Prolactin does not Mediate the Suppressive Effect of the Suckling Stimulus on Luteinizing Hormone Secretion in Ovariectomized Lactating Rats

KEI-ICHIRO MAEDA, EMI UCHIDA, HIROKO TSUKAMURA, NAGANARI OHKURA, SATOSHI OHKURA AND AKIRA YOKOYAMA

School of Agriculture, Nagoya University, Nagoya 464-01, Japan

Abstract

The plasma LH concentration in ovariectomized lactating rats is low for 14 days postpartum, while the prolactin concentration is high during this period. We examined the effect of the inhibition of increased prolactin secretion with bromocriptine (CB-154) on the LH secretion in lactating rats ovariectomized on day 2 (day 0 = day of parturition). Blood samples were collected through an indwelling atrial cannula every day. LH levels were kept low until day 9 in lactating rats injected daily with CB-154 (0.6 mg/day, s. c.). The duration of the period during which LH secretion was suppressed was shorter in lactating rats treated with CB-154 than in saline-injected controls. The replacement with ovine prolactin by means of a mini-osmotic pump (0.3 mg/day, s. c.) in CB-154-treated lactating rats restored the duration of LH suppression. In rats deprived of their pups on day 2, the LH concentration rose immediately after removal of the pups and the LH level was not significantly different between rats treated with CB-154, ovine prolactin and saline, indicating that neither the CB-154 treatment nor the high level of prolactin alone has any effect on LH secretion in rats deprived of their pups. The present results clearly demonstrate that prolactin does not mediate the suppressing effect of the suckling stimulus on LH secretion in early lactation and support our theory that the suckling stimulus controls the LH and prolactin secretion independently at the hypothalamic level.

Received December 19, 1989
Correspondence to: KEI-ICHIRO MAEDA
related to the suppression of LH secretion during lactation (McNeilly, 1984) because of the antigonadal action of the high level of prolactin (Cohen-Becker et al., 1986; Smith and Bartke, 1987). Although the abolition of prolactin secretion resulted in an earlier resumption of the estrous cycle in intact lactating rats (Hansen et al., 1983), it is difficult to discriminate between the effect of prolactin on progesterone secretion from lactational corpora lutea and on gonadotropin secretion because progesterone secreted from the corpus luteum by the stimulation of prolactin is also responsible for suppressing the gonadotropin (Smith & Neill, 1977). Smith (1978b) was the first to report that the abolition of prolactin secretion by bromocriptine (CB-154) in lactating rats, which were acutely ovariectomized either on day 2 or 11 of lactation, increased the plasma LH level 5 days after ovariectomy and concluded that compared to the suckling stimulus, the relative contribution of prolactin in suppressing LH secretion increased as lactation advanced. However, little is known about changes in the potency of the suckling stimulus itself in suppressing the LH secretion throughout lactation.

In the present study, we reevaluated the effect of the abolition of prolactin secretion with CB-154 treatment on the suppressed LH secretion throughout lactation using our model, ovariectomized lactating rats.

**Materials and Methods**

**Animals and treatments**

Female Wistar-Imamichi rats weighing 250–300 g were kept under the condition of 14L : 10D (Lights on at 0500 h) and 22±2°C with food (Labo MR-RO, Nihon-Nohsan, Yokohama, Japan) and water supplied ad libitum. The animals were mated with males and housed individually before the expected day of parturition. The day on which newly-born pups were found in the morning was designated day 0. The litter size was adjusted to 8 on day 1. On day 2, all rats were bilaterally ovariectomized and a silicone cannula (i. d. 0.5 mm, o. d. 1.0 mm, No. 00, Sinetsu Polymer, Tokyo) was inserted via the jugular vein into the right atrium. All surgical procedures were performed aseptically under ether anesthesia.

A dopamine agonist, 2-Br-α-ergocriptine mesylate (CB-154, Sandoz, Basel, Switzerland) was dissolved in a small amount of ethanol, diluted with saline and injected into the animals to lower prolactin secretion. Ovine prolactin for biological use (NIADDK-oPRL-18), provided by the National Hormone and Pituitary Program, was dissolved in a small amount of 0.03 M NaHCO3, diluted with saline (12.5 mg/ml) and stored at -20°C until use.

