Plasma Gonadotropins and Prolactin in Pseudopregnancy in the Rat (35800)

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Pseudopregnancy in the rat is characterized by persistent corpus luteum function and a failure of cyclic ovulation (1). Prolongation of the life of the corpus luteum can be accomplished in the hypophysectomized rat by injection of prolactin (2), and the continued function of this organ during pseudopregnancy has been attributed to continued secretion of prolactin by the adenohypophysis (1). On the other hand, the failure of ovulation to occur is thought to be caused by a suppression of the release of gonadotropins (1). Because of the limitations of the bioassays previously used to measure gonadotropins and prolactin, it has been impossible, heretofore, to measure plasma levels of these hormones during pseudopregnancy. These levels determined by radioimmunoassay (RIA) are reported below.

Methods. Virgin female rats of the Holtzman strain, weighing 200–240 g, were maintained in conditions of controlled temperature and lighting (lights on 7:00 a.m. to 7:00 p.m.) and fed laboratory chow. After determining that the animals had 4-day estrus cycles by daily examinations of vaginal smears for at least two cycles, they were used for experiments. An attempt to induce pseudopregnancy was made either by repeated probing of the cervix for approximately 2 min on the day of estrus or by placing the female with a vasectomized male on the night of proestrus.

On day 4 of presumed pseudopregnancy, the rats were anesthetized with ether, laparotomized, and a uterine horn was traumatized by scratching the antemesometrial surface with a burred needle. On day 9, the uteri were examined for the presence of deciduomata. Other presumably pseudopregnant rats were ovariectomized 4 days after mating with vasectomized bucks.

One-milliliter blood samples were quickly drawn from the jugular vein while the rats were lightly anesthetized with ether at the times indicated. The plasma concentration of FSH and prolactin was estimated by RIA using the kits supplied by NIAMD.[3] The RIA for LH was carried out in the O-O system of Niswender *et al.* (3).[4] Results were expressed in terms of the standards supplied with the kits in the case of FSH and prolactin and in terms of the NIH LH-S-1 standard in the case of LH. Significance of differences between means was determined by Student's *t* test.

Results. Effect of cervical probing on plasma prolactin and LH levels. A blood sample was removed on the morning of estrus to obtain a control value for the hormones. After 3 hr, to allow recovery from this procedure, cervical stimulation was carried out and another blood sample was obtained 15 min later. There was a doubling of plasma prolactin levels in the poststimulation samples ($p<.01$) (Fig. 1). There was considerable variability in the magnitude of the rise, but a rise in plasma prolactin was exhibited

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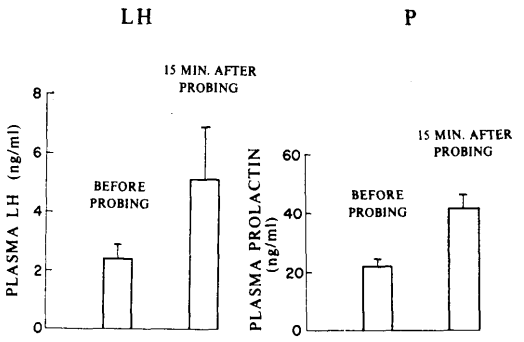

Fig. 1. Plasma LH and prolactin (P) concentrations before, and 15 min after, cervical probing. In Figs. 1–3, the height of the bar represents the mean and the vertical lines represent one SEM.

by 15 of the 16 rats stimulated. Plasma LH levels were not significantly elevated following cervical stimulation. Only one of the 16 animals stimulated developed pseudopregnancy as evidenced by a clear prolongation of diestrus.

