PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 175, 398–405 (1984)

Autofeedback Effects of Prolactin on Basal, Suckling-Induced, and Proestrous Secretion of Prolactin[1] (41812)

MICHAEL SELMANOFF[2] AND KAREN A. GREGERSON[3]

Department of Physiology, University of Maryland, School of Medicine, Baltimore, Maryland 21201

Abstract. Subcutaneous injections of ovine prolactin (oPRL, 4 mg/kg) were utilized to study the negative feedback effect of PRL on its own secretion in lactating and 4-day estrous cycling female rats. Basal PRL secretion in the 10-day postpartum lactating rat is not suppressed by acute or 48 hr of exposure to oPRL. In contrast, basal PRL secretion on the morning of proestrus is inhibited following 48 hr of exposure to oPRL. Thus, the lactating rat appears to be unresponsive to the negative feedback action of PRL compared with the adult female rat in regards to basal PRL secretion. The suckling-induced PRL response is partially suppressed by exposure to oPRL in 10-day postpartum lactating rats. Considering the areas under the PRL response curves, the suckling-induced release is blunted by 6 (decreased 49%), 12 (decreased 46%), and 48 (decreased 77%) hr of oPRL exposure compared to controls. In contrast, in cycling animals, 48 hr of exposure to elevated oPRL levels dramatically abolished the proestrous PRL surge. Elevated PRL levels, by a direct action on the brain and/or indirectly by altering ovarian function, inhibit or override the hypothalamic mechanism(s) mediating the proestrous PRL surge while having a lesser effect on the mechanism(s) mediating the suckling-induced PRL response. Taken together, the present data point to probable differences in the mechanism(s) mediating basal, suckling-induced, and the proestrous secretion of prolactin.

Prolactin is thought to exert an autoregulatory, negative feedback effect on its own secretion (1). This feedback action probably is exerted within the hypothalamus since direct inhibition of pituitary PRL secretion by PRL usually has not been observed *in vitro* (2–7) and autosuppression is demonstrable in the absence of the gonads in male (8) and female (4) rats. Hypothalamic implants of prolactin in the median eminence region terminate PRL-dependent states such as pregnancy, pseudopregnancy, and lactation (1) and inhibit the circulating titers of PRL associated with suckling (9), pregnancy (10), pseudopregnancy (11), and the proestrous surge (9). In addition, injection of differing amounts of various prolactin and placental lactogen preparations have been reported to inhibit basal (4, 7, 8, 12–14), stress-induced (7, 12, 15), TRH-induced (7), and suckling-induced (15) PRL secretion.

We previously have shown that the basal secretion of rPRL in adult male rats is suppressed within 3–4 hr of an injection of oPRL (4 mg/kg sc) and that this suppression ceases about 8–10 hr later when the oPRL has been substantially cleared from blood (14). Other instances of PRL release may be more or less sensitive to this negative feedback effect. In the present study we have determined the ability of exogenous oPRL administration to suppress endogenously secreted rPRL in four other instances: basal secretion in the cycling and lactating female, the proestrous surge, and suckling-induced release. In our experiments, known amounts of purified oPRL were administered and the circulating levels of oPRL were monitored in the same rats that endogenous secretion was evaluated. In addition, the delivery of oPRL to the medial basal hypothalamus (MBH) via the circulation probably approximates the *in vivo* situation better than intracerebroventricular PRL administration (i.e., delivery via the cerebrospinal fluid) or invasive implantation of PRL pellets into the MBH.

[1] Supported by NIH Grants NS-14611 to M.S. and HD-15955 to M.S. and P. M. Wise. These data have been presented in abstract form at the 12th Annual Meeting of the Society for Neuroscience, Minneapolis, Minn., October 31st to November 5th, 1982 (8:51, Abstract 18.3).

[2] Recipient of NIH Research Career Development Award NS-00731 and to whom reprint requests should be addressed.

[3] Recipient of NIH National Research Service Award HD-06481.

