Lactogenic Hormones of the Placenta and Pituitary Inhibit Suckling-Induced Prolactin (PRL) Release but Not the Ante-Partum PRL Surge (44094)

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Abstract. **Prolactin (PRL) and other lactogenic hormones feed back at the hypothalamus to inhibit PRL release. At midpregnancy, high circulating levels of placental lactogens (PL) terminate the mating-induced biphasic PRL surges in female rats. In the dark period preceding parturition, however, an ante-partum PRL surge occurs despite continuously high levels of PL. This study examined whether the lactogenic hormone negative feedback loop is altered during the ante-partum surge using two models: (i) pregnant rats given a hypothalamic implant of albumin, ovine PRL, or recombinant rat PL-I on Day 19 or 20 of pregnancy; and (ii) pregnant rats bearing a transplant of a rat choriocarcinoma cell line, Rcho-1 (PL-secreting), or HRP-1 (non–PL-secreting). Serial blood samples were taken *via* carotid cannula from all rats. Although lactogenic hormones placed in the hypothalamus reduced suckling-induced PRL release by 89%, hypothalamic implants of oPRL or recombinant rPL-I did not attenuate the ante-partum PRL surge. Rcho-transplanted rats also did not have a significantly reduced ante-partum PRL surge (peak PRL level, 131 ng/ml) compared with HRP-bearing rats (peak PRL level, 107 ng/ml). Northern blot analysis revealed that the Rcho-1 tumors expressed both PL-I and PL-II, while the HRP-1 tumors did not express either PL. The inability of the Rcho-1 transplants to inhibit the ante-partum PRL surge suggests that lactogenic hormone negative feedback is disrupted during the ante-partum period, possibly by the changing steroid profile associated with parturition.**
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Prolactin (PRL) release is regulated in part by a negative short-loop feedback inhibition mediated by dopamine (DA) from the hypothalamus (1). Thus, hypothalamic implants of PRL have been shown to inhibit the proestrous PRL surge (2), the PRL surges of pregnancy (3, 4) and pseudopregnancy (5, 6), and suckling-induced PRL release (2, 7). Others have shown that pituitaries grafted in the hypothalamus block the PRL surges of pregnancy (8, 9). While elevation of cerebroventricular PRL levels increased tyrosine hydroxylase activity in the hypothalamus during lactation, this was effective only during early stages but not at midlactation (10). Plasma PRL levels were attenuated in pup-exposed dams on Day 6 of lactation, but not Day 13, following PRL treatment (10). This suggests that PRL short-loop feedback is not fully effective after early lactation.

Physiologically, the PRL surges of pregnancy are terminated by placental lactogens secreted at midpregnancy (11–13). Both placental lactogen–I (PL-I) and placental lactogen–II (PL-II) can bind and activate the PRL receptor (14, 15) enabling these hormones to participate in PRL feedback inhibition. A rat choriocarcinoma tumor cell line, Rcho-1, which secretes PL-I when transplanted *in vivo* (16), caused premature termination of the PRL surges during pregnancy (17) and inhibited suckling-induced PRL release (18). Yet, an ante-partum PRL surge occurs in the dark period preceding parturi-

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tion (19, 20) in spite of continuing high plasma levels of PL-II. Both human PL injected intracerebroventricularly (21) and intrahypothalamic pituitary grafts (22) were unable to inhibit the ante-partum PRL surge.

The goal of this study was to compare the ability of lactogenic hormones from the pituitary and placenta to inhibit PRL secretion under two different physiological conditions. The aims were 2-fold: (i) to compare the ability of lactogenic hormones from the pituitary and the placenta to inhibit suckling-induced release; and (ii) to determine the ability of pituitary and placental hormones to attenuate the ante-partum PRL surge.

