Lactational Anovulation in Rats and Its Dependency on Progesterone (43168)

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Abstract. **The relationship between prolactin (PRL) secretion and anovulation in lactating rats was studied. Normal lactating rats and lactating rats treated with antiserum against luteinizing hormone-releasing hormone at the time of postpartal ovulation were used. Normal lactating rats were treated with either a dopamine agonist (CB-154, 150 μg/rat) on Day 10 or 13, or pups removal on Day 7 or 10, and thereafter luteolysis and inhibition on PRL secretion were assessed. With the CB-154 treatment, the incidence of luteolysis increased as the lactational period advanced (42% vs 72%), whereas it decreased (73% vs 14%) with the pups removal. Thus, dopamine effectively inhibited PRL secretion during the later lactational stage, but could not do so during the earlier stage when there were mechanisms other than dopamine stimulating PRL secretion. Following luteal regression induced by CB-154, ovulation did not occur if the rats were treated with CB-154 on Day 10, whereas 50% of the rats ovulated within 4 days if treated on Day 13. Furthermore, in the lactating rats treated with anti-luteinizing hormone-releasing hormone serum during late pregnancy, ovulation was not observed until Day 10 of lactation. Since the serum progesterone levels were low in these rats due to the absence of ovulation and lactational corpora lutea, the blockade of ovulation was not due to elevated circulating progesterone during the early lactational period. The mechanism of ovulation blockade during lactation thus seems to shift from being progesterone independent to progesterone dependent at a similar period when the neuroendocrine control of PRL secretion shifts from dopamine independent to dependent.** [P.S.E.B.M. 1991, Vol 196]

In rats, the anovulatory state during postpartum lactation and pseudopregnancy is characterized by an endocrine milieu with a high secretion rate of progesterone, which may be responsible for the inhibition of ovulation (1, 2). In lactation, in addition to the high level of circulating progesterone, the suckling stimulus is believed to play a central role in the blockade of ovulation (3). The suckling stimulus effectively suppresses the basal secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in intact lactating rats (4), inhibits the postcastration rise in both LH and FSH for 15 days postpartum (5–7), and decreases the pulsatile release of LH in ovariectomized lactating rats (3, 8).

It has been reported previously that ovulation did not occur for at least 7 days after postpartum in lactating rats with baseline levels of progesterone (9). Although this finding indicates a progesterone-independent mechanism for the blockade of ovulation, it remains unclear whether the progesterone-dependent mechanism was functional, particularly in the later lactational period.

In the present study, we investigated the progesterone-dependent mechanism of ovulation blockade during lactation in rats whose serum progesterone had been reduced either by treatment with a dopamine agonist (bromocriptine mesylate), pups removal, or inhibition of lactational corpora lutea formation.

Materials and Methods

Adult Wistar rats (250–300 g) bred in our laboratory were maintained under a lighting schedule of 14-hr light (0500–1900 hr) and 10-hr dark and fed commercial rat chow and water *ad libitum*. Vaginal smears were taken daily, and rats that showed regular 4-day cycles were selected. The day of sperm detection and that of parturition were designated as Day 1 or preg-

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nancy and Day 0 of lactation, respectively. Litter size was adjusted to eight pups on Day 1 of lactation.

Treatment with Bromocriptine Mesylate (CBT) or Pups Removal (PR) at Various Times during the Lactational Period. Bromocriptine mesylate (CB-154; Sigma Chemical Co., St. Louis, MO) was dissolved in 0.3% tartaric acid to make a concentration of 1.5 mg/ml. CB-154 solution (100 μl) was administered subcutaneously at 0600 hr on Day 7, 10, or 13 of lactation (CBT animals: Day 7, $n = 8$; Day 10, $n = 7$; Day 13; $n = 11$). This dose of CB-154 was sufficient to cause luteolysis in pseudopregnant rats with the administration at 1200 hr on Day 7 of pseudopregnancy ($n = 8$). In another group, pups were removed at 0600 hr on Day 7 or 10 of lactation (PR animals: Day 7, $n = 30$; Day 10, $n = 7$). Each rat was serially bled once daily between 1530 and 1630 hr for 3 consecutive days after CB-154 treatment (CBT) or pups removal (PR). Blood samples were taken by cardiac puncture under ether anesthesia unless otherwise described. Ovulation was judged by the presence of a swollen oviductal ampulla a laparotomy on the day of estrus.