0037-9727/84 $1.50

Materials and Methods. Adult lactating and cycling female Sprague–Dawley rats (Zivic-Miller, Allison Park, Pa.) were used in this study. Lactating rats weighing 275–350 g were received from the supplier at 5 days postpartum and were used for experiments at 10 days postpartum. The day of birth was designated Day 1 postpartum by the supplier. On Day 10 postpartum, pups were removed from their mothers at 0900, 1000, or 1100 hr and were kept in a separate room (16). Care was taken to minimize sudden noises, movements or other disturbances which could provoke stress responses in the mothers (1). Four hours later, eight pups were returned to each mother and the onset of suckling (i.e., Time 0) was defined as the time when six or more of the eight pups started to suckle. This occurred with a latency of 0.5–7 min (mean ± SEM = 3.2 ± 0.5, N = 49) from the time of pup return in 18 × 9.5 × 7 (l × w × h)-in. cages. Pups were allowed to suckle for 40 min and then were removed to allow us to monitor the attenuation of the PRL response. In order to study the proestrous PRL surge, cyclicity of virgin female rats weighing 200–250 g was determined by daily vaginal lavage and rats exhibiting at least two consecutive 4-day estrous cycles were used. All rats were housed under 14-:10-hr (L:D) lighting conditions with lights on at 0400 hr. Rats were fitted with indwelling right atrial cannulae under ether anesthesia by a simplified version (17) of the method of Terkel and Urbach (18). Lactating rats were cannulated on Day 8 or 9 postpartum and cycling rats on the morning of diestrous Day 2.

In lactating rats at the time of the first blood sample, a long piece of PE-50 polyethylene tubing (Clay Adams, Parsippany, N.J.) was connected to the indwelling catheter with a 24-gauge piece of stainless-steel tubing (Small Parts Inc., Miami, Fla.) and exteriorized out of the cage so that the experimentor could be well removed from the cage for these samplings. Eight or ten serial blood samples (0.25–0.4 ml each) were taken from these animals and the blood volume was replaced with 0.9% saline. Thus, about 3.5 ml or about 14% (lactating) or 19% (cycling) of the estimated blood volumes were removed over 2-, 4-, 8-, or 10-hr periods. The cannulae were kept patent with heparinized saline whenever blood was not being removed. Plasma from these serial samples was obtained after centrifugation at 4°C and was stored frozen at −20°C until assayed.

Ovine PRL was provided by Dr. Albert Parlow through the NIADDK Rat Pituitary Hormone Distribution Program (NIADDK-oPRL-14; biopotency, 31 IU/mg). It was dissolved in 0.01 M $NaHCO_3$ (pH 8.6) and administered as sc injections (4 mg/kg body wt). Rat and ovine prolactin were determined in plasma samples by homologous double-antibody radioimmunoassays after the method of Niswender and co-workers (19). Reagents for these PRL assays were provided by the Rat Pituitary Hormone Distribution Program. oPRL was radioimmunoassayed with the final concentration of reagents being 1:450,000, 1:750, and 1:400 for the first antibody (NIADDK-anti-oPRL-1), second antibody (goat-ARGG), and normal rabbit serum, respectively. This resulted in the binding of 40–50% of 10,000 cpm of ^{125}I-oPRL which were added per assay tube. Nonspecific binding ranged 1–3%. The sensitivity of this 3-day, room-temperature assay ranged from 50–200 pg (least detectable dose defined as 15% displacement). The oPRL standard used was oPRL-I-1 (biopotency, 35 IU/mg). In the case of rPRL, some of these experiments entailed measuring baseline PRL levels and suppressions from baseline. To accurately quantitate these changes, the assay was maximized for sensitivity (20) and 50- or 100-μl plasma aliquots were assayed (total assay volume = 0.5 ml). The sensitivity of the assay ranged from 20–50 pg (least detectable dose defined as 15% displacement) and the aliquot volumes allowed us to determine unknown values from that portion of the standard curve exhibiting the greatest precision (i.e., 50 ± 20% displacement). The rPRL standard used was rPRL-RP-2 (biopotency, 30 IU/mg) which is 2.8 times more potent than RP-1.

Cochran's test for heterogeneity of variance revealed that the raw PRL data was heterogeneous, hence, natural log (ln) transformation of the data was performed to stabilize the variance. The ln-transformed data were statistically analyzed by one- or two-way analyses of variance (time and group) with repeated measures on one factor (time) followed by F tests or the Newman–Keuls multiple range test at P = 0.05 (21).