Materials and Methods

Animals. Female Sprague-Dawley rats (Sasco, Omaha, NE), weighing 200–225 g on arrival, were housed in a controlled temperature (22°C) and an alternating 12:12-hr light:dark environment (lights on at 0600 hr), with food and water available *ad libitum*. For the pregnancy studies, estrous cycles were followed by daily vaginal smears, and a proestrous female placed with a single male. Presence of vaginal sperm marked Day 0 of pregnancy. Rats were individually housed throughout the experiments.

Surgery. Rats received a transplant of 1×10^6 Rcho-1 or HRP-1 cells/50 μl injected under the right kidney capsule. Animals used in the first experiment received cell transplants on Day 10 of pregnancy. Ovariectomies were performed through two bilateral dorsal incisions on nulliparous rats. These rats also received tumor cells at the time of ovariectomy.

Since all experiments required multiple blood samples, each animal received a carotid artery cannulation 1 day prior to blood sampling. PE50 tubing filled with heparinized saline (20 IU/ml) was advanced 1 in. into the left carotid artery, exteriorized at the back of the neck, and protected by a stainless steel spring extended outside the cage. Surgery was performed under Ketamine (120 mg/kg)/acepromazine maleate (1 mg/kg) anesthesia injected intramuscularly.

Lactogenic hormones were placed in the median eminence of the hypothalamus using a Kopf stereotaxic instrument. A mixture of cocoa butter and 25 μg of oPRL, recombinant rPL-I, or albumin (control) was tamped into a 23-gauge capillary tube. A second control group received bone wax in the tube as a control for nonspecific effects of the proteins. The tube was placed 5.5 mm anterior of the intra-aural line, 0.5 mm lateral from the midline, and 0.9 mm above the floor of the cranial cavity. Implants were fixed in place by three skull screws and dental cement. Recombinant rPL-I was a gift from Dr. M. Robertson (23).

Blood Sampling and Radioimmunoassay. Serial blood samples and trunk blood samples were collected in heparinized centrifuge tubes. For the serial samples, 0.3-ml volumes of blood were taken and replaced with an equal volume of heparinized saline. The final blood sample from all rats was trunk blood obtained by rapid decapitation. Plasma PRL levels were determined by radioimmunoassay using RP-3 as the standard reference and ^{125}I-PRL as the labeled antigen. Plasma was separated from whole blood by centrifugation and then stored at −20°C. Samples were assayed at 5 and 25 μl plasma volume. The lower limit of sensitivity was 50 pg; the interassay coefficient of variation was 9.03%.

Cell Cultures. Rcho-1 cells, which secrete PL-I *in vivo*, were cultured in RPMI-1640 culture media supplemented with 20% heat-inactivated fetal bovine serum at 37°C under humidified atmosphere of 96% air/5% CO_2. Cells were passaged by brief exposure (60 sec) to 0.25% trypsin–0.02% EDTA, followed by mechanical scraping of cells from the culture dish. Cells were subsequently plated at a density of 1×10^6 cells/75 cm^2 tissue culture flask. Cells were briefly trypsinized, scraped, centrifuged, and resuspended in culture medium before they were injected under the kidney capsules.

HRP-1 cells (24), a cell line that does not secrete any members of the PRL-GH family, served as a control for several of the experiments. The HRP-1 cells were maintained, passaged, and transplanted as described for the Rcho-1 cells.

mRNA Analysis. Rcho-1 and HRP-1 tumors were immediately frozen in liquid nitrogen and stored at −70°C. Tumor RNA was extracted by the modified method of Chomczynski and Sacchi (25) as described by Xie and Rothblum (26). In brief, approximately 0.10 g of tumor was lysed using a single-step acid guanidinium thiocyanate-phenol-chloroform extraction. RNAs were precipitated in isopropanol and stored as precipitates at −70°C until ready for use, then washed with 70% ethanol, dried under reduced pressure, and diluted in TE. Total RNA (10 μg/well) was fractionated on 1% agarose-formaldehyde gel and transferred to a Nytran membrane by capillary transfer (27). Denatured DNA probes were hybridized with prehybridized Nytran membranes containing RNA samples for 24 hr at 42°C. Blots were then washed with high stringency (0.1 × SSC and 0.5% SDS) at 65°C, air-dried, and autoradiographed.

