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Natural and Artificially Induced Ovulatory Models Related to Lactation in the Rat: Role of Prolactin (42738)

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Abstract. The presence and the importance of a preovulatory prolactin (PRL) peak was determined in four, natural or artificially induced, ovulatory models related to lactation in the rat. Gonadotrophin peaks were determined in the afternoon preceding ovulation in four models: postpartum ovulation (PPO), ovulation after the lactational period (AL) (natural models), ovulation after litter removal at midlactation (ML), and ovulation in lactating rats (LR) (artificially induced models). In PPO, AL, and ML rats a preovulatory PRL surge was detected, showing that its presence is a common characteristic of ovulation in the rat. Bromocriptine inhibition of PRL levels in PPO and AL rats did not modify the percentage of rats which ovulated. In contrast, this treatment was able to significantly increase ovulation percentage in ML rats. Moreover, in LR rats strong dopaminergic inhibition of PRL levels, induced by pergolide, was necessary for ovulation to take place, but if pergolide-treated rats were injected with ovine PRL ovulation was completely inhibited. These data suggest that while a PRL surge seems to be always present in natural ovulatory models, it is not essential for ovulation to take place. On the other hand, in artificially induced ovulatory models, suppression of prolactinemia is able to induce ovulation or to increase the percentage of rats which ovulated. This effect of PRL on ovulation may be direct or indirect.

Lactation delays the reinitiation of estrous cyclicity in rats, resulting in a relative physiological sterility for the duration of suckling. During this period, the occurrence of ovulatory peaks of pituitary gonadotrophins is suppressed (1, 2). When lactation ends (around Day 23 postpartum), ovulatory cycles are resumed (3, 4). Furthermore, it has been well established that ovulation also occurs during lactation shortly after delivery (within 24–48 hr) (5, 6). It is suggested that this event takes place in response to a release of luteinizing hormone. This surge is the consequence of an increment in serum estrogen concentration and a decrease of serum progesterone concentration, which occurs postpartum (7).

Besides these two physiological examples, ovulation can be artificially induced in lactating rats by early removal of the litters (8) and by administration of dopaminergic drugs, which reduce the high serum prolactin levels present in these animals (9).

In the ovulation occurring at midcycle, a proestrous prolactin peak has been determined. In these ovulatory models related to lactation, the coexistence of a PRL peak with the LH and FSH preovulatory surges has not been described. Thus, the aim of the present work is to study, in a comparative way, the endocrine profiles in four ovulatory models in lactating rats and to evaluate the role of PRL in ovulation and in the lactational anovulation.

Materials and Methods. *Animal models.* Adult virgin female rats (250–300 g body wt) of a Wistar-derived strain were housed in groups in an air-conditioned room with lights on at 0700 and off at 1900 hr. They were given free access to laboratory chow and tap water. The groups consisted of:

(i) Postpartum ovulation: Vaginal smears of regular cycling females were taken daily. On the day of proestrus, males were introduced into the cages and mating was verified by observation of sperm in the vaginal smears the next morning. This day was designated as Day 1 of pregnancy. Beginning on Day 22 pregnant rats were observed for delivery and the day and time of its occurrence were noted. Only 2 out of 33 (2/33) rats delivered on Day 22 of pregnancy and both had ended their delivery before 1600; the rest (31/33) did so on Day 23 and 10/10 of these animals, whose parturition time was determined, had already ended their delivery by 1400 hr.

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On the day of expected delivery (Day 23 of pregnancy), animals were injected with solvent or bromocriptine, half a dose (1.5 mg/kg) at 0930 and 1315 and one dose (3 mg/kg) at 1715 hr. For blood samplings, rats were momentarily separated from their pups. Jugular vein blood samples were taken at 1300, 1700, and 0900 hr of the following morning. Rats ovulated 24 to 48 hr after delivery. Vaginal smears could not be taken in these animals, as they showed large amounts of erythrocytes due to parturition.