Two groups of postpartum rats were daily injected subcutaneously with either CB-154 (0.6 mg/day, CB-L) or saline (S-L) at 1800 h from day 2. Since the CB-154 treatment would cause a decrease or cessation of milk production (Pozo, 1972), little or no increase in the litter weight could be expected over 24 hours. To assure similar strength of the suckling stimulus in both CB-L and S-L mothers, four litters were rotated every day among 4 mothers in the following order: the CB-L mother, two intact mothers and the S-L mother. Therefore, the CB-L mother daily received 8 pups which had been attached to the S-L mother on the previous day. The S-L mother similarly received a litter that had been nursed by an intact mother. In this way, each litter was nursed by each mother every fourth day and the litters could be kept in good condition throughout the experiment.

Another group of postpartum rats with their pups (P-L), treated with CB-154 same as the CB-L group, were infused with ovine prolactin (0.3 mg/day) by means of a mini-osmotic pump (Alza, California, model No. 2001) implanted under the dorsal skin on day 2. The mini-osmotic pump was replaced with a new one on day 10. Litters were weighed every day throughout the experiments. The suckling behavior of the pups was observed twice a day throughout the experiment to ensure that the mothers in all groups were given a similar suckling stimulus.

Postpartum rats deprived of their pups on day 2 were allocated to the following 3 groups. In the first group, the rats were injected with...
CB-154 (0.6 mg/day, s. c.) at 1800 h every day to determine whether CB-154 affects the LH secretion (CB-NL group). In the second group, the rats were infused with ovine prolactin (0.3 mg/day) by means of a mini-osmotic pump to examine the effect of prolactin on LH secretion (P-NL group). In the third group, the rats were injected with saline (1 ml/day, s. c.) at 1800 h every day (S-NL group). All treatments were started on day 2.

Blood samples (250 µl) were collected every day between 1100 h and 1300 h through the indwelling atrial cannula to determine the change in the plasma LH concentration. Blood samples (100 µl) for the estimation of the prolactin concentration in plasma were also collected every third day between 1300 and 1400 h, taking special care not to increase the prolactin concentration through stress. The plasma was separated by immediate centrifugation at 4°C for 20 min and stored at −20°C until assayed for LH and prolactin.

**Hormone assay**

Plasma LH concentrations were determined by heterologous radioimmunoassay as previously described (Fox & Smith, 1984). Antiovine LH serum was generously supplied by Dr. G. D. Niswender. Rat LH for reference and iodination was provided by the National Hormone and Pituitary Program. Values are expressed in terms of NIADDK-rLH-RP-2. The least detectable concentration for 50 µl plasma was 38 pg/ml. The intra- and inter-assay coefficients of variation were 6.2% at the level of 20.5 pg/tube and 11.3% at the level of 22.7 pg/tube, respectively.

Plasma prolactin concentrations were determined by a double-antibody radioimmunoassay with a rat prolactin RIA kit supplied by the National Hormone and Pituitary Program. The reference standard was NIADDK-rPRL-RP-3. The intra- and inter-assay coefficients of variation were 5.1% at the level of 1.07 ng/tube and 2.7% at the level of 1.08 ng/tube, respectively. The limit of detection was 6.25 ng/ml for 10 µl plasma.

**Statistical analysis**

The statistical difference was determined by ANOVA followed by Duncan’s multiple range test.

**Results**

**Plasma Prolactin levels**

The plasma prolactin level in the S-L group was higher throughout the experiment than in any other group (Fig. 1). The level of prolactin in the CB-L group was

Fig. 1. Profiles of plasma prolactin during the puerperal period in ovariectomized rats. The day on which newly born pups were discovered is designated day 0. The number of pups per litter was adjusted to 8 on day 1. All rats were bilaterally ovariectomized on day 2. Two groups of rats were injected daily with either saline (S-L) or CB-154 (CB-L) subcutaneously and their pups were exchanged daily at 1800 h for those which had been attached to the foster mother for 3 days. Two other groups of rats deprived of their pups on day 2 were injected daily with either saline (1 ml/day, s. c., S-NL) or CB-154 (0.6 mg/day, s. c., CB-NL) at 1800 h. Values are means±SEM. * P<0.05 (vs S-L group, Duncan’s multiple range test).

CB-154 (0.6 mg/day, s. c.) at 1800 h every day to determine whether CB-154 affects the LH secretion (CB-NL group). In the second group, the rats were infused with ovine prolactin (0.3 mg/day) by means of a mini-osmotic pump to examine the effect of prolactin on LH secretion (P-NL group). In the third group, the rats were injected with saline (1 ml/day, s. c.) at 1800 h every day (S-NL group). All treatments were started on day 2.

Blood samples (250 µl) were collected every day between 1100 h and 1300 h through the indwelling atrial cannula to determine the change in the plasma LH concentration. Blood samples (100 µl) for the estimation of the prolactin concentration in plasma were also collected every third day between 1300 and 1400 h, taking special care not to increase the prolactin concentration through stress. The plasma was separated by immediate centrifugation at 4°C for 20 min and stored at −20°C until assayed for LH and prolactin.

**Hormone assay**

Plasma LH concentrations were determined by heterologous radioimmunoassay as previously described (Fox & Smith, 1984). Antiovine LH serum was generously supplied by Dr. G. D. Niswender. Rat LH for reference and iodination was provided by the National Hormone and Pituitary Program. Values are expressed in terms of NIADDK-rLH-RP-2. The least detectable concentration for 50 µl plasma was 38 pg/ml. The intra- and inter-assay coefficients of variation were 6.2% at the level of 20.5 pg/tube and 11.3% at the level of 22.7 pg/tube, respectively.

Plasma prolactin concentrations were determined by a double-antibody radioimmunoassay with a rat prolactin RIA kit supplied by the National Hormone and Pituitary Program. The reference standard was NIADDK-rPRL-RP-3. The intra- and inter-assay coefficients of variation were 5.1% at the level of 1.07 ng/tube and 2.7% at the level of 1.08 ng/tube, respectively. The limit of detection was 6.25 ng/ml for 10 µl plasma.

**Statistical analysis**

The statistical difference was determined by ANOVA followed by Duncan’s multiple range test.

**Results**

**Plasma Prolactin levels**

The plasma prolactin level in the S-L group was higher throughout the experiment than in any other group (Fig. 1). The level of prolactin in the CB-L group was

Fig. 1. Profiles of plasma prolactin during the puerperal period in ovariectomized rats. The day on which newly born pups were discovered is designated day 0. The number of pups per litter was adjusted to 8 on day 1. All rats were bilaterally ovariectomized on day 2. Two groups of rats were injected daily with either saline (S-L) or CB-154 (CB-L) subcutaneously and their pups were exchanged daily at 1800 h for those which had been attached to the foster mother for 3 days. Two other groups of rats deprived of their pups on day 2 were injected daily with either saline (1 ml/day, s. c., S-NL) or CB-154 (0.6 mg/day, s. c., CB-NL) at 1800 h. Values are means±SEM. * P<0.05 (vs S-L group, Duncan’s multiple range test).
kept low throughout the experiment by the treatment with CB-154.

**Daily weight gain of litters**

The litters which were rotated every day among the set of 4 mothers (one CB-L mother, one S-L mother and two intact mothers), increased in weight constantly throughout the experiment (Fig. 2), although they obtained little or no milk every 4th day from the CB-L mother. The daily gain in these litters was not significantly different from that in litters nursed by the ovariectomized lactating mother throughout the experiment. The daily weight gain of litters nursed by P-L mothers was significantly less than that of litters nursed by ovariectomized lactating mothers from day 13 of lactation onward.

**Plasma LH level**

The plasma LH level in the S-L group was kept low between days 3 and 13, and then increased to reach the maximum value of $2.7 \pm 0.6$ ng/ml on day 20 (Fig. 3). Plasma LH in the CB-L group remained low until day 9. It began to increase on day 10 and remained at a significantly...
higher level than in the S-L group. In the P-L group, plasma LH remained low up to day 11 and was kept an intermediate level thereafter. There was no significant difference in the plasma LH concentration in these 3 groups between days 3 and 10, except for the difference on day 9 between the CB-L and S-L and between the P-L and S-L groups.