cDNAs for members of the rat placental PRL family were generously provided by Dr. D. Linzer (PL-I; Northwestern University, Evanston, IL) (28) and Dr. M. Soares (PL-II; University of Kansas, Kansas City, KS) (29). The cDNA inserts were used as templates for the synthesis of [^{32}P]-labeled cDNA probes using the Prime-It II Random Primer Labeling Kit (Stratagene).

Experiments. The first experiment determined the ability of hypothalamic implants of lactogenic hormones to inhibit suckling-induced PRL release during early lactation. Dams received a hypothalamic implant of bone wax or 25 μg oPRL, recombinant rPL-I or albumin

mixed in cocoa butter on Day 4 of lactation. They were also cannulated at this time. On Day 5 of lactation, litters were removed in the morning for 6 hr, then returned, and blood samples taken at 0, 5, 15, 30, and 60 min of suckling. The dams and litters were observed and pups weighed daily until Day 10 of lactation to determine the dam's lactational competency. On Day 10, each dam was sacrificed, the hypothalamus exposed, and the location of each implant verified.

The second experiment examined the ability of hypothalamic implants of lactogenic hormones to inhibit the ante-partum PRL surge. Pregnant rats were cannulated and received a hypothalamic implant of 25 μg of oPRL, recombinant rPL-I or albumin mixed in cocoa butter on Day 19 or 20 of pregnancy. Serial blood samples were taken at 0600, 0900, 1100, and 1600 h beginning on Day 20, until parturition. Time of parturition and size of litters was also recorded. The dams were sacrificed by rapid decapitation 2–3 days following parturition. The hypothalamus was then exposed and the location of each implant verified.

The third experiment examined the ability of Rcho-1 tumor secretions to attenuate the ante-partum PRL surge. Rats received an Rcho-1 or HRP-1 transplant of 1×10^6 cells under the right kidney capsule on Day 10 of pregnancy. They were cannulated on Day 19 of pregnancy. Serial blood samples were taken at selected times beginning on Day 20, until parturition. Time of parturition and size of litters was also recorded. Two to three days following parturition, dams were sacrificed by rapid decapitation, and the tumors collected for mRNA analysis.

The fourth experiment examined the expression of PL-I and PL-II mRNA by the Rcho-1 tumor under various hormonal conditions. Tumors were grown under the following conditions: (i) under the kidney capsule of pubertal female rats; (ii) under the kidney capsule of an ovariectomized rat treated with long-term progesterone and estrogen; (iii) inside the spleen of an ovariectomized rat treated with long-term progesterone and estrogen; and (iv) under the right kidney capsule in the female rats described in the third experiment. The expression of Rcho-1 tumors was compared with the expression of HRP-1 tumors grown under similar conditions.

Statistics. Data are expressed as the means ± SEM. A statistics program, StatView 512+ (Brainpower, Agoura Hills, CA), was used to analyze data, by a two-way analysis of variance for repeated measures. Differences were considered significant if $P < 0.05$ by Fisher's t test.

Results

Prolactin Release in Day 5 Lactating Rats. Lactating dams given bone wax or albumin hypothalamic implants exhibited a strong PRL response to suckling on Day 5 of lactation (Fig. 1). Blood samples were taken at 0, 5, 15, 30, and 60 min of suckling after 6 hr of pup separation. Plasma PRL levels reached 173 ng/ml in bone wax–implanted dams and 192 ng/ml in albumin-implanted rats at 60 min of suckling. In contrast, suckling-induced PRL release was significantly reduced ($P < 0.05$) compared with albumin-implanted dams, at 15, 30, and 60 min in PRL-treated rats (60 min, 24 ng/ml) and at 30 and 60 min in PL-I–treated dams (60 min, 26 ng/ml).

