Maintenance of Luteal Function in Rats by Rat Prolactin[1] (35341)

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In rats bearing autografted pituitaries, luteal maintenance may be extended for as long as 3 or 4 months (1). Exogenous prolactins purified from heterologous pituitary sources and administered to hypophysectomized rats are much less effective in maintaining luteal function. The purest preparations sustain such function for only 17 to 19 days (2, 3). Macdonald *et al.* (3) demonstrated this trophic restriction resides in the antigenicity of heterologous prolactin. The present study was undertaken to test whether purified homologous rat prolactin would maintain progesterone secretion for a period greater than did the heterologous prolactins. This study was made possible by the recent availability of purified rat prolactin.

Methods and Materials. Mature cycling female, rats weighing over 200 g, were purchased from the Charles River Company, Wilmington, Massachusetts. They were housed in our light-, temperature-, and humidity-controlled room where food and water are supplied *ad libidum*. The estrous cycles were observed for 1 week prior to hypophysectomy to establish the day of metestrus. On that day, rats were hypophysectomized parapharyngeally under ether anesthesia. The animals were assigned to 4 or 5 dosage groups of 5 or 6 animals each by formal randomization. Two like experiments were conducted. The data from each were similar and were combined for final presentation. Rat prolactin, sheep prolactin, and saline were injected subcutaneously twice daily and vaginal smears were examined every morning throughout the experimental period. The first injection was given immediately after operation. Both prolactins were totally dissolved in saline made slightly alkaline with NaOH. Rat prolactin (11 IU/mg) was administered at a dose of 1.0, 1.5, or 3.0 IU/0.4 ml of saline each day. Sheep prolactin NIH-P-S9 (30 IU/mg) was given as 3.0 IU in 0.4 ml of saline. These doses of prolactin were divided equally and administered morning and evening. Estradiol, 20 μg/0.2 ml of sesame oil was given the day of hypophysectomy and 10 μg/0.1 ml on alternate days thereafter. This treatment tests luteal function. In the presence of progesterone from the corpus luteum the vaginal smear remains predominately leukocytic but in the absence or cessation of progesterone secretion the vaginal smear becomes filled with cornified epithelial cells. Three consecutive days of vaginal cornification were taken as a sign of the absence or cessation of progesterone secretion. The duration of luteal function was expressed in days counting from the day of hypophysectomy to the day prior to 3 consecutive days of vaginal cornification. Animals were sacrificed after the prolactin injected groups showed cornified vaginal smears. The ovaries and uterus were examined macroscopically, weighed, and fixed in Bouin's solution for histological examination. The data were analyzed by the Duncan-Bonner multiple range test (4).

Results and Discussion. Rat prolactin (1.5 IU b.i.d.) maintained progesterone secretion from the corpora lutea of rats hypophysectomized on the day of metestrus. The duration of this function judged by persistent leukocytic vaginal smears was 25.2 days (Table I) which is longer than the normal pregnancy period of 21–22 days and may have been extended further if greater amounts of prolactin had been available. The smaller doses of rat prolactin were less than optimal; 0.25 IU b.i.d. was as ineffective as saline, the vehicle; and 0.75 IU b.i.d. provided marginal maintenance. Two rats in this latter group

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TABLE I. Luteal Maintenance by Rat and Sheep Prolactin in Hypophysectomized Rats.

Treatment[a] (IU)	No. of rats	Days[b]
Saline vehicle	11	3.0 ± 0.1
Sheep prolactin, 1.5	9	16.9 ± 2.1
Rat prolactin, 0.25	5	3.6 ± 0.1
0.75	6	11.2 ± 3.5
1.50	10	25.2 ± 1.4

[a] Prolactin was given in 0.2 ml of 0.9% NaCl at the IU specified, twice a day. Estradiol was administered on the day of hypophysectomy at a dose of 20 μg in 0.2 ml of sesame oil and on alternate days thereafter at a dose of 10 μg in 0.1 ml of sesame oil.

[b] Days until cornification of vaginal smears; mean values ± SE.

of 6 had no demonstrable luteal function, having totally cornified vaginal smears by day 3, the same as saline injected controls; and another displayed function for only 6 days. Sheep prolactin administered at 1.5 IU b.i.d. maintained luteal function for 16.9 days in essential agreement with results found with similar doses of sheep prolactin in previous experiments (3, 5). At autopsy the ovaries of all rats weighed essentially the same, but less than those from intact rats of similar age. The uteri of all saline injected rats contained fluid, a sure sign of the absence of progesterone secretion (6). However, prolactin-injected rats displayed varying quantities of uterine fluid without relation to dose. The fluid volumes observed may have been due to differing amounts of residual progesterone secretion.

The comparison between sheep and rat prolactin is valid since both were tested in parallel. This study shows that rat prolactin is capable of maintaining progesterone secretion in rats longer than sheep prolactin ($p<0.01$). Thus, homologous prolactin purified from rat pituitaries maintained luteal function for a longer period than did the antigenic sheep prolactin.

The duration of luteal function maintained in hypophysectomized rats with sheep prolactin was decreased by concomitant administration of luteinizing hormone (LH) in an earlier study (3). Two observations suggest that cessation of function in the current study was not due to LH contamination. Parlow reports this product free from LH by the OAAD assay (personal communication). N. R. Moudgal assayed the purified rat prolactin used in these experiments against a well-characterized LH antiserum by Ouchterlony double diffusion test and found no evidence of any immunologically crossreacting material (personal communication). This suggests that the prolactin used was free of LH contamination.

A span of luteal function equivalent to that maintained by pituitary autografts was not observed in the present study. Since the effect of an administered purified protein hormone is superior when given twice a day compared to once a day, particularly in a saline vehicle, it is conceivable that injection of prolactin would not approach the results derived from the continuous release of prolactin from a pituitary graft. Nevertheless, luteal function was sustained longer in animals receiving rat prolactin than sheep prolactin. This supports the contention that prolactin is luteotropic, that the corpus luteum has no inherent life span and that homologous prolactin is more conducive to lasting luteal function than heterologous prolactin.

Summary. Administration of prolactin to hypophysectomized rats maintains luteal function. When rat and sheep prolactins were given at the same dose (IU) rat prolactin was found to maintain luteal function for a greater number of days. This duration exceeded the length of pregnancy in the rat.

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