Blockade of Postpartum Ovulation by Injection of Anti-Luteinizing Hormone-Releasing Hormone (LHRH) Serum. The anti-LHRH serum was prepared by immunizing rabbits with LHRH conjugated with bovine serum albumin in our laboratory. Anti-LHRH serum (500 μl) was injected intravenously daily from Day 21 of pregnancy until the day of parturition according to the procedure reported previously (9). This volume of anti-LHRH serum completely blocks ovulation in cycling rats when administered at noon on the day of proestrus. All rats ($n = 8$) were bled between 1130 and 1230 hr on Days 5 and 10 of lactation.

Radioimmunoassay for Progesterone. The serum samples (0.1 ml) frozen for progesterone assay were thawed and extracted with 2 ml of ethyl ether. One-tenth of each sample was analyzed by radioimmunoassay in duplicate using specific antibodies generated in our laboratory (10).

Statistical Analysis. The data were analyzed by one-way analysis of variance followed by the Wilcoxon-Mann-Whitney U test when differences were significant, and chi-square analysis was used as appropriate. Differences at $P < 0.05$ were considered to be statistically significant.

Results

Changes in Serum Progesterone Levels after CBT or PR. Figure 1A shows the changes in progesterone levels after CBT on Day 7, 10, or 13 of lactation. Progesterone levels decreased significantly within 2 days and 1 day after CBT on Days 10 and 13, respectively, but the same treatment on Day 7 did not cause any significant decrease. Progesterone levels after PR on Day 7 or 10 are shown in Figure 2A. PR on Day 7 caused a significant decrease in progesterone levels within 2 days, but PR on Day 10 caused no significant decrease (Fig. 2A).

As shown in Figures 1B and 2B, either CBT or PR animals were divided into two groups according to the response of their serum progesterone levels: Group 1,