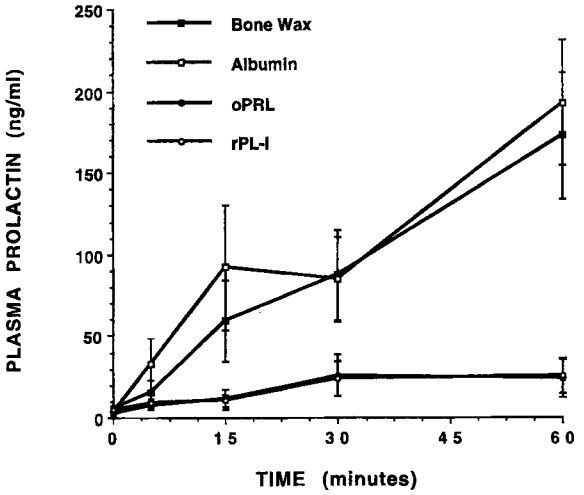

FIGURE 1. Plasma PRL levels measured in Day 5 lactating dams bearing a hypothalamic implant of bone wax or 25 μg of albumin, oPRL, or rPL-I. Pups were separated from the dams for 6 hr, then suckled for 1 hr. The implants of oPRL significantly ($P < 0.05$) reduced plasma PRL levels compared with the albumin controls at 15, 30, and 60 min. The rPL-I implants significantly ($P < 0.05$) lowered PRL levels at 30 and 60 min. Each value represents a mean ± SEM of determinations from 6–14 rats.

Daily litter weight gains were reduced in all groups following surgery (Fig. 2). The most dramatic reduction was in the litters of PRL-implanted dams, which were significantly different ($P < 0.05$) than control litters on Days 5, 6, and 7 of lactation. Litter weight gains of PL-I–treated dams were significantly lower than controls ($P < 0.05$) on Days 5 and 6 of lactation.

Prolactin Release in Late Pregnant Rats. The ante-partum PRL surge was not significantly reduced by any of the treatments given. Plasma PRL levels peaked at 0300 hr in the dark period preceding parturition in albumin-implanted (77 ng/ml), PRL-implanted (124 ng/ml), and PL-I–implanted (116 ng/ml) animals (Fig. 3). Time to onset of parturition and size of litters was not significantly altered. Half of the PL-I– (3/6) and PRL-implanted (5/10) dams, however, had low litter survival rates due to a reduced lactational competency. Only 20% (2/10) of the albumin-implanted dams had a reduced lactational competency.

The presence of Rcho-1 and HRP-1 tumors also did not significantly alter the ante-partum PRL surge (Fig. 4). Plasma PRL levels peaked at 0300 hr in the pre-partum dark period in Rcho-1–transplanted (131

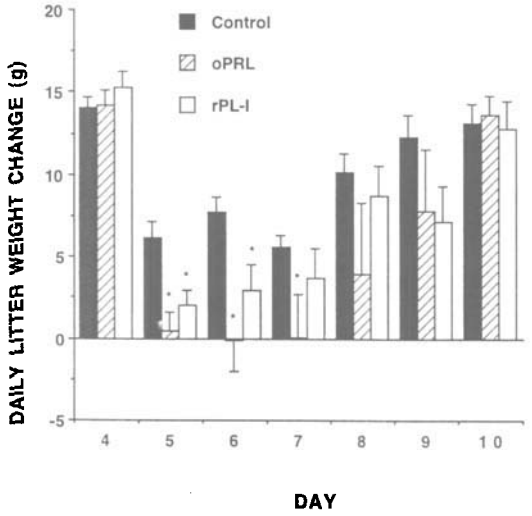

FIGURE 2. Daily litter weight change of eight pups suckled by dams given hypothalamic implants on Day 4 of lactation. Litter weights from albumin- and bone wax–implanted dams were combined. Compared with the control group, dams with rPL-I implants had significantly ($*P < 0.05$) lower weight gains on Days 5 and 6, and dams with oPRL implants had significantly lower litter weight gains on Days 5, 6, and 7. The litter weight change of rPL-I– and oPRL-implanted dams was not significantly different at any time. Each value represents the mean ± SEM of 6–14 litters.