(ii) First ovulation after litter removal at the end of the lactational period: When rats delivered naturally, they were left with 8 to 10 pups per mother. On Day 23 postpartum litters were removed and the occurrence of the first proestrus was checked by vaginal smears every morning: 15 rats which ovulated out of 19 (15/19) had proestrous smears 48 hr after litter removal, 3/19 rats after 24 hr, and 1/19 after 72 hr. Some rats (17%) had to be discarded because on the day of litter removal they already had an estrous smear, which was indirect evidence that the first proestrus had already taken place.

On the morning of proestrus, animals were injected with solvent or bromocriptine, half a dose at 0900 and 1315 hr and a dose at 1715 hr. Jugular vein blood samples were taken at 1300 and 1700 hr of proestrus and 0900 hr of the following day. Ovulation occurred within 24 hr of proestrus.

(iii) First ovulation after litter removal at midlactation: Three days after delivery each litter size was adjusted to 5 female pups and on Day 13 of lactation litters were removed at 0900 hr (8). Daily vaginal smears were taken from this day onward until the first proestrus was evident. Thirty-six out of 40 rats showed a proestrous smear 48 hr after litter removal while 4 out of 40 showed it after 24 hr. On the day of proestrus, animals were injected at 0915 and 1315 hr with half a dose of solvent or bromocriptine and at 1715 hr with one dose; blood samples were taken at 1100 and 1700 hr and 0900 hr of the following morning. Ovulation occurred within 24 hr of the proestrous smears.

(iv) Ovulation in lactating rats: This is an original model developed in this study. Rats were treated as in (iii), but pups were not withdrawn on Day 13 postpartum; instead, mothers were injected with solvent or pergolide, a dopamine agonist, on Days 13, 14, and 15 at 0900 hr (7 and 11 animals, respectively). For blood sampling, dams were momentarily separated from their pups. Jugular vein blood samples were taken on Day 15 at 1100 and 1700 hr and 0900 hr on Day 16, when ovulation was investigated. Pups were weighed on Day 13 before the mothers were injected and on Day 16 after the experiment ended.

In a further series of experiments, 16 rats were treated with pergolide as described and 7 of these rats were also injected with oPRL (NIADKK), 75 μg/rat, on Days 13, 14, and 15 at 0900, 1300, and 1700 hr. The remaining 9 animals were injected with solvent. Eight other animals, that received only solvent, served as controls. On Day 16 ovulation was investigated.

Thus, of the four models described, the first two (postpartum ovulation, after lactation) could be considered "natural" since no treatment was necessary in order to observe ovulation. The last two models (midlactation, lactating rats) should be considered as "artificially induced" since some treatment was needed in order to trigger ovulation.

Ovulation, as an index of the biological effect of hormones, was determined as described (10).

Blood samples and hormone radioimmunoassays. Sampling was maximally reduced because frequent blood extractions can alter ovulation (11). The timing was selected according to previous laboratory experience in order to obtain one sample before, one sample during, and one sample after the time of the preovulatory LH surge preceding ovulation. All samples were taken from the jugular vein under light ether anesthesia.

Serum hormone determinations. PRL and FSH were determined by RIA using kits provided by the NIADDK; results were expressed in terms of RP_2 rat PRL standard and RP_1 rat FSH standard, respectively. LH was determined with the RIA developed by Niswender *et al.* (12) and results are expressed in terms of RP_1 rat LH.

Drugs. Bromocriptine mesylate (Sandoz, Buenos Aires), a dopaminergic drug, was dissolved in its own weight of tartaric acid in

$\frac{1}{3}$ 70% ethanol and $\frac{2}{3}$ saline to a final concentration of 3 mg/ml. It was injected ip. The total amount per day was 6 mg/kg.

Pergolide (Eli Lilly, Indianapolis), a potent, long lasting, dopaminergic drug was dissolved to a final concentration of 0.05 mg/ml in distilled water; the dose of 0.05 mg/kg was administered daily sc (13).

Ovine prolactin (NIADDK o-PRL-17, biological potency 31.0 UI/mg) was dissolved in 0.03 M CO_3HNa in saline at a concentration of 2.5 mg/ml. It was then diluted in phosphosaline buffer (pH 7.4) to a final concentration of 150 μg/ml and was injected subcutaneously at a dose of 75 μg/0.5 ml per animal, three times a day.

Statistical analysis. Results were evaluated using Student's paired t test to compare the means at different times with basal values within the same group. Percentages were compared by the test of difference of two percentages (14). Differences of means between two different groups were analyzed by Student's t test; differences of means of more than two groups were analyzed by variance analysis followed by Duncan's test. $P < 0.05$ was considered significant.

Results. *(i) Postpartum rats.* Control postpartum rats (Fig. 1, left) showed significantly elevated values of LH, FSH, and PRL at 1700 when compared to levels at 1300 ($P < 0.05$). In bromocriptine-treated rats the LH peak was maintained ($P < 0.02$), and FSH levels were increased, though the difference did not attain significance and PRL remained consistently low. There were no differences in the ovulation percentages or in the number of ova between groups (Fig. 3).

(ii) First ovulation after litter removal at the end of the lactational period. The first ovulation after lactation was accompanied in control rats by afternoon peaks of the three hormones tested (Fig. 1, right) ($P < 0.05$). Those animals treated with bromocriptine showed significantly elevated LH values at 1700 hr ($P < 0.01$), high FSH levels at 1700 and 0900 hr, and low PRL values throughout the experiment. There were no differences in ovulation occurrence or in ova number, between solvent- and bromocriptine-treated animals (Fig. 3).

(iii) First ovulation after litter removal at midlactation. Rats separated from their litters at midlactation ovulated 24–72 hr later. In control rats this ovulation was preceded by peaks of LH ($P < 0.01$), FSH ($P < 0.01$), and PRL ($P < 0.02$) in the first proestrous afternoon (Fig. 2, left). Bromocriptine-treated animals showed significantly elevated LH and FSH values at 1700 ($P < 0.01$) and PRL levels remained at basal values during the whole experiment.

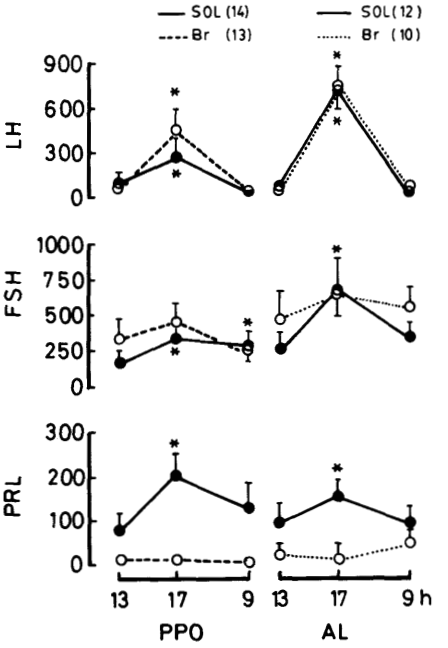

FIG. 1. Postpartum ovulation (PPO) and first ovulation after litter removal at the end of the lactational period (AL). LH RP_1 (ng/ml), FSH RP_1 (ng/ml), and PRL RP_2 (ng/ml) profiles of ovulating rats are represented. Means and error standards are indicated for hormone values. *$P < 0.05$ or less, comparing the value at each time vs the initial level. SOL, Solvent-injected controls. Br, Bromocriptine. Numbers of rats are in parentheses.

The incidence of ovulation was significantly higher ($P < 0.05$) in rats which had received bromocriptine (88%) compared to controls (59%), at the time tested, though we cannot discard the possibility that rats that failed to ovulate could have done so later. There was no difference in ova number (Fig. 3).

(iv) Ovulation in lactating rats. Rats which suckled their litters showed very low LH levels, normal unvarying FSH levels, and an afternoon peak of prolactin ($P < 0.05$) (Fig.

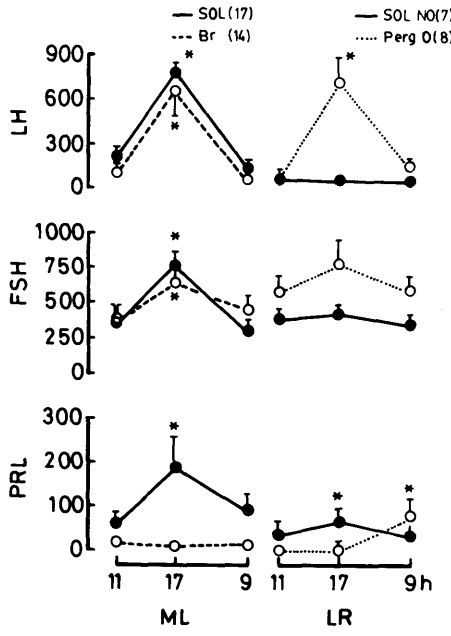

FIG. 2. First ovulation after litter removal at midlactation (ML) and ovulation in lactating rats (LR). SOL NO, Solvent-injected controls which did not ovulate. Perg O, Pergolide-treated ovulating rats.

2). Only $\frac{1}{15}$ of the solvent-treated rats ovulated spontaneously. Pergolide-injected animals showed significantly elevated LH values at 1700 hr ($P < 0.01$). FSH increased from 548 ± 90 ng/ml at 1100 to 756 ± 152 ng/ml at 1700 though the difference was not statistically significant and PRL was very low during the afternoon of proestrus and rose significantly ($P < 0.01$) in the morning of estrus. Interestingly enough, it was possible to induce ovulation in 67% of the pergolide-injected animals; this percentage was significantly higher ($P < 0.05$) than the 7% obtained in saline-treated rats. All rats that received pergolide and oPRL exhibited an anestrous vaginal smear on the expected days of proestrus and estrus. None of these animals ovulated.

In order to investigate if the treatment with pergolide had altered milk yield, pup weight gain was analyzed. While pups from solvent-injected mothers increased 6.3 ± 0.2 g in 3 days, pups from pergolide-injected mothers increased only 2.9 ± 0.7 g in the same period, the difference being significant ($P < 0.05$). These results suggest that PRL, and hence milk secretion, was partially suppressed, though a possible action on oxytocin cannot be discarded. But even if the weight gain was smaller in litters from pergolide-injected mothers, in these dams, the neuroendocrine suckling reflex was still activated (15, 16).

Discussion. All natural or artificially induced ovulatory models here tested showed LH and FSH peaks on the afternoon preceding ovulation. Besides, in postpartum ovulation (PPO), ovulation after lactation (AL), and ovulation at midlactation (ML) a preovulatory PRL surge was determined for the first time. This shows that its presence is a common characteristic of ovulation in the rat. Pergolide-treated rats (LR) ovulated, but, the PRL peak was not observed because pergolide is a strong dopaminergic drug.

In order to establish the importance of the PRL peak on the following ovulation, the first three groups were also tested in the presence of a dopaminergic drug, which sup-

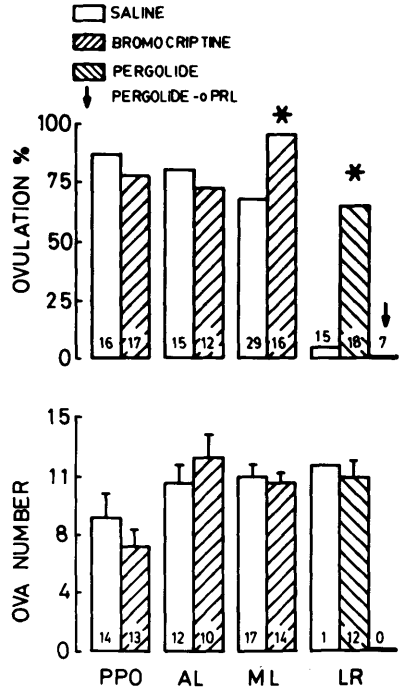

FIG. 3. Ovulation percentages (upper panel) and OVA number (lower panel) in four ovulatory models related to lactation. Numbers inside the columns indicate number of animals per group. *$P < 0.05$ between groups.

presses this surge. Bromocriptine treatment lowered PRL titers but did not alter gonadrotrophin levels or ovulation either in PPO rats or in AL animals, as described in normal cycling rats (17, Lux *et al.*, unpublished).

Apparently, the PRL peak seems not to be of critical importance in the normal sequence of neuroendocrine events leading to ovulation in natural models. Its presence could be the result of the increase in estradiol levels (18, 19). On the other hand, some authors point out that the increase in the frequency of LHRH secretion might be accompanied by a decrease of dopamine turnover on proestrus (20–24). If so, the PRL peak could be related to such an event. On the other hand, PRL could most probably play other roles in the natural ovulatory process: it may act at the hypothalamic–pituitary unit as an autofeedback regulator (25), interact with dopaminergic neurons that control pituitary secretion (26, 27), modify LHRH-induced gonadotrophin release (3, 28–30), act directly at the ovary, modifying corpus luteum functions (31), or play a role in sexual receptivity (32).

In the artificially induced ovulatory models described herein, PRL seems to influence the outcome of ovulation. When litters were removed at midlactation, ovulation took place in only 59% of solvent-injected animals, at the time tested. In contrast, rats treated with bromocriptine on proestrous showed a significant increment ($P < 0.05$) in ovulation percentages (88%). In this way, PRL levels could be involved in the low ovulation percentage obtained in solvent-treated rats. As PRL has been described to have antigonadotrophic effects (33–35), the time lapse between the abrupt separation of the litters, when PRL levels and suckling were still important, and the estrous smears may not have been enough for the reinstatement of the neuroendocrine mechanisms triggering ovulation in all animals. However, PRL effects could be indirect, by altering progesterone levels, as it has been repeatedly described that PRL enhances progesterone secretion (31). High progesterone levels can block ovulation completely, as happens in pregnancy and pseudopregnancy (7). In this case bromocriptine treatment caused an abrupt fall in PRL levels, which in turn induced a rapid fall in progesterone titers, thus increasing ovulation percentage.

To further investigate the participation of PRL in anovulation, pergolide, a potent long-lasting dopaminergic agent, or solvent, was administered to lactating rats. Only one of the control animals ovulated spontaneously. In contrast, pergolide-injected dams showed cyclical vaginal smears and ovulation occurred in 67% of the cases. The critical role of PRL is shown by the hormone replacement study in pergolide-treated rats. Exogenous oPRL completely blocked ovulation. This indicates that the effect of pergolide is due to its action on PRL secretion rather than to the dopaminergic effect itself.

Data from the literature indicate that lactational anovulation is the result of high PRL levels and of the suckling stimulus (8, 9, 33–37). The present results stress the role of PRL at this time of lactation, as suckling stimuli were present in both our experimental groups. In fact, pups from pergolide-treated rats were probably hungrier than pups from saline-injected mothers, because low prolactin levels lower milk secretion and so were likely to provide a more intense suckling stimulation, as has been proved (15, 16). Even so, PRL inhibition, in the presence of this strong suckling stimulation, was able to induce ovulation. PRL can alter gonadotrophin secretion through different mechanisms. It was suggested that high PRL levels are able to reduce LHRH secretion and also inhibit LH secretion from the pituitary (6, 33–53). PRL could also be acting at the gonadal level inhibiting estradiol secretion or increasing progesterone levels, and both procedures are known to have antigonadrotrophic effects (37).

In summary, these results suggest that while a PRL peak is a common characteristic of ovulation in the rat, its presence in natural ovulatory models is not fundamental for the next ovulation to take place. In contrast, in artificially induced models, inhibition of prolactinemia by dopaminergic drugs is able to increase or induce ovulation in lactating mothers. This effect of PRL on ovulation may be direct or indirect. The possibility that PRL may play an important role in later events in natural models and the exact way in which a decrease in PRL contributes im-

proved ovulation in artificial models remain to be determined.

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