Results. *Experiment 1.* The results of the first experiment are shown in Fig. 1. oPRL was injected at Time 0 and the endogenous rPRL response was evaluated in serial blood samples obtained from 10-day postpartum lactating rats whose pups had been removed 4 hr prior to Time 0. The circulating titers of oPRL achieved by the single sc injection at Time 0 (0900 hr) in the lactating rat are shown in the top panel. The Time-0 sample was undetectable in the oPRL assay. Thereafter, peak levels were achieved by 2 hr and then returned to low levels by 8 hr postinjection. The oPRL values at 1 and 4 hr are similar to those published by Advis and co-workers (12) using a similar oPRL preparation and the overall profile is very much like the one we documented in intact adult male rats (14). The oPRL injection had no effect on endogenous basal rPRL secretion in 10-day postpartum female rats when compared with diluent injected controls (Fig. 1, lower panel). As stated in the Materials and Methods section, individual rats were hooked up for sequential bleeding at Time 0. This hook up involved cage disturbance, hook up of the extension cannula, and threading of the extension cannula out of the cage. This was followed by a blood sampling and then an sc injection of oPRL or diluent. This hook up and handling resulted in small, transient stress-induced PRL release in some rats. We think this accounts for the increased rPRL values at Time 0 and the subsequent declines 30 and 60 min thereafter which occur in both oPRL- and diluent-treated rats.

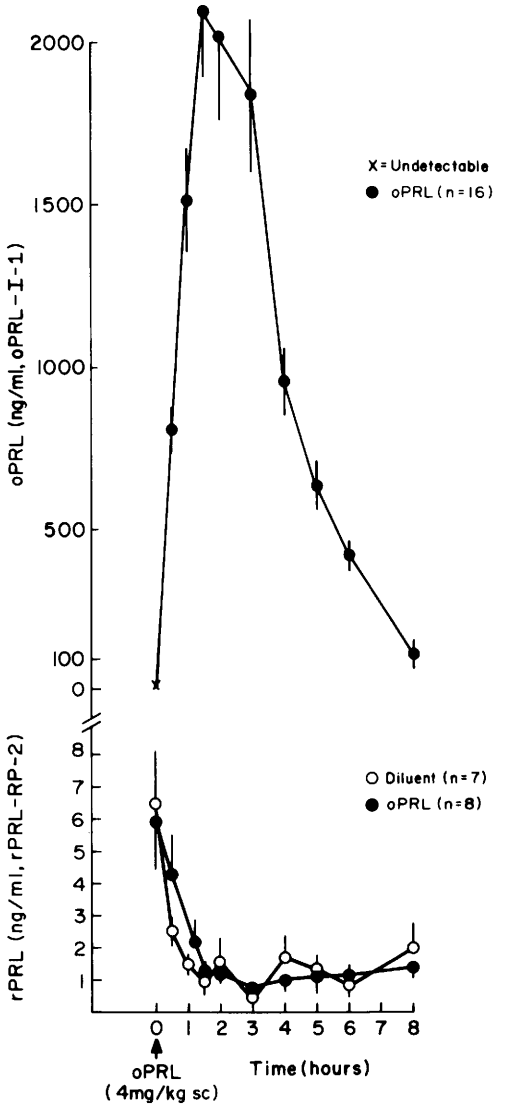

FIG. 1. Lack of negative feedback response of basal rPRL secretion to an sc injection of oPRL (4 mg/kg) at 0900 hr in 10-day postpartum lactating rats.

Experiment 2. In Experiment 2 (Fig. 2), oPRL or diluent was injected at 6 hr (6 hr exposed) or 12 and 6 hr (12 hr exposed) before the onset of suckling at Time 0. There is a significant suppression of rPRL at both 30 and 40 min of suckling for both oPRL-treated groups. Considering the areas under the response profiles, the 6-hr exposed area is 49% less and the 12-hr exposed area is 46% less than controls. The three Time-0 points are not different from one another indicating that 6 and 12 hr of oPRL exposure do not suppress basal rPRL secretion in these lactating rats. The oPRL titers in the two experimental groups at Time 0 (left histogram) and at 120 min (right histogram) may be read on the right-hand scale. These levels are in the expected range considering the overall oPRL profile documented in Fig. 1.