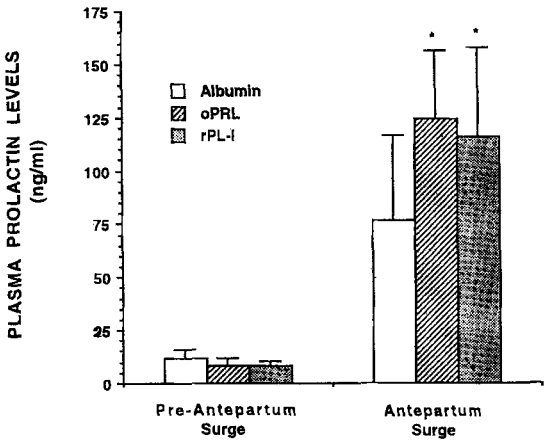

FIGURE 3. Plasma PRL levels measured in pregnant rats bearing 25-μg albumin, oPRL, or rPL-I hypothalamic implants. Blood was sampled during the 2 days preceding parturition. Plasma PRL levels on the day preceding parturition were averaged to show basal levels of PRL and compared with peak plasma PRL levels during the ante-partum PRL surge. Neither the oPRL nor the rPL-I implant was effective in inhibiting the ante-partum PRI surge. Peak PRL levels during the ante-partum surge were significantly higher ($*P < 0.05$) than basal levels in oPRL- and PRL-implanted animals. Each value represents a mean ± SEM of determinations from four or five rats.

ng/ml) and HRP-1–transplanted animals (121 ng/ml). The length of pregnancy, the litter size, and the lactational competency in the two groups were not significantly different in transplanted animals.

mRNA Content of Rcho-1 Tumors. Messenger RNA expression of several PRL-GH family members by the Rcho-1 cell line was evaluated by Northern blot analysis (Figs. 5 and 6). Rcho-1 transplants grown under the kidney capsules of primiparous and steroid-treated

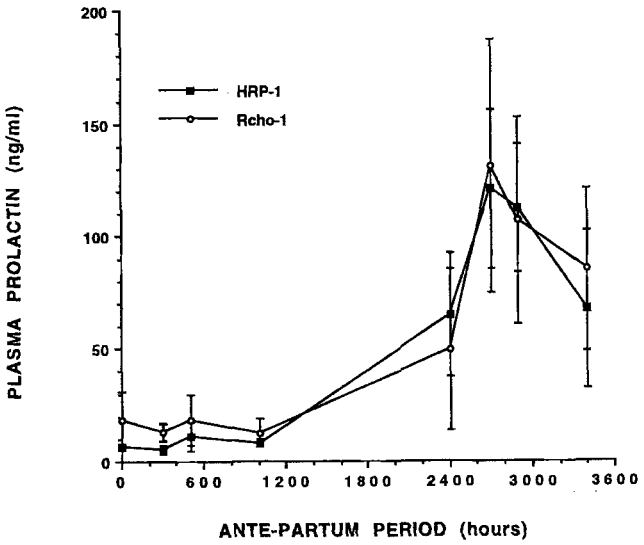

FIGURE 4. Plasma PRL levels measured in pregnant rats bearing an HRP-1 or Rcho-1 transplant. Blood was sampled during the 2 days preceding parturition. The PRL levels during the ante-partum surge in Rcho-transplanted rats were not significantly ($P < 0.05$) different than PRL levels in HRP-transplanted animals at any time point. Each value represents a mean ± SEM of determinations from five to seven rats.