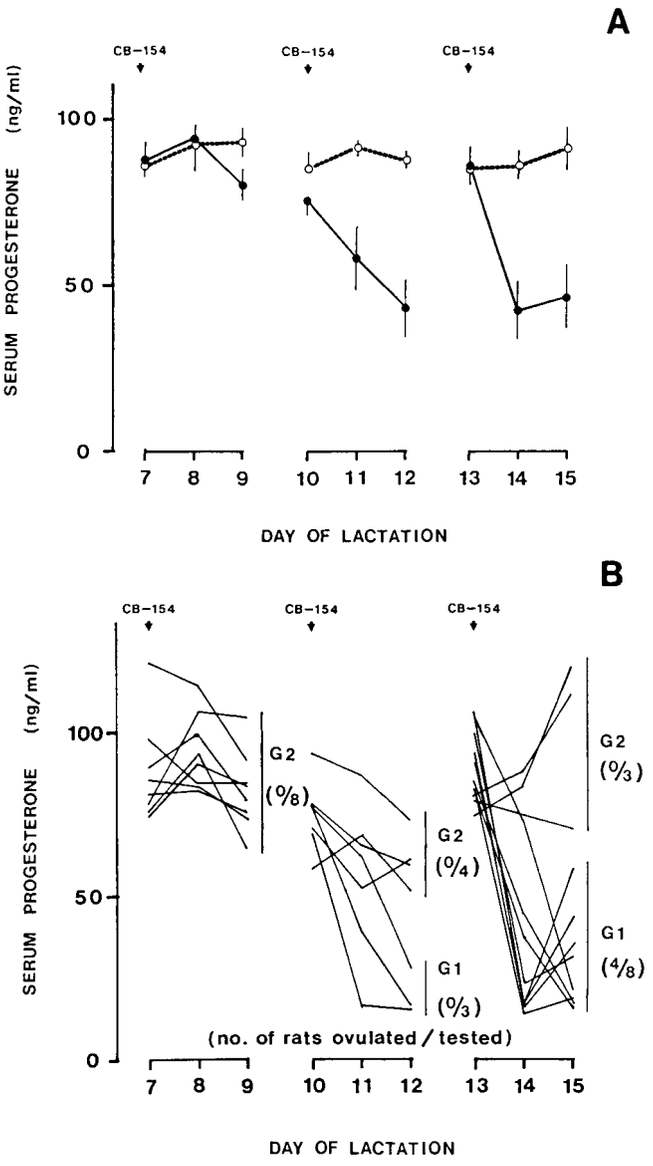

Figure 1. (A) Serum concentration of progesterone in rats treated with CB-154 (150 μg sc) on Day 7, 10, or 13 of lactation (●——●) and in normal lactating rats (O---O). Day of parturition was designated as Day 0 of lactation. The arrow indicates day of administration of CB-154. Each point represents the mean ± SE of progesterone concentration. (B) Individual changes in serum progesterone levels in rats treated with CB-154 (150 μg sc) on Day 7, 10, or 13 of lactation (these means are represented in A, ●) and the occurrence of ovulation within 4 days after CB-154 treatment. Animals treated with CB-154 were divided into two groups according to the response of their serum progesterone levels. Group 1 (G1), animals in which progesterone was reduced to basal level. Group 2 (G2), animals in which progesterone was maintained at high levels. Fractions in parentheses represent the ratio of rats that ovulated to those tested in G1 or G2, respectively.

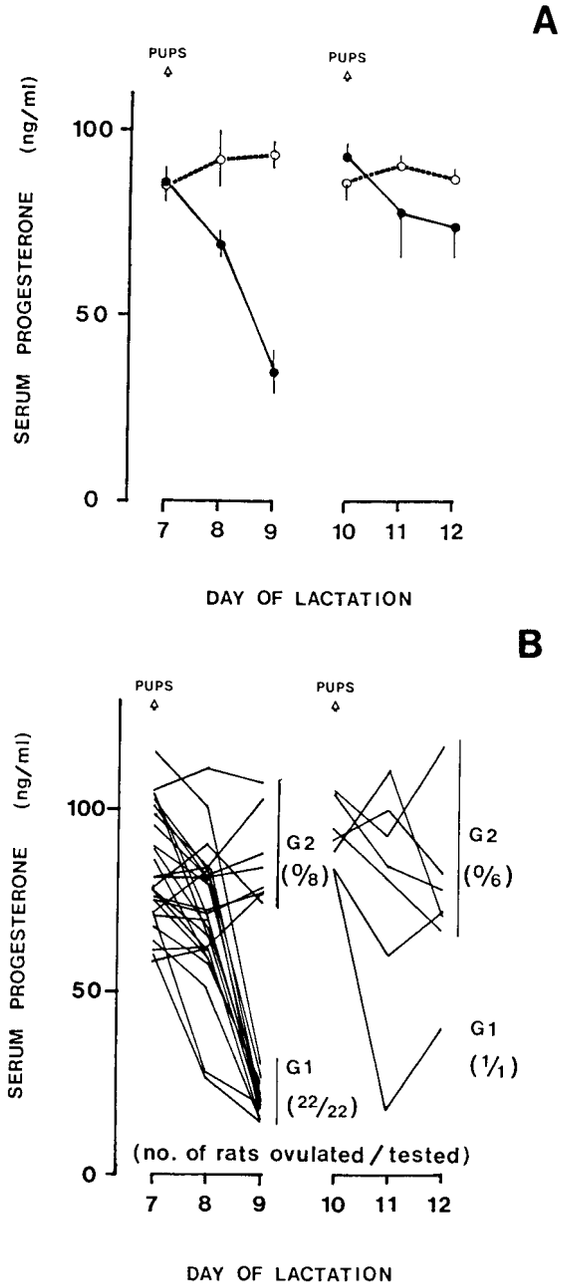

Figure 2. (A) Serum concentration of progesterone in rats deprived of pups on Day 7 or 10 (●——●) and in normal lactating rats (O---O). The arrow indicates the day of pups removal. Each point represents the mean ± SE of progesterone concentration. (B) Individual changes in serum progesterone levels in rats deprived of pups on Day 7 or 10 (these means are represented in A, ●) and the occurrence of ovulation within 4 days after pups removal. Group 1 (G1), animals in which progesterone was reduced to basal levels. Group 2 (G2), animals in which progesterone was maintained at high levels. Fractions in parentheses represent the ratio of rats that ovulated to those tested in G1 or G2, respectively.