Experiment 3. In experiment 3 (Fig. 3), oPRL or diluent was administered every 8 hr for 48 hr prior to the onset of suckling at Time 0. It was considered possible that 48 hr of exposure to oPRL could decrease milk production which, in turn, might affect the intensity of pup suckling during the 40-min suckling episode. Hence, two precautions were taken. First, pups from diluent-treated moth-

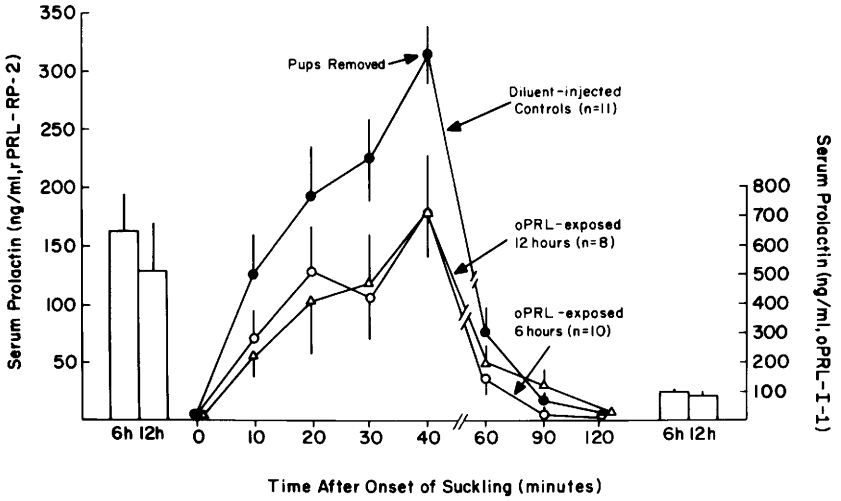

FIG. 2. Partial suppression of the suckling-induced prolactin response by 6 and 12 hr of exposure to exogenously administered oPRL in 10-day postpartum lactating rats.

ers were randomized and used for suckling of both oPRL and diluent-treated mothers. Second, the litters of eight pups were weighed to the nearest one-tenth of a gram before and after the 40-min suckling episode. The increased weight/litter for pups suckling diluent-treated mothers was 3.1 ± 0.7 g (mean ± SEM, N = 10 litters) while the weight gain/litter in pups suckling oPRL-treated mothers was 3.4 ± 0.9 g (N = 8 litters). These means were not significantly different. In oPRL-exposed rats, the suckling-induced PRL response was significantly suppressed at the 10-, 20-, 30-, 40-, 60-, and 90-min time points. The area under the oPRL-exposed response profile is 77% less than the control area. At −120, −60, and 0 times there was no suppression of basal rPRL secretion in these rats treated with oPRL for 46–48 hr. Again, the oPRL titers in the experimental group are illustrated as histograms (Time 0 on left, 120-min sample on right). Reading off the right-hand scale indicates values comparable to those seen previously in Experiments 1 and 2. Unexpectedly, the peak PRL levels attained in control rats in this experiment were about one-half as great as in

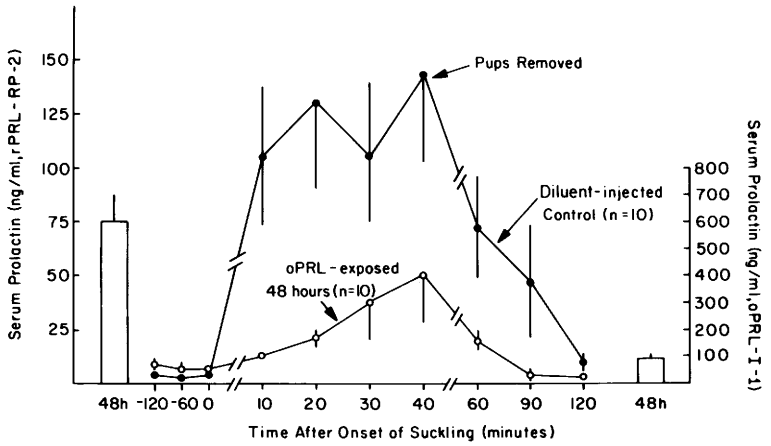

FIG. 3. Partial suppression of the suckling-induced prolactin response by 48 hr of exposure to exogenously administered oPRL in 10-day postpartum lactating rats.

the previous experiment (Experiment 2). We cannot account for this discrepancy except to note that the two experiments were done at different times of the year, about 6 months apart, and entailed different rats and assays.

Experiment 4. In Experiment 4, 4-day cycling rats received oPRL every 8 hr starting at 0900 hr on diestrous Day 1 with the last injection being given at 0900 hr of the day of proestrus. This oPRL treatment totally abolished the proestrous PRL surge normally seen during the afternoon hr (Fig. 4). Seen in the inset of Fig. 4 are the 0900-, 1030-, and 1200-hr times plotted on an expanded scale. The oPRL treatment significantly suppressed basal rPRL secretion at all three times in these cycling rats. Each of the afternoon rPRL values in oPRL-treated rats is also significantly suppressed below the morning values of diluent-treated rats.

Discussion. These results demonstrate that administration of oPRL (4 mg/kg/injection sc) suppresses endogenous rPRL secretion under three circumstances: (i) basal release in cycling rats, (ii) suckling-induced release, and (iii) the proestrous surge. In previous work in the adult male rat (14), we showed that suppression of basal rPRL secretion was first demonstrable 3–4 hr following a single oPRL injection, that this effect was reversible some 8–10 hr later when the oPRL was substantially cleared from the blood, and that continued oPRL administration maintained this suppression over a 48-hr period. The present data revealed that basal secretion in the cycling rat is similarly suppressed following 48 hr exposure. In contrast, neither acute (Fig. 1) nor 48 hr (Fig. 3) of exposure was able to inhibit basal secretion in the 10-day postpartum lactating rat. This is in agreement with Whitworth and co-workers (15) who also did not suppress basal secretion in the lactating rat 4 hr after oPRL administration (3 mg/rat sc). This suggests that the neural mechanism(s) mediating negative feedback is relatively refractory during lactation. It is reasonable that there not be a sensitive negative feedback relationship operating during lactation, since this would presumably compromise the lactational state. Alternatively, it is possible that basal PRL secretion is so low in the lactating rat that no further suppression can be shown.

The several lines of evidence supporting the role of dopamine as the PIH responsible for the tonic regulation of basal PRL secretion have been reviewed recently (22–24). Furthermore, increased tuberoinfundibular dopamine synthesis, turnover and release is pro-

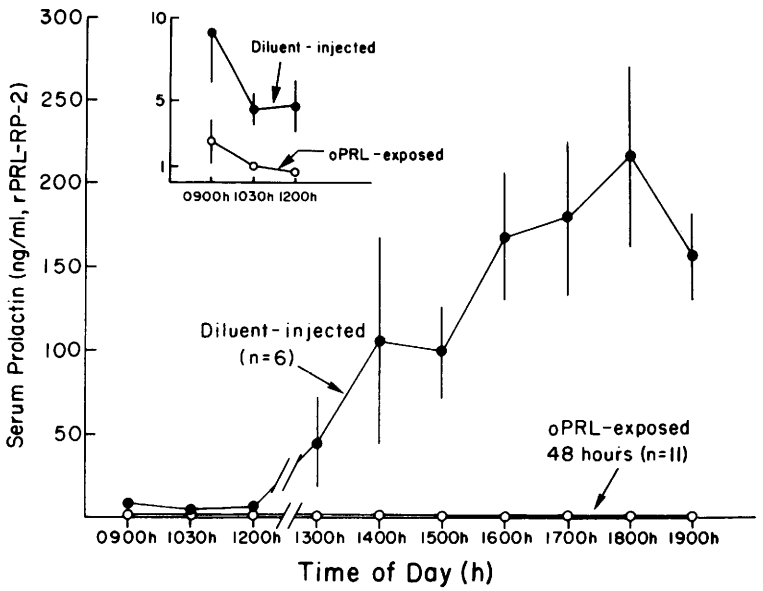

FIG. 4. Suppression of basal and proestrous surge levels of prolactin by 48 hr of exposure to exogenously administered oPRL in 4-day cycling rats.

voked by intracerebroventricular or peripheral administration of PRL (25, 26). In male (14) and female (27) rats treated with the present oPRL regime, dopamine turnover in the TIDA neurons was increased at the first time interval assessed, that is, after 2 and 6 hr of oPRL exposure respectively. In contrast, in the lactating rat, Demarest and co-workers (28) have not found exogenous PRL to increase dopaminergic activity, a finding which could account for the inability of oPRL to suppress basal PRL secretion in the lactating rat.