FIGURE 5. Northern blot analysis of PL-I expression by Rcho-1 tumors grown in the spleen or under the kidney capsule (KC) of female rats. Total RNA (10 μg/lane) was fractionated on a 1% agarose gel by electrophoresis and transferred to a Nytran membrane. Hybridizations were carried out using [^{32}P]-labeled PL-I probe. Autoradiograph was exposed for 86 hr. Lane A, postconfluent cell culture; Lane B, Rcho-1 cells from the KC of a pubertal rat; Lane C, Rcho-1 cells from the KC of a postpartum rat; Lane D, HRP-1 cells from the KC of a postpartum rat; Lane E, Rcho-1 cells from the KC of an ovx rat; Lane F, Rcho-1 cells from the KC of an ovx, steroid-treated rat; Lane G, HRP-1 cells from the KC of an ovx, steroid-treated rat; Lane H, Rcho-1 cells from the spleen of an ovx rat; Lane I, Rcho-1 cells from the spleen of an ovx, steroid-treated rat; Lane J, HRP-1 cells from the spleen of an ovx, steroid-treated rat. Note the absence of PL-I expression by the HRP-1 tumors.

nulliparous animals expressed PL-I and PL-II. Rcho-1 tumors grown within the spleen also expressed PL-I and PL-II, suggesting that the site of tumor growth does not alter Rcho-1 expression of lactogenic hormones. HRP-1 transplants did not express either of the PRL-like placental hormones.

Discussion

In this study we have shown that hypothalamic implants that are capable of inhibiting suckling-induced prolactin (PRL) release do not inhibit the ante-partum PRL surge. Furthermore, rat choriocarcinoma (Rcho-1)

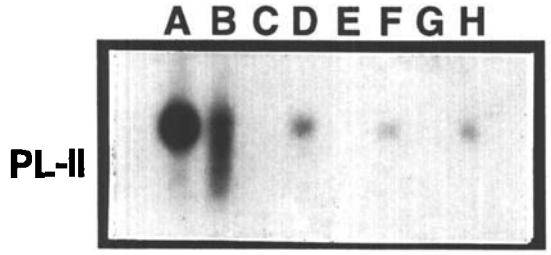

FIGURE 6. Northern blot analysis of PL-II expression by Rcho-1 tumors grown in the spleen or under the kidney capsule (KC) of female rats. Total RNA (10 μg/lane) was fractionated on a 1% agarose gel by electrophoresis and transferred to a Nytran membrane. Hybridizations were carried out using [^{32}P]-labeled PL-II probe. Autoradiograph was exposed for 115 hr. Lane A, postconfluent cell culture; Lane B, Rcho-1 cells from the KC of a pubertal rat; Lane C, HRP-1 cells from the KC of an ovx, steroid-treated rat; Lane D, Rcho-1 cells from the KC of an ovx, steroid-treated rat; Lane E, HRP-1 cells from the spleen of an ovx, steroid-treated rat; Lane F, Rcho-1 cells from the spleen of an ovx, steroid-treated rat; Lane G, HRP-1 cells from the KC of a postpartum rat; Lane H, Rcho-1 cells from the KC of a postpartum rat. Note the absence of PL-II expression by the HRP-1 tumors.

transplants that secrete high levels of PLs into the circulation do not inhibit the ante-partum PRL surge.

In a dam that is allowed to suckle freely her litter, PRL levels remain at fairly high levels as rats nurse for a total of 12–18 hr each day (30). Under most physiological conditions high circulating PRL levels stimulate tuberoinfundibular DA neurons to synthesize and release DA that will then inhibit further PRL secretion (31). PRL short-loop feedback has been demonstrated in cycling female rats given hypothalamic implants of PRL. PRL placed in the median eminence of cycling rats blocks the proestrous PRL surge (2). Pituitary weight and PRL content is also reduced in rats bearing a hypothalamic implant of PRL (31). Although PRL feedback inhibition appears to be less effective during midlactation (10), hypothalamic implants of PRL significantly inhibit the amount of PRL released after 1 hr of suckling (20). The reduced levels of circulating PRL ultimately result in a reduction in litter weight gains (7).