progesterone reduced to basal levels; and Group 2, progesterone maintained at high levels. All, 57.1%, or 27.3% of CBT animals treated on Day 7, 10, or 13 were classified as Group 2, respectively (Fig. 1B). The percentages of animals that could be classified as Group 2 among PR animals on Day 7 or 10 were 26.7% and 85.7%, respectively (Fig. 2B). As the lactational period advanced, the percentage of animals in Group 1 increased among CBT animals on Day 13 and decreased among PR animals on Day 10 significantly. The effects of CBT and PR on progesterone secretion from lactational corpora lutea showed a reciprocal relation (Fig. 3).

Ovulation after Both Treatments. The occurrence of ovulation within 4 days after either CBT or PR is indicated in Figures 1B and 2B according to the groups categorized above. In animals that maintained high progesterone levels (Group 2), none ovulated. However, in animals with decreased progesterone levels (Group 1), ovulation occurred in all PR animals and in half of CBT animals treated on Day 13.

In CBT animals, their pups ceased to gain body weight only on the day of CB-154 treatment, but regrowth started 1 day later. Suckling behavior was observed during this period.

Anti-LHRH-treated Animals (Anti-LHRH Rats). Lactating rats without functional corpora lutea were prepared by blocking the postpartum ovulation with anti-LHRH serum, and the effect of the suckling stimulus on ovulation was examined (Table I). In lactating anti-LHRH rats, ovulation was not observed until Day 10 of lactation. In four of eight rats, however, ovulation occurred between Days 11 and 15. Progesterone levels on Days 5 and 10 in anti-LHRH rats were near the baseline (Table I). When pups were removed from anti-LHRH rats on Day 1 of lactation, ovulation occurred in all (n = 4) animals within 5 days. Thus, ovulation could be blocked by the suckling stimulus until Day 10 of lactation without any increase in serum progesterone levels. This anti-LHRH treatment did not interfere with lactation, since pups continued to gain weight normally.

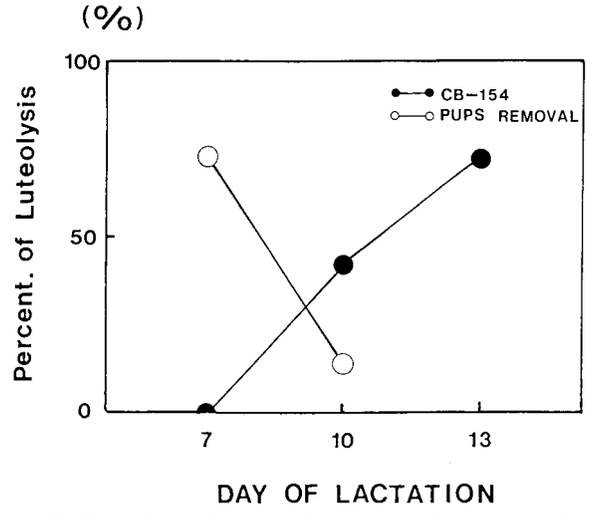

Figure 3. Percentage of animals showing a decline in progesterone level after treatment with CB-154 on Day 7, 10, or 13, or after pups removal on Day 7 or 10.

Table I. Percentages of Ovulators and Concentrations of Serum Progesterone in Lactating or Postpartal Rats Treated with Anti-LHRH Serum

Treatment	Percentage of ovulating rats on lactation days (%)			Serum progesterone on lactation day (ng/ml)	
	1–5	6–10	11–15	5	10
Anti-LHRH[a]	0 (0/8)	0 (0/8)	50 (4/8)	19.3 ± 1.0[b]	19.3 ± 1.5
Anti-LHRH + pups removal[c]	100 (4/4)				

[a] Anti-LHRH was injected once a day from Day 21 of pregnancy to the day of parturition.
[b] Mean ± SE.
[c] Pups were removed on Day 1 of lactation.

