Maintenance of Progesterone Secretion By Ovine Prolactin or Pituitary Autografts in the Absence of Endogenous LH[1] (37712)

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The ability of prolactin to maintain luteal function in the rat as demonstrated by the early work of Astwood (1) and Evans *et al.* (2) has been confirmed repeatedly (2–4). Lately, however, this concept of prolactin as the sole luteotropic agent in the rat has been called into question by several lines of evidence. Armstrong (5) has shown that LH also can stimulate the secretion of progesterone by rat luteal tissue on an acute basis. Recently, Moudgal (6) and Madhwa Raj and Moudgal (7) advanced the involvement of LH in luteal function by showing that neutralization of LH with LH antiserum (LH A/S) during the first half of pregnancy will cause abortion due to reduction of progesterone production. The work of Behrman *et al.* (8) has indicated that LH and prolactin may both be involved in the stimulation of progesterone output by the rat ovary.

The present study was undertaken to determine whether the secretory function of corpora lutea maintained in hypophysectomized rats by exogenous or endogenous prolactin can be altered by LH A/S, the rationale being that the antisera would neutralize any LH contaminating the administered prolactin or secreted by the autograft. It has already been demonstrated that antisera to rat prolactin do not block decidual formation if given on the morning of estrus (9) in the rat. The present data furnish additional necessary evidence pertinent to the solution of the difficult puzzle as to what maintains the secretory function of rat corpora lutea under different circumstances.

Methods and Materials. Animals. Charles River (CD) rats were housed in temperature- and humidity-controlled rooms and fed Purina rat chow and water *ad libitum*. The lighting schedule was 14 hr of light and 10 hr of darkness. Following two complete estrous cycles as determined by vaginal lavage, the animals were hypophysectomized by the parapharyngeal approach on the morning of metestrus (day 1). The autotransplantation of the anterior pituitary was accomplished by exposing the kidney through a dorso-lateral incision; the kidney capsule was incised, and the tissue placed under the capsule with the aid of forceps. The sella turcica was examined at autopsy, and only completely hypophysectomized animals were included in this report.

Hormones and antisera. Prolactin (NIH-P-S9) in 15% gelatin was administered at the rate of 2 IU/0.2 ml twice daily from the time of hypophysectomy until sacrifice. The LH antiserum was prepared by immunizing rabbits with NIH ovine LH carried in Freund's complete adjuvant. Contaminating antibodies to serum specific proteins, as well as other pituitary hormones, were adsorbed as described earlier (7). The biological potency of this antiserum was assessed by determining the amount required to induce abortion in rats when given on day 8 of pregnancy; 0.2 ml of antiserum I and 0.4 ml of antiserum II induced abortion when given as one sc injection. Antiserum I (LH A/S I) was administered as 0.3 ml daily (1.5 times the aborting dose) and LH A/S II as 0.5 ml daily (1.25 times the aborting

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dose) on days 5 and 6 after hypophysectomy. Control animals received equivalent amounts of normal rabbit serum (NRS). In Experiment I, all animals received 17-β-estradiol in corn oil (sc) as 20 μg on day 9 and 10 μg on succeeding days to test luteal function (1) as determined by the maintenance or failure to maintain vaginal diestrous smears.

Blood samples and progesterone assay. Serial blood samples (1 ml) were obtained by cardiac puncture while the animals were under ether anesthesia. Each animal was bled on days 5, 6, 7, and 9 or days 5, 6, 7, 9, and 15 following hypophysectomy. The samples taken on days 5 and 6 were obtained immediately before the administration of either LH A/S or NRS. All blood was allowed to clot at room temperature for 2 hr, centrifuged, the serum removed to individual vials, and frozen for future analysis. Each serum sample was thawed, and the progesterone isolated from 100- to 500-μl aliquots employing petroleum–ether extraction and thin layer chromatography according to Neill *et al.* (10). Tritiated progesterone (2000 cpm/sample, 50 counts/mM; NEN, Boston, MA) was added to correct for procedural losses. Recoveries ranged from 60 to 75%. Radioimmunoassay of the isolated progesterone was done by the method of O'Grady *et al.* (11). Ovariectomized monkey serum charged with 2 ng/500 μl showed a value of 2.3 $\pm$ 0.07 (SE)/500 μl in this assay system. Hypophysectomized rat serum assayed below 0.4 ng/ml. In Experiment III, column chromatography using LH Sephadex 20 (12) was employed to replace thin layer chromatography with the modification that 17.5 $\times$ 0.8 cm columns were used and 0.8 g of LH Sephadex 20 was carried in 3.0 ml of benzene:methanol (90:10) to pack the column. The column was then washed with 6 ml iso-octane:benzene:methanol (90:5:5) prior to adding the sample in the same vehicle.

Statistical analysis. Analysis of variance was performed on each experiment.

Results. Experiment I. Hypophysectomized prolactin-treated rats ($n = 3$) receiving LH A/S I or NRS ($n = 4$) on days 5 and 6 after hypophysectomy had peripheral serum progesterone values which were not statistically different on given days (Table I). The peripheral progesterone values in both control and LH A/S treated groups fell to low levels by day 15. All animals in this experiment were treated with 17-β-estradiol beginning on day 9. By day 15, they had all developed cornified vaginal smears which correlate with the low progesterone values found at this time.

TABLE I. Serum Progesterone Levels (ng/ml) from Prolactin-Treated Rats[a] Given LH A/S or NRS on Days 5 and 6 After Hypophysectomy.