This study followed the time course of suckling-induced PRL release in postpartum rats given hypothalamic implants of ovine PRL. Plasma PRL levels were significantly reduced compared with controls at 15, 30, and 60 min following the onset of suckling. Litter weight gains were reduced on Days 5–7 of lactation when dams were implanted on Day 4. This suggests that a PRL short-loop feedback mechanism is intact at this time of lactation. We found temporary reductions in lactational competency, as evidenced by reduced plasma PRL levels and pup weight gains, in dams given a hypothalamic implant of 25 μg of rat PL-I instead of ovine PRL. Since PL-I is capable of binding to and activating the PRL receptor (15), it is most likely acting through the PRL short-loop feedback mechanism to inhibit suckling-induced PRL release. Since, however, this inhibition of pup weight gain is only temporary, it is possible that the PRL feedback system becomes desensitized over time. Another possible explanation for the recovery of weight gain by the pups after 3–4 days of treatment may be due to a loss of hormone from the implant.

During a normal pregnancy, rat placental lactogens act to maintain the pregnancy while terminating the PRL surges of early pregnancy. The trophoblast giant cells of the rat placenta express PL-I on Days 7–12 of pregnancy (32). Circulating PL-I levels peak on day 12, then dramatically drop (33). Several studies have correlated the rise in circulating levels of PL-I with cessation of the mating-induced PRL surges (11–13). On Day 11, the trophoblast giant cells initiate expression of PL-II (32) followed by expression of PRL-like protein A and PRL-like protein-C on Day 13 (34, 35). These latter three hormones are co-expressed until parturition. It is likely that PL-II continues the suppression of the twice daily PRL surges as evidenced by experiments in which removal of the placentas after midpregnancy temporarily restored the surges (36). The ability of the PLs to inhibit the PRL surges of pregnancy is associated with their ability to bind and activate the PRL receptor (15). By activating the PRL receptor, PL-I and PL-II can participate in PRL feedback inhibition.

Although circulating PL-II levels remain high until parturition (37, 38), PRL levels increase during the dark period preceding parturition. Grattan and Averill were unable to inhibit this ante-partum PRL surge by intracerebroventricular injection of human PL (21) or by intrahypothalamic pituitary grafts (22). The present experiments demonstrate the inability of hypothalamic implants of PRL and rat PL-I to inhibit the ante-partum surge. Although the ante-partum PRL surge was not inhibited, lactation was impaired in 50% of these animals. It may be that PRL release was inhibited following parturition, resulting in reduced levels of milk synthesis. It also suggests that suckling-induced PRL release and the ante-partum PRL surge are differentially regulated.

In order to determine if a more chronic exposure to placental secretions would be effective in attenuating the ante-partum PRL surge, the Rcho-1 cell line was used. Rcho-1 cells phenotypically resemble trophoblast giant cells and maintain trophoblast giant cell sequential gene expression of PL-I, PL-II, PRL-like protein-A, and PRL-like protein-C (16, 39) *in vitro*. But, unlike the co-expression of PL-I and PL-II by the trophoblast giant cells *in vivo*, which is limited to 24 hr (32), co-expression of the PLs is maintained since PL-I expression is not terminated (40). When Faria and Soares maintained Rcho-1 transplants in pubertal female rats, the cells expressed only PL-I and none of the other members of the PRL-GH family (16). In this study, Rcho-1 cells transplanted under the kidney capsule on Day 10 of pregnancy were unable to inhibit the PRL surge which precedes parturition. In a previous study, plasma levels of PL-I averaged 133 $\pm$ 60 ng/ml in lactating rats that

bore Rcho-1 tumors and were undetectable in HRP-1–bearing dams (18). Thus, a significant amount of PL-I is secreted *in vivo* from the Rcho-1 tumors. This suggests that the induction of the ante-partum surge does not require a reduction in PL levels. Furthermore, the sensitivity of PRL shortloop feedback to PLs is reduced during the ante-partum period. Serotonergic neurons may play a role in this feedback alteration. Although serotonin stimulates the nocturnal surge of early pregnancy (41), the ante-partum surge is inhibited by treatment with 5-hydroxytryptophan, a serotonin precursor (42). The actions of other hypothalamic factors may be altered in the ante-partum period.