Plasma gonadotropins and prolactin on the fourth and ninth days of pseudopregnancy. Pseudopregnancy was established in rats by mating with vasectomized males on the night of proestrus. On the fourth day of the ensuing pseudopregnancy, the rats were anesthetized and a blood sample was drawn. Following the removal of blood one horn if the uterus was traumatized. Controls were normal rats on the first day of diestrus. Plasma prolactin in the pseudopregnant group was similar to that observed in the controls (Fig. 2), whereas the level of LH was significantly lower ($p<.05$) and that of FSH was significantly higher ($p<.05$) in the pseudopregnant animals. All of the rats that were mated with vasectomized males became pseudopregnant as evidenced by persistent diestrus and development of large deciduomata on examination on day 9 of pseudopregnancy. The weight of the control uterine horn at sacrifice was 153 ± 8 (mean ± SEM) mg while that of the traumatized horn was 1510 ± 204 mg.

On day 9 of pseudopregnancy, plasma prolactin was significantly lower than at day one of diestrus ($p<.001$); whereas plama FSH was elevated ($p<.01$) and plasma LH was unchanged.

Effect of ovariectomy on plasma gonadotropins and prolactin in pseudopregnancy. On day 4 of presumed pseudopregnancy produced by mating with vasectomized bucks and just prior to ovariectomy, plasma FSH, LH, and prolactin were not significantly different from levels observed in the controls which were to be ovariectomized on the first day of diestrus (Fig. 3). Five days after spaying, on day 9, LH levels had risen in both groups but the degree of rise was much greater in the rats which had been mated ($p<.02$) than in the controls. The FSH concentrations also rose and to an equal degree in both groups. Prolactin levels were not significantly altered in either group following castration.

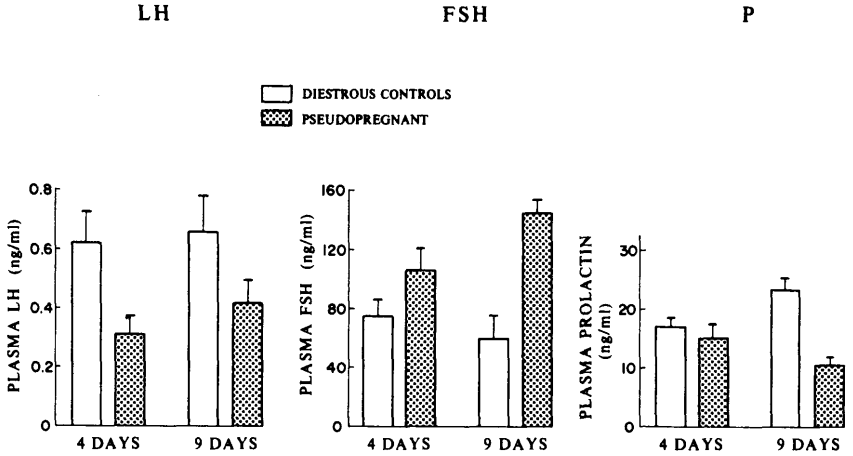

FIG. 2. Plasma LH, FSH, and prolactin (P) concentrations on days 4 and 9 of pseudopregnancy.

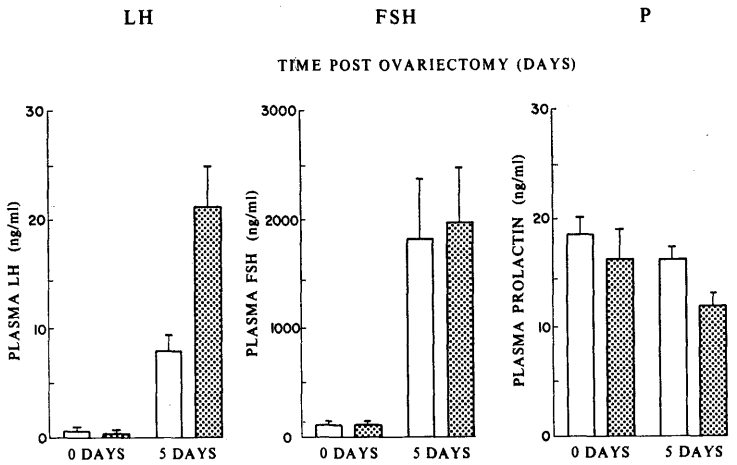

FIG. 3. Plasma gonadotropin and prolactin concentrations just prior to, and 5 days after, ovariectomy of normal (open bars); and pseudopregnant rats (stippled bars).