We observed that oPRL exposure blunts but does not entirely abolish the suckling-induced PRL response. Our suppression is comparable to that reported by Whitworth and co-workers (15) who found a 61% inhibition of the suckling-induced response determined after 30 min of suckling in rats administered oPRL (3 mg/rat) 4 hr prior to the onset of suckling. One interpretation of our results is that a decrease in TIDA neuronal activity mediates the suckling-induced PRL response (17) and that oPRL exposure does not allow this decrease to occur. The fact that the response is not totally abolished may be due to a relative insensitivity of the TIDA neurons to PRL in the lactating rat (28) or to a PRF-mediated component of the response. Alternatively, the suckling-induced response may be solely PRF-mediated and the present results show that the response may be partially overridden by increasing the braking action of the PIH dopamine or by directly inhibiting PRF release.

In marked contrast to the suckling-induced PRL response, the proestrous surge of PRL was totally abolished by oPRL administration. Indeed, in oPRL-treated rats, not only is the rPRL surge abolished in every rat but the afternoon rPRL values, like those values in the morning, are further suppressed below the morning baseline levels in diluent-treated rats. This inhibition may be due to a direct, feedback action of PRL on the brain, to an indirect brain effect secondary to an action of PRL on the steroid output of the ovary or adrenal gland, or to both. It is probable that the reason the proestrous afternoon PRL values are so thoroughly suppressed is because oPRL exerts effects at both the brain and the ovary. Dopamine turnover is increased and basal rPRL secretion decreased within 6 hours of the initial oPRL injection, that is, at 1500 hr of diestrous Day 1 (27). This evidence of rapid brain autoregulation of PRL secretion could account for the subsequent suppression of the proestrous PRL surge. On the other hand, this oPRL treatment inhibits the preovulatory rise in estradiol (27) which is essential to the occurrence of the proestrous PRL surge (29). Progesterone secretion in these rats also is increased starting on diestrous day 2 (27), an effect observed by others (30–32) and which is indicative of these rats entering into a pseudopregnant state. The altered steroid pattern could account for the complete suppression of the proestrous PRL surge. In any event, the results demonstrate that the mechanism(s) regulating the proestrous surge of PRL is very sensitive to hyperprolactinemia relative to the mechanism(s) regulating the suckling-induced PRL response.

The physiological significance of these negative feedback effects remains an enigma. Blood-borne PRL should have ready access to hypothalamic PIH (dopaminergic) and PRF neurosecretory terminals since the median eminence resides outside the blood–brain-barrier. Prolactin could reach the median eminence via the general circulation or by retrograde flow in portal vessels (33). Indeed, Olivier and co-workers (34) have reported that the prolactin concentration in portal blood delivered to the median eminence is about 4900 ng/ml in the adult male rat. This prolactin, delivered from the anterior pituitary gland to the brain, may perfuse not only the median eminence but also the adjacent arcuate nucleus and retrochiasmatic area via capillaries which branch off of the primary portal plexus and course dorsally and rostrally into these portions of the medial basal hypothalamus (35, 36). Hence the dendrites, cell bodies, axons and terminal boutons of the neurons comprising the tuberoinfundibular dopamine tract may be continuously exposed to microgram concentrations of prolactin. Considering the physiological range of PRL in the peripheral circulation, the oPRL injections administered every 8 hr in this study result in PRL exposure to the animal that may be considered supraphysiological because of the peak levels attained (around 2000 ng/ml), the continuous nature and the duration of the exposure. Only in lactating mammals and in animals with PRL secreting pituitary tumors are the circulating PRL levels anywhere near as high.

The manner in which our exogenously administered PRL superimposes or interacts with the microgram concentrations of PRL thought to be delivered to the brain in the portal blood is at present a matter of speculation.

It is apparent from these and other results that substantial amounts of exogenous PRL need to be administered for a minimum of 3–4 hr to demonstrate the autofeedback suppression of endogenous PRL secretion. A milligram dose of oPRL given 30 min prior to suckling did not affect the response nor did nanogram amounts given 4 hr prior to suckling (15). Similarly, a small dose of oPRL infused into cows for 2.5 hr did not diminish milking-induced PRL release (37). Advis and co-workers (12) blunted the stress-induced PRL response with a milligram oPRL dose administered 4 hr prior to ether stress but not when the same dose was administered with insufficient time for it to have its effect (1 hr) or with enough time for the oPRL to be cleared from the circulation (12 hr). Acute exposure to crude placental lactogen preparations has been ineffective in inhibiting the nocturnal and diurnal PRL surges in pseudopregnant rats (38), the nocturnal surges in pregnant rats (10) and the suckling-induced PRL response (6) probably because of these dose and time constraints. However, 4 days of exposure to crude placental extracts recently has been shown to produce a dose-dependent suppression of basal rPRL release in intact or castrated male rats (8).