The Rcho-1 tumors grown under the kidney capsule in rats used for this study co-expressed PL-I and PL-II, as indicated by Northern blot analysis. HRP-1 tumors did not express either PL. These results are similar to those found by Faria and Soares (16). Although these authors could not detect PL-II expression in Rcho-1 tumors maintained in pubertal rats, the Rcho-1 cells they cultured expressed both PL-I and PL-II *in vitro* (16). While mRNA levels of PL-I and PL-II were not quantitated for this study, it appears that PL-I expression is greater than that of PL-II in the Rcho-1 tumors. Possibly, the PL-II mRNA levels in tumors analyzed by Faria and Soares were too low to be detected by Northern blot analysis. Alternately, the ability of the Rcho-1 cells to secrete PL-II *in vivo* may have been acquired by further differentiation of the PL-I cells over time and passaging of the cell line.

This study gives further support to the hypothesis that suckling-induced PRL release and the ante-partum PRL surge are regulated differently. While short-loop feedback is evident in early lactation, in the late-pregnant rat PRL feedback inhibition does not appear to be operational. Control of the ante-partum PRL surge also differs from the regulation of the nocturnal PRL surges of early pregnancy. The placental secretions which had previously inhibited the biphasic PRL surges of early pregnancy do not inhibit the ante-partum PRL surge.

Since ovariectomy precipitates a rapid drop in circulating P levels, removal of the ovaries will also advance parturition. The ante-partum surge is often absent under these circumstances. For example, none of the rats ovariectomized by Bridges and Goldman on Day 21 of pregnancy exhibited a PRL surge (20). In a study by Grattan and Averill, the ante-partum surge was present in only two of nine rats ovariectomized on Day 19 of pregnancy (43). Estrogen treatment restored the ante-partum surge, while further advancing parturition in rats ovariectomized on Day 19. Furthermore, the PRL surge in ovariectomized rats receiving estrogen replacement therapy was present at the same amplitude as the surge exhibited by intact animals (43).

Estrogen may be acting at the hypothalamus and/or pituitary to stimulate PRL release. Alternately, estrogen may alter gene expression by the placenta in pregnant rats, and by Rcho-1 tumors in ovariectomized animals. As a result, newly transcribed and released factor(s) may then affect PRL release. An indirect mechanism of PRL inhibition is supported by a recent study by Grattan and Averill (22). They were able to inhibit the ante-partum PRL surge by a dopamine agonist, bromocriptine, demonstrating that the lactotrophs are still responsive to DA (22). This suggests that the PRL shortloop feedback has probably been altered at the hypothalamic level.

To summarize, the regulation of PRL release changes over the course of pregnancy and lactation. In particular, PRL shortloop feedback inhibition is temporarily disrupted during late pregnancy. Neither ovine PRL nor rat PL-I placed in the median eminence, nor Rcho-1 tumors, are capable of inhibiting the ante-partum PRL surge, even though these treatments have been shown to attenuate suckling-induced PRL release. Because the disruption of PRL shortloop feedback is limited to the dark period preceding parturition, it seems likely that the ante-partum PRL surge, and/or the neuronal changes that trigger it, has some physiological importance. Currently, however, the physiological function of the ante-partum PRL surge is unknown. Further understanding of the acute neuronal changes that occur in the ante-partum period may provide insight into the mechanisms of PRL release during early pregnancy and lactation.

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