Plasma LH in S-NL, CB-NL and P-NL groups increased immediately after the removal of pups on day 2 and reached a maximum detectable levels by day 18 (Fig. 4). Plasma LH in these 3 groups was not significantly different, but a slight difference was found on day 17 between S-L and CB-L and between S-L and P-NL groups.

**Discussion**

The present findings clearly showed that the LH secretion was suppressed in early and mid-lactation in ovariectomized lactating rats. The CB-L mothers seemed to receive the same vigorous suckling stimulus as the saline-injected controls, because the daily weight gain of litters nursed in rotation was not significantly different from that of litters nursed by non-treated ovariectomized lactating mothers throughout lactation (Fig. 2), and the material behavior of CB-L mothers was not different from that of S-L mothers. Since the treatment with CB-154 did not affect the LH secretion in non-lactating ovariectomized rats (Fig. 4) and Smith (1978a) reported that the dose of CB-154 used in the present study failed to affect LH secretion in cycling female rats, CB-154 might exert its effects only on prolactin secretion and not on LH secretion. These facts support our theory that the suckling stimulus can suppress the LH secretion at the hypothalamic level through neural pathways without any mediation of prolactin secretion in early and mid-lactation.

In lactating sows, treatment with CB-154 failed to affect plasma LH on day 12 of lactation but increased it from day 16 onward (Bevers et al., 1983), suggesting that prolactin did not mediate the suppressive effect of the suckling stimulus on LH secretion in lactating sows during
early lactation. Smith (1978b) reported that the CB-154 injection on day 8 of lactation increased plasma LH in ovariectomized lactating rats. The difference between our results and hers could be ascribed to the different litter changing procedure. The litter was switched back and forth between the control and the CB-154 treated females every 12 h in her experiment. In our preliminary experiment in which the litter was exchanged between the saline and the CB-154-treated mothers every 24 h, plasma LH in saline-injected ovariectomized mothers began to increase two days earlier than in ovariectomized mothers without any treatment and the weight of the litters did not increase smoothly. In the present experiments we used a rotation system among 4 mothers and the weight gain in litters was not significantly different from that in litters nursed by non-treated mothers.

The replacement of ovine prolactin in rats injected with CB-154 decreased LH in late lactation compared with lactating rats injected with CB-154 (Fig. 3). The LH level in P-L group was significantly lower than in the CB-L group from day 10. The prolactin infusion alone had no effect on LH secretion. There was no significant difference between the LH levels in the P-NL and S-NL groups (Fig. 4). These results suggest, therefore, that the LH secretion is suppressed by the cooperative action of the suckling stimulus and prolactin in late lactation, and that the suppressive effect of the suckling stimulus alone observed in early lactation decreased in late lactation. The prolactin infusion in rats injected with CB-154 did not completely suppress the LH secretion. This might be caused by the lower intensity of the suckling stimulus in the P-L group than in the S-L group, because the weight gain of the litter decreased in the P-L group from day 10 (Fig. 2). The dose of prolactin infused in the present study is enough to maintain milk production in the former half of lactation, but not in the latter half. (Fig. 2). Another possibility is that the long-term infusion of ovine prolactin in the P-L group could produce an antibody to prolactin in these rats which attenuated the effect of ovine prolactin on LH secretion.

In conclusion, the present results clearly demonstrate that the suckling stimulus itself can suppress the LH release without any contribution of prolactin early lactation in rats.

Acknowledgments

We are grateful to the National Hormone and Pituitary Program for the gift of the LH assay kit, to Dr G. D. Niswender for the antiserum to LH, to Sandoz for the gift of CB-154, to Dr. T. Imamichi, Imamichi Institute for Animal Reproduction for the gift of animals, and to Ms. Y. Niwa for her technical assistance. Our thanks are also due to Dr. J. S. Tindal for reading the original manuscript. The radioimmunoassay was performed at the Nagoya University Radioisotope Center. This study was partly supported by Grants-in-aid Nos. 61480076 and 01790482 from the Ministry of Education, Science and Culture, Japan, and by the Ishida Foundation, Nagoya, Japan.

References


Neuroendocrinol 42, 328–333.