Taken together [(14) and the present results], our findings show that PRL release in five instances (i.e., basal secretion in the male, cycling female and lactating female, the proestrous surge and suckling-induced release) is differentially sensitive to the negative feedback action of PRL which suggests that the PIH/PRF mechanism(s) controlling PRL secretion in these various circumstances probably differ.

The authors gratefully acknowledge the expert technical assistance of Jeffrey I. Mechanick and the diligence of our manuscript typist Miss Maria Tate.

1. Meites J, Lu KH, Wuttke W, Welsch CW, Nagasawa H, Quadri SK. Recent studies on functions and control of prolactin secretion in rats. Recent Progr Horm Res **38**:471–526, 1972.
2. Nicoll CS. Aspects of the neural control of prolactin secretion. In: Martini L, Ganong WF, eds. Frontiers in Neuroendocrinology. Oxford Univ Press, Cambridge, pp291–330, 1971.
3. Voogt JL, Ganong WF. *In vitro* evidence against the anterior pituitary as a site of negative feedback of prolactin. Proc Soc Exp Biol Med **147**:795–797, 1974.
4. Nagasawa H, Yanai R, Yamanouchi K. Inhibition of pituitary prolactin secretion by human placental lactogen in rats. J Endocrinol **71**:115–120, 1976.
5. Vician L, Lieberman ME, Gorski J. Evidence that autoregulation of prolactin production does not occur at the pituitary level. Endocrinology **110**:722–726, 1982.
6. Yogev L, Terkel J. Endogenous inhibition of prolactin secretion in pregnant lactating rats. Endocrinology **110**:158–162, 1982.
7. Bybee DE, Nakawatase C, Szabo M, Frohman LA. Inhibitory effects of prolactin on its secretion involve central nervous system dopaminergic mediation. Neuroendocrinology **36**:27–32, 1983.
8. Laherty RF, Budd JM, Srebnik HH. Inhibition of prolactin secretion by rat placental extract. Proc Soc Exp Biol Med **172**:29–33, 1983.
9. Voogt JL, Meites J. Suppression of proestrous and suckling-induced increase in serum prolactin by hypothalamic implant of prolactin. Proc Soc Exp Biol Med **142**:1056–1058, 1973.
10. Voogt JL. Regulation of nocturnal prolactin surges during pregnancy in the rat. Endocrinology **106**:1670–1676, 1980.
11. Dang BT, Voogt JL. Termination of pseudopregnancy following hypothalamic implantation of prolactin. Endocrinology **100**:873–880, 1977.
12. Advis JP, Hall TR, Hodson CA, Mueller GP, Meites J. Temporal relationship and role of dopamine in "short loop" feedback of prolactin. Proc Soc Exp Bio Med **155**:567–570, 1977.
13. Nicholson G, Greeley GH Jr, Humm J, Youngblood WW, Kizer JS. Prolactin in cerebrospinal fluid: A probable site of prolactin autoregulation. Brain Res **190**:447–457, 1980.
14. Selmanoff M. Hyperprolactinemia increases dopamine turnover rates in both medial and lateral aspects of the median eminence. Fed Proc **40**:415, Abstract 1046, 1981.
15. Whitworth NS, Grosvenor CE, Mena F. Autofeedback regulation of prolactin (PRL) secretions: Effect of prolactin before suckling on the subsequent nursing-induced release of PRL in the lactating rat. Endocrinology **108**:1279–1284, 1981.
16. Terkel J, Damassa DA, Sawyer CH. Ultrasonic cries from infant rats stimulate prolactin release in lactating mothers. Horm Behav **12**:95–102, 1979.
17. Selmanoff M, Wise PM. Decreased dopamine turnover in the median eminence in response to suckling in the lactating rat. Brain Res **212**:101–115, 1981.

18. Terkel J, Urbach L. A chronic intravenous cannulation technique adapted for behavioral studies. Horm Behav **5**:141–148, 1974.
19. Niswender GD, Chen CL, Midgley AR, Meites J, Ellis S. Radioimmunoassay for rat prolactin. Proc Soc Exp Biol Med **130**:793–797, 1969.
20. Selmanoff M, Selmanoff C. The role of pup age, estradiol-17β and pituitary responsiveness in the differences in the suckling induced prolactin response during early and late lactation. Biol Reprod **29**:400–411, 1983.
21. Winer BJ. Statistical Principles in Experimental Design. New York, McGraw-Hill, 2nd ed, pp21–26, 149–167, 191–196, 208, and 518–539, 1971.
22. Weiner RI, Cronin MJ, Cheung CY, Annunziato L, Faure N, Goldsmith PC. Dopamine: A prolactin inhibitory hormone. In: Müller EE, Agnoli A, eds. Neuroendocrine Correlates in Neurology and Psychiatry. Amsterdam, Elsevier, pp41–55, 1979.
23. Neill JD. Neuroendocrine regulation of prolactin secretion. In: Martini L, Ganong WF, eds. Frontiers in Neuroendocrinology. New York, Raven Press, Vol 6:pp129–156, 1980.
24. Weiner RI, Bethea CL. Hypothalamic control of prolactin secretion. In: Jaffe RB, ed. Prolactin. Amsterdam, Elsevier, pp19–56, 1981.
25. Moore KE, Demarest KT, Johnston CA. Influence of prolactin on dopaminergic neuronal systems in the hypothalamus. Fed Proc **39**:2912–2916, 1980.
26. Moore KE, Demarest KT. Tuberoinfundibular and tuberohypophyseal dopaminergic neurons. In: Ganong WF, Martini L, eds. Frontiers in Neuroendocrinology. New York, Raven Press, Vol 7:pp161–190, 1982.
27. Wise PM. Effect of hyperprolactinemia on estrous cyclicity, plasma LH, estradiol and progesterone and catecholamine turnover rates (CA T/O) in microdissected brain areas. Program of the 65th Annual Meeting of The Endocrine Society, San Antonio, Texas, p167, Abstract 347, 1983.
28. Demarest KT, McKay DW, Riegle GD, Moore KE. Biochemical indices of tuberoinfundibular dopaminergic neuronal activity during lactation: A lack of response to prolactin. Neuroendocrinology **36**:130–137, 1983.
29. Neill JD, Freeman ME, Tillson SA. Control of the proestrus surge of prolactin and luteinizing hormone secretion by estrogens in the rat. Endocrinology **89**:1448–1453, 1971.
30. Nikitovitch-Winer M, Everett JW. Comparative study of luteotropin secretion by hypophysial autotransplants in the rat. Effects of site and stages of the estrus cycle. Endocrinology **62**:522–532, 1958.
31. Schwartz NB. A model for the regulation of ovulation in the rat. Recent Progr Horm Res **25**:1–55, 1969.
32. Smith MS, McLean BK, Neill JD. Prolactin: The initial luteotropic stimulus of pseudopregnancy in the rat. Endocrinology **98**:1370–1377, 1976.
33. Mezey E, Palkovits M. Two-way transport in the hypothalamohypophyseal system. In: Ganong WF, Martini L. Frontiers in Neuroendocrinology. New York, Raven Press, Vol 7:pp1–30, 1982.
34. Oliver C, Mical RS, Porter JC. Hypothalamic-pituitary vasculature: Evidence for retrograde blood flow in the pituitary stalk. Endocrinology **101**:598–604, 1977.
35. Ambach G, Palkovits M, Szentágothai J. Blood supply of the rat hypothalamus. IV. Retrochiasmatic area, median eminence, arcuate nucleus. Acta Morphol Acad Sci Hung **24**:93–118, 1976.
36. Ambach G, Palkovits M. The blood supply of the hypothalamus in the rat. In: Morgane PJ, Panksepp J, eds. Handbook of the Hypothalamus, Vol 1, Anatomy of the Hypothalamus. New York, Dekker, pp 267–378, 1979.
37. Tucker HA, Convey EM, Koprowski JA. Milking-induced release of endogenous prolactin in cows infused with exogenous prolactin. Proc Soc Exp Biol Med **142**:72–75, 1973.
38. Smith MS, Neill JD. Termination at midpregnancy of the two daily surges of plasma prolactin initiated by mating in the rat. Endocrinology **98**:696–701, 1976.

Received September 2, 1983. P.S.E.B.M. 1984, Vol. 175.
Accepted December 1, 1983.