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Evidence of Prolactin Short Loop Feedback in the Postpartum Lactating Rat (41668)

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Abstract. Serum prolactin was studied in lactating and nonlactating rats after restraint stress, chronic estradiol benzoate treatment, and acute L-tryptophan treatment. As expected, these treatments evoked increases in serum prolactin in nonlactating rats. In lactating rats these treatments did not increase basal serum prolactin when litters were removed for 4 hr. Daily estradiol benzoate treatment increased pituitary gland prolactin content after 14 days, but there was no effect on basal serum prolactin. Daily estradiol treatment did not augment prolactin release in response to 30 min of suckling. It is suggested that the short loop feedback by prolactin (i.e., prolactin inhibiting its own secretion) makes the lactating rat refractory to these stimuli of prolactin secretion.

Previous reports suggest the existence of a short loop feedback of prolactin, in which prolactin acts on the hypothalamus to inhibit its own secretion. Injections of prolactin have been shown to increase dopamine (DA) turnover in the hypothalamus, while causing a decrease in its own secretion by this mechanism (1). Associated with the increased hypothalamic DA turnover that was evoked by prolactin are reduced LH secretion (2) and reduced prolactin release during restraint stress (3). Implants of prolactin in the median eminence (ME) also suppress prolactin release from the pituitary since pseudopregnancy (4) and lactation are terminated (5). Median eminence prolactin implants also prevent increases in pituitary prolactin levels in response to estradiol benzoate (EB) treatment (6). These reports indicate that a short loop feedback of prolactin exists and suggest its mechanism involves increased tuberoinfundibular dopamine system activity. During lactation hypothalamic dopamine turnover is increased (7) suggesting that the short loop feedback of prolactin might be exerted between suckling periods. Recent data also suggest that placental lactogen may prevent prolactin release in pregnant rats in response to estrogen treatment (8).

In the present study, changes in serum prolactin levels in response to restraint stress and EB injection, and changes in serum prolactin levels in response to L-tryptophan were compared in lactating rats removed from their litters to nonlactating rats. Since prolactin release by these stimuli was altered in lactating rats and similar alterations of prolactin were noted in nonlactating rats treated with prolactin, it is suggested that the short loop feedback occurs to regulate prolactin release during lactation.

Materials and Methods. *Animals.* Sprague–Dawley rats were used in this study. The rats were housed in an artificially lighted (lights on 0700–2100 hr) temperature-controlled (25 ± 1°C) animal room. Purina Rat Chow (Ralston Purina Co., St. Louis, Mo.) and water were provided *ad libitum.*

Nonlactating rats (250–300 g) were housed in groups of four. Rats used in lactation experiments were bred in group cages and were placed in individual plastic cages when pregnancy was apparent. The day of parturition was designated Day 0. On Day 1, postpartum, the litter size was adjusted to six pups.

Prolactin release after restraint stress. Prolactin release in response to 15 min of physical restraint was studied: in male rats 4 hr after sc injection of 1 mg ovine prolactin (NIH-P-S11) or 1 mg bovine serum albumin (BSA), in diestrous cycling rats, and in lactating rats 4 hr after litter removal on Day 14 postpartum. The rats were stressed 15 min as described by Advis *et al.* (3) and were decapitated at the end of the stress period. The trunk blood was collected for RIA of rat prolactin.

Prolactin release after L-tryptophan. Changes in serum prolactin were studied after ip injection of L-tryptophan (100 mg/kg) (Sigma Chemical Co., St. Louis, Mo.) in an aqueous 1% methyl cellulose vehicle, or after injections of vehicle in: male rats 4 hr after sc injection of 1 mg ovine prolactin (NIH-P-

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S11) or 1 mg BSA, and in lactating dams 4 hr after pup removal on Day 14 postpartum. The rats were killed by decapitation 30 min after L-tryptophan injection and the trunk blood was collected for RIA of rat prolactin.

Prolactin release after EB treatment. The effect of daily sc injection of 1 μg EB (Nutritional Biochemical Co., Cleveland, Ohio) or the corn oil vehicle was studied in ovariectomized nonlactating, and in both intact lactating, and lactating rats ovariectomized on Day 1 postpartum, eight rats were used per experimental group. Injection of EB or corn oil was started on Day 1 postpartum or on the day of ovariectomy of nonlactating rats. Blood samples of 1.0 ml were collected by orbital sinus puncture under light ether anesthesia 3, 6, 10, and 14 days after the start of EB treatment. In the lactating rats two blood samples were collected each day, the first sample was taken 4 hr after the pups were separated from the dams at 1200 hr. At 1530 hr the pups were returned to the dams and the second blood sample was collected at 1600 hr after 30 min nursing. In nonlactating rats blood samples were collected at 1600 hr. Administration of estrogens to lactating rats can impair milk production (9). Ovariectomy, however, reduces the inhibitory effect of exogenous estrogens (9). In the present study, litter weights were taken daily except on days 3, 6, 10, and 14 to monitor potential inhibitory effects of estrogen treatment. Average litter weights on Days 2, 5, 9, and 13 were computed and are presented in Table I.

In a second study EB (1 μg/day, sc) or the corn oil vehicle were administered to lactating rats ovariectomized on Day 1 postpartum for 14 days. The dams were separated from their litters (six pups each) for 4 hr and were killed by decapitation. The trunk blood and anterior pituitary glands were collected for hormone measurement.

Assays. Serum was separated from the blood and stored at −20°C until the RIAs were performed. Anterