose. This dosage was chosen since preliminary experiments (Garris and Rodway, unpublished observations) indicated it to be a minimal effective dose for protecting the CL from LHAS-induced luteolysis in the hysterectomized rat. PRL was injected at a concentration of 5.25 IU (150 μg)/0.2 ml saline (S-12, ovine PRL) sc. This concentration was selected since it approximates the amount of PRL secreted in a 24-hr period by the PSP rat pituitary gland (12). Both LH antiserum (LHAS) and normal horse serum (NHS: vehicle control) were injected sc in a 0.5-ml volume. The biological and immunological characteristics of the antiserum have been previously described (13, 14).

A 0.5-ml sample of blood was collected from ether-anesthetized rats by direct jugular puncture. Blood samples were allowed to clot at 5°, serum collected and stored at −20° until assayed.

Hormone assays. Serum progesterone and estradiol levels were estimated by radioimmunoassay as previously described (6, 15). All values are expressed uncorrected for procedural loss (≤7%).

Luteal and extraluteal ovarian tissue samples were thawed in 1.0 ml of saline (0.1 *M*)−PBS buffer and homogenized using a Polytron grinder. For progesterone analyses, a 20-μl aliquot of the homogenate was extracted with 4.5 ml of petroleum ether. A known concentration of [^{3}H]progesterone was added to identical samples to serve as an indication of extraction efficiency. The extract was dried, redissolved in 1.5 ml of PBS solution, and aliquots of 20- and 50-μl volumes were assayed as previously described (6) for progesterone concentrations.

Tissue estradiol levels were estimated following extraction of 100 μl of the PBS tissue homogenate with 4.5 ml of anhydrous diethyl ether. Samples with tracer solution were treated in a similar manner. Samples were frozen in a dry ice−ethanol bath for aqueous phase separation. The aqueous phase was dried and resuspended in 200 μl of PBS and assayed by radioimmunoassay for estradiol concentrations as previously described (15).

Statistical analysis. All group data were expressed as the mean ± SEM. Intergroup differences were analyzed by Student's *t* test and Newman−Keuls test, where appropriate.

Experimental protocol. Rats were hysterectomized on Day 5 of PSP in order to extend the duration of PSP and insure that the CL would become LH dependent (2, 3, 16). On Day 9, rats were randomly assigned to groups for either oil, E-1, or PRL injections. Half of the rats in each group simultaneously received either an LHAS or NHS injection. Blood samples were collected at 0, 24, and 72 hr postinjection for analysis of serum progesterone levels. At 72 hr postinjection rats were decapitated, the ovaries collected, and the luteal and extraluteal tissue separated for steroid analysis. Collection of the ovaries at 72 hr postinjection was performed to avoid compromising the results of tissue analysis with retained hormone from the injections, and to analyze the chronic effects of the E-1 and PRL treatments on luteal and extraluteal steroid levels which were recognized to override the requirements for LH by the rat CL. The acute effects of either treatment regime were not tested in this study.

Results. *Effects of LHAS and NHS in-*

jections on serum progesterone levels in Day 5 hysterectomized-PSP rats treated with oil, estradiol, or prolactin. Serum progesterone levels remained constant between Days 9 and 12 of PSP in all rats receiving an NHS injection and simultaneously treated with oil, E-1, or PRL (Fig. 1). In contrast, rats treated with LHAS and oil on Day 9 exhibited a dramatic fall in serum progesterone levels by Day 12. The luteolytic actions of LHAS were overcome by both E-1 and PRL treatment. In these rats, serum P levels were maintained at NHS-treated, control levels through Day 12 of PSP.

Effects of oil, estradiol, and prolactin treatment on Day 12 luteal and extraluteal ovarian tissue concentrations of progesterone and estradiol in the Day 5-hysterectomized-PSP rat following NHS or LHAS treatment on Day 9. Comparison of Day 12 luteal and extraluteal ovarian estradiol concentrations is depicted in Fig. 2 (A and B). Rats treated with LHAS and oil exhibited a significant reduction in estradiol

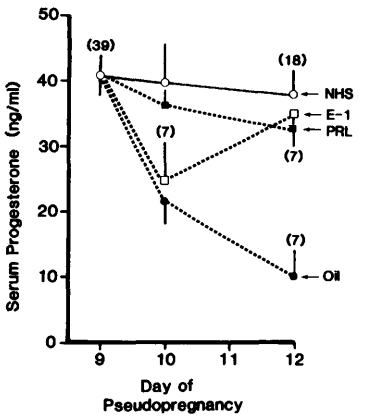

FIG. 1. Effects of oil, 1 μg of estradiol (E-1), or prolactin (PRL) injections on serum progesterone levels following treatment of Day 5-hysterectomized rats with either NHS (solid lines) or LHAS (dashed lines). All NHS-treated rats (i.e., oil, E-1, and PRL) are represented as a single group mean. Serum progesterone levels are depicted as the group mean ± SEM; numbers in parentheses indicate rats per group. Statistical analyses on Day 12 of PSP: NHS vs LHAS/oil-treated rats, $P \leq 0.001$; NHS vs E-1- or PRL/LHAS-treated rats, $P \geq 0.05$; LHAS/oil vs E-1- or PRL/LHAS-treated rats, $P \leq 0.01$.

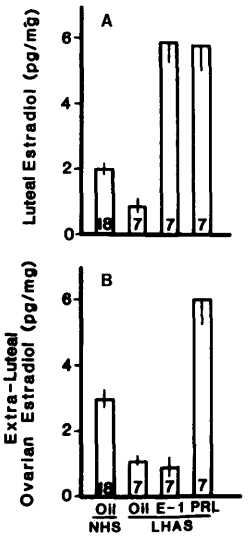

FIG. 2. Luteal (A) and extraluteal, ovarian (B) estradiol levels on Day 12 of PSP in Day 5-hysterectomized rats treated with either NHS or LHAS and oil, estradiol (E), or prolactin (PRL) on Day 9. All NHS-treated (i.e., oil, E-1, and PRL) rats are represented as one group, since no significant intergroup differences were found. All values are expressed as group means ± SEM; numbers in parentheses indicate rats per group. Statistical analyses: Luteal estradiol—NHS/oil vs LHAS/oil, $P \leq 0.01$; NHS/oil vs LHAS/E-1 or PRL, $P \leq 0.001$; LHAS/oil vs LHAS/E-1 or PRL, $P \leq 0.001$; LHAS/E-1 vs LHAS/PRL, $P \geq 0.05$. Extraluteal, ovarian estradiol—NHS/oil vs LHAS/oil, $P \leq 0.01$; NHS/oil vs LHAS/E-1, $P \leq 0.01$; NHS/oil vs LHAS/PRL, $P \leq 0.01$; LHAS/oil vs LHAS/E-1; $P \geq 0.05$; LHAS/oil or E-1 vs LHAS/PRL; $P \geq 0.001$.

concentrations in both ovarian compartments compared to NHS-treated rats injected with oil, E-1, or PRL. In contrast, E-1 treatment resulted in a dramatic rise in the luteal estradiol content of LHAS-treated rats. Similar treatment had no effect on the estradiol concentrations of extraluteal tissue. However, PRL treatment in the absence of LH induced a significant elevation of the estradiol concentration in both luteal and extraluteal ovarian compartments compared to similar NHS-treated controls. The effect of PRL on the luteal levels of estradiol equaled those of the E-1 treatment, while PRL treatment was the only effective inducer of a rise in

extraluteal, ovarian tissue estradiol concentrations in LHAS-treated rats.

The effects of combined NHS or LHAS and either oil, E-1, or PRL treatment on luteal or extraluteal, ovarian progesterone concentrations are depicted in Fig. 3 (A and B). Luteal progesterone levels were greater than those of the extraluteal ovarian compartment. Neither E-1 nor PRL in combination with NHS treatment altered luteal progesterone concentrations compared to oil-treated controls. In contrast, PRL treatment decreased extraluteal progesterone levels in NHS-treated rats while E-1 had no effect. LHAS treatment resulted in nearly a 50% decrease in luteal progesterone levels of oil-treated rats but had no effect on the progesterone concentration in the extraluteal compartment. Both E-1 and PRL treatment effectively maintained the luteal progesterone concentrations of LHAS-treated rats at NHS control levels. Both E-1 and PRL induced a slight suppression of extraluteal, ovarian progesterone levels in LHAS-treated rats.

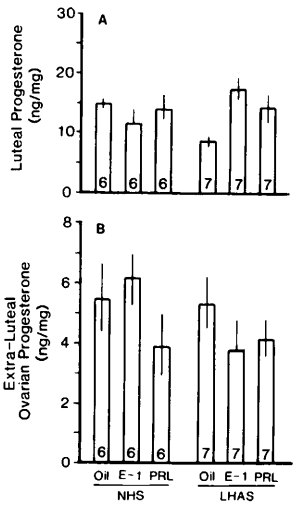

FIG. 3. Day 12 luteal (A) and extraluteal, ovarian (B) progesterone concentrations in Day 5-hysterectomized, PSP rats treated with either NHS or LHAS and oil, 1 μg of estradiol (E-1) or prolactin (PRL) on Day 9. All values are represented as group means ± SEM; numbers in parentheses indicate rats per group. Statistical analyses: Luteal progesterone—NHS/oil vs NHS/E-1 or PRL, $P \geq 0.05$; NHS/oil vs LHAS/oil, $P \leq 0.01$; LHAS/oil vs LHAS/E-1, $P \leq 0.01$; NHS/oil vs LHAS/E-1, $P \leq 0.05$; LHAS/oil vs LHAS/PRL, $P \leq 0.01$; NHS/oil vs LHAS/PRL, $P \geq 0.05$. Extraluteal, ovarian tissue—NHS/oil vs NHS/E-1, $P \geq 0.05$; NHS/oil vs NHS/PRL, $P \leq 0.05$; NHS/oil vs LHAS/oil, $P \leq 0.05$; NHS/oil vs LHAS/E-1 or PRL, $P \leq 0.05$.

Discussion. The results of the present study confirm and extend those of previous reports (6–11, 17–20) indicating that estrogens can serve a luteotrophic function in the rat. Prior studies have focused mainly on the luteotrophic effects of estrogen during pregnancy in the rat (6–8, 17) and have employed large doses of the steroid to reportedly induce an elevated intraluteal concentration of the hormone (17) and override the requirement for LH by the CL (6, 7, 17). The present study indicates that estradiol can also accomplish this result in the PSP rat and at much lower doses than needed in the hypophysectomized-hysterectomized pregnant animal. Furthermore, the results demonstrate that E-1 acts mainly on the luteal compartment of the ovary, as evidenced by the induced increase in luteal estradiol concentrations. Also, the effects of PRL on maintaining both serum (2, 3) as well as both luteal and extraluteal ovarian progesterone levels suggest that an estradiol-induced release of PRL may participate in luteal maintenance in the LH-deprived rat.

Extension of pseudopregnancy by hysterectomy is believed to occur as a result of removing the luteolytic effects of the uterus and by removing the influence of the uterus on the development of a need for LH by the CL (i.e., LH-dependent progesterone secretion) (3). In the present study, this model system was used since it extends the diestrus period to nearly that of normal pregnancy (2, 16), maintains constant serum progesterone levels (2, 3) and allows for the examination of the factors influencing LH-dependent progesterone secretion (3). Interestingly, neither E-1 nor PRL affected serum progesterone levels in NHS-treated rats. It was only after the removal of LH from circulation by LHAS treatment that the luteotrophic activities of these hormones became obvious (Fig. 1). These results indicate that normally both PRL and

LH are necessary for maintaining luteal function, and that PRL alone, at normal circulating levels, is not enough to maintain progesterone secretion after Day 9 of PSP. However, in the absence of LH, both estradiol and PRL are able to maintain normal serum and luteal progesterone levels as well as normal luteal levels of estradiol. Since LHAS treatment did not induce ovulation, as assessed by visual inspection of the ovaries, the effects of estradiol treatment on luteal function are interpreted as being specific. These results suggest that LH and PRL, in concert, serve to maintain luteal estradiol levels which, in turn, maintain CL function.

Exactly how these alterations in the luteotrophic complex occur in the rat remains to be determined. It is well recognized that PRL is essential for CL function during the first 8 days of pregnancy and throughout PSP (4, 14, 21). The fact that hysterectomy prolongs the normal occurrence of diurnal prolactin surges from the rat pituitary (22) may explain, in part, how the CL is maintained following uterine removal. Also, the timing of hysterectomy is critical for the proper expression of LH dependency in the rat (3). While hysterectomy early during PSP delays the appearance of LH-dependent progesterone secretion, removal of the uterus on or after Day 5 insures that the CL becomes LH dependent on Day 9 (2, 3). Using the Day 5-hysterectomized rat, it was demonstrated that elevated PRL levels (in the form of pituitary homotransplants) effectively delayed LH dependency (3, 23) unless the uterus was allowed to remain in place until Day 8. Then, even very high levels of PRL did not affect the timing of the appearance of LH dependency. While the effects of hysterectomy late in PSP on the maintenance of PRL surges has not been reported, it seems likely that they would be extended, since removal of the uterus at this stage of PSP prolong vaginal diestrus (16) which is dependent upon PRL maintained, luteal function (4, 21). The results of the present study suggest that PRL in the absence of LH can maintain luteal progesterone secretion and that PRL may serve its role by stimulating an intraluteal increase in estradiol levels. The possibility that the luteotrophic properties of estradiol are a result of increased systemic PRL concentrations (24) is currently under investigation.

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