Discussion

The present study confirmed previous results showing that ovulation can be suppressed by the suckling stimulus independently of the peripheral level of progesterone (9), and revealed that this mechanism, manifested during the early period of lactation, disappeared as lactation advanced. Since the removal of the suckling stimulus before Day 10 always caused ovulation in the presence of basal progesterone level, the mechanism of ovulation inhibition during this period appears to depend totally on the neuronal signal originating from suckling. However, after Day 10 of lactation, high peripheral progesterone levels are necessary for the inhibition of ovulation, as was shown in lactating rats with depleted levels of progesterone induced by CB-154 or anti-LHRH treatment.

Thus, the mechanism of ovulation inhibition during the lactational period shifts from a progesterone-independent to a progesterone-dependent one. Interestingly, in anti-LHRH rats, whose serum progesterone stayed at low levels from the beginning of lactation, this shift also occurred at a period similar to that in CBT rats whose serum progesterone remained at high levels until the treatment. Therefore, progesterone levels do not seem to affect the timing of this shift. A low rate of ovarian estrogen production seems to be one of the factors preventing ovulation during lactation, because the injection of a large amount of estrogen into lactating rats has been found to induce a gonadotropin surge (10, 11). Smith and Neill (7) showed that in ovariectomized lactating rats, the FSH secretion responsible for estrogen production began to increase on Day 5 of lactation and reached a level comparable with that in diestrous rats on Day 10. Thus, acquisition of the ability to secrete a basal amount of FSH in lactating rats seems to occur coincidentally with the shift from a progesterone-independent to dependent mechanism of ovulation blockade.

Treatment with the dopamine agonist, CB-154, in the early period of lactation (around Days 7–10) did not lower progesterone levels, even though the dosage was sufficient to induce luteolysis in pseudopregnant rats, showing that there is a different control in luteal function in lactating rats, especially of early period, from that in pseudopregnant rats. The response against pups removal also changed between Days 7 and 10 and showed a reciprocal relation with the response after CB-154 treatment. These results suggested that the neuroendocrine control on luteal function shift from dopamine-independent to dependent mechanism, because the response against dopamine agonist changed around Days 7–10. The mechanism that has been reported to regulate prolactin-releasing factor-mediated prolactin (PRL) secretion during lactation (13, 14) may be dominant in early lactation. However, the possibility that the corpora lutea's sensitivity to or responsibility for PRL changes has not been ruled out yet. Further study is needed to define this mechanism of the shift.

We previously found that administration of progesterone can induce pseudopregnancy-like PRL surges in cycling rats (15, 16). van der Schoot *et al.* (17) showed that a pseudopregnancy-like PRL secretion appeared in lactating rats if pups were removed. Therefore, progesterone must be involved in the release mechanism of PRL late in the lactational period.

Controversy exists about the possible stimulatory involvement of serotonin in PRL secretion during lactation. Kordon *et al.* (18) and Parisi *et al.* (19) affirmed this possibility, whereas de Greef *et al.* (20) obtained negative results. The former two groups obtained data using lactating rats on Days 5–8 and the latter group, on Days 10–14. If the PRL-releasing mechanism changes during the lactational period, as we have proposed, then the discrepancy seems to be due to the difference in the lactational period and PRL-releasing mechanism whereby serotonergic input may play a major role in the early period of lactation (before day 10), and dopaminergic input in the later period of lactation.

The data presented in this article suggest that the mechanism responsible for ovulation inhibition shifts from a progesterone-independent to a progesterone-dependent one, and that the neuroendocrine control of luteal function shifts from a dopamine-independent to a dopamine-dependent one. Furthermore, the shift of

these mechanisms occurs at a similar period of postpartum lactation, suggesting a common mode of regulation for these mechanisms.

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