	Experiment I[b]	
Days[c]	Control[d]	Experimental[d]
5	43.7 $\pm$ 0.8	48.9 $\pm$ 4.5
6	35.1 $\pm$ 4.5	51.1 $\pm$ 3.7
7	27.6 $\pm$ 13.8	46.0 $\pm$ 1.8
9	19.9 $\pm$ 15.2	45.2 $\pm$ 16.5
15	3.9 $\pm$ 1.9	12.9 $\pm$ 6.6

[a] Rats given prolactin (NIH-P-S9) 2.0 IU/0.2 ml 15% gelatin twice daily from day 1 to day of autopsy.

[b] Control rats ($n = 3$) received 0.3 ml NRS daily. Experimental rats ($n = 4$) received 0.3 ml LH A/S I daily.

[c] Days after hypophysectomy.

[d] Mean $\pm$ SE, ng/ml serum. Experimental animals were not statistically different from controls on given days.

Experiment II. In hypophysectomized pituitary-autografted rats treated with LH A/S I ($n = 3$) and NRS ($n = 4$) according to the same schedule as in Experiment I, the serum progesterone values were uniformly high and unchanged by the antiserum treatment (Table II). The administration of estradiol beginning on day 10 did not elicit vaginal cornification in these animals, attesting to the maintenance of luteal function by the pituitary autografts.

Experiment III. Further confirmation of the observation that LH A/S does not influence progesterone secretion under these circumstances was obtained in a repeat of Experiment II using LH A/S II. Blood samples were obtained on days 5 (pretreatment), 6, 7, and 9. The resulting serum progesterone values were not different (Table

TABLE II. Serum Progesterone Levels (ng/ml) from Pituitary-Autotransplanted Rats Treated with LH A/S or NRS on Days 5 and 6 After Operation.

	Experiment II		Experiment III	
Days[a]	Control[b,d]	Experimental[b,d]	Control[c,d]	Experimental[c,d]
5	71.6 ± 18.0	73.6 ± 21.7	135.0 ± 8.6	145.8 ± 15.1
6	51.5 ± 21.1	60.8 ± 13.1	135.3 ± 18.3	141.3 ± 15.5
7	48.6 ± 16.4	60.7 ± 18.2	129.6 ± 14.3	114.0 ± 10.9
9	54.2 ± 14.4	79.5 ± 19.0	105.0 ± 12.3	94.5 ± 12.4

[a] Operated on the day of metestrus, day 1.

[b] Control rats ($n = 3$) received 0.3 ml NRS daily. Experimental rats ($n = 4$) received 0.3 ml LH A/S I daily.

[c] Control rats ($n = 8$) received 0.5 ml NRS daily. Experimental rats ($n = 11$) received 0.5 ml LH A/S II daily.

[d] Mean ± SE, ng/ml serum. Experimental animals were not statistically different from controls on given days.

II) between the group treated with antisera and that given NRS.

Discussion. In the present experiments, using a potent LH antibody to totally neutralize the contribution of any circulating LH, the ability of endogenous and exogenous prolactin to sustain luteal function was demonstrated. Prolactin maintained progesterone secretion, and LH antiserum was without effect. While prolactin is not known to acutely stimulate progesterone synthesis, is has been shown by Behrman *et al.* (13) to maintain the enzymes necessary for luteal steroidogenesis and to inhibit 20α-hydroxy steroid dehydrogenase activity (14). These actions imply a role of prolactin in sterol turnover and modulation of the conversion of progesterone to 20α-hydroxypregn-4-en-3-one.

The present data leaves no doubt that prolactin can, in the complete absence of LH, stimulate the rat corpus luteum to secrete progesterone. This exclusive stimulus of prolactin is sufficient to promote progesterone secretion adequate to maintain diestrous smears in the face of a challenge with estrogen. Interestingly, Maneckjee and Moudgal (15) have observed that the minimal effective dose of progesterone to maintain vaginal diestrous smears in rats treated with 0.01 μg estradiol is 50 μg, that for decidualization is 250 μg, and that for pregnancy is 4 mg. The ability of the corpus luteum of the intact rat to secrete progesterone varies depending upon whether the rat is cycling, pseudopregnant, or pregnant, the least amount of progesterone being secreted during the luteal phase of the estrous cycle (16, 17).

Recent data from this laboratory (18–20), however, shows that in pseudopregnancy, pregnancy, and lactation, where progesterone secretion is maximal, neutralization of LH leads to a drastic reduction in progesterone output from the ovary. In this context, mention should be made of the observation of Madhwa Raj and Moudgal (7) that pituitary homotransplants are unable, in the presence of LH antiserum, to maintain pregnancy. Thus, it would appear that for the maintenance of a low to moderate level of progesterone secretion, prolactin alone is an adequate stimulus, but, under circumstances requiring maximal progesterone output, the supporting role of LH is also involved. This will explain why prolactin alone maintains luteal function in the pseudopregnant but not in the pregnant or lactating rat.

Summary. Adult cycling rats hypophysectomized on the morning of metestrus (day 1) were pituitary-autotransplanted or treated with 2.0 IU of prolactin twice daily by subcutaneous injection for 15 days. These two types of animals received either LH antiserum (LH A/S) or normal rabbit serum (NRS) on days 5 and 6 after hypophysectomy. Multiple blood samples were drawn by heart puncture from individual animals

throughout the study, and the sera were analyzed for progesterone by radioimmunoassay. The results show that LH A/S was not effective in reducing serum progesterone levels maintained by exogenous or endogenous prolactin.

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