Discussion. It has long been assumed that the pattern of hormone release from the adenohypophysis during pseudopregnancy in the rat was characterized by a sustained release of prolactin and a sustained suppression of gonadotropin discharge (1). The data of this study indicate that an increased release of prolactin does occur immediately after cervical probing in the rat. These findings agree well with earlier reports of a depletion of pituitary prolactin following cervical stimulation (4, 5).

Pseudopregnancy resulted from cervical probing in only one of 16 rats in the current experiments. The probable cause for the relative inefficacy of cervical stimulation was the use of ether anesthesia to obtain blood samples both before and after cervical stimulation. It has earlier been reported that ether anesthesia can block the induction of pseudopregnancy in the rat (6).

Our data indicate that the increased release of prolactin during pseudopregnancy is relatively brief since prolactin levels were not significantly elevated above those of diestrous controls by day 4 and on day 9 they were actually significantly lower than those of the control group. Additional experiments are required to determine the exact duration of the release, but it is clear that the corpora remain functional for a considerable period after prolactin levels have returned to normal or even subnormal levels since they were capable of supporting the development of deciduomata from days 4–9.

As expected, the levels of LH were even lower than those of diestrous controls on day 4 of pseudopregnancy. This lowering of LH may be brought about by the suppressive action of ovarian progesterone acting together with small amounts of estrogen. Although no significant elevation in LH followed cervical probing, it is noteworthy that an elevation of LH was reported to follow copulation in rats (7). Surprisingly, FSH levels were elevated at both 4 and 9 days of pseudopregnancy on comparison with the diestrous controls. We speculate that this may be accounted for by a lesser sensitivity of FSH to the inhibitory effect of progesterone than was the case for LH.

Following removal of the inhibition from ovarian steroids by ovariectomy in pseudopregnant rats, the plasma levels of both FSH and LH rose as expected, but the degree of LH rise was much greater than was observed in the spayed diestrous controls. A possible explanation for this unexpected finding is a rebound of LH from suppression by ovarian steroids, presumably principally progesterone. FSH did not exhibit this behavior possibly because it was less suppressed in the pseudopregnant rats than in the normals.

In conclusion, our findings indicate that pseudopregnancy in the rat is characterized by a shorter release of prolactin than previ-

ously suspected, a decreased release of LH, and an enhanced release of FSH. This enhanced release of FSH presumably accounts for the follicular development which is observed in late pseudopregnancy prior to the ensuing ovulation. Both prolactin and LH are thought to be important in the maintenance of the corpus luteum in the rat (8). Therefore, the continued function of the corpus during late pseudopregnancy when levels of both LH and prolactin were low is puzzling. It appears that once enhanced secretion by this gland has been triggered it may be self-sustaining for a period of several days.

Summary.

1. Cervical probing of estrous rats resulted in an elevation of plasma prolactin 15 min later.

2. Plasma prolactin was not elevated on day 4 of pseudopregnancy and was actually lower on day 9 than in diestrous controls.

3. On day 4 of pseudopregnancy plasma LH levels were lower than those observed in the controls; where FSH levels were elevated on day 4 and 9.

4. Following castration on day 4 of pseudopregnancy, plasma FSH and LH levels both rose, but plasma LH rose to a higher value in the pseudopregnant castrates than in controls on the 5th day after spaying. Prolactin levels remained unchanged following spaying.

5. It is concluded that the discharge of prolactin which initiates pseudopregnancy in the rat is not maintained throughout pseudopregnancy and is associated with a suppression of LH and an augmentation of FSH release from the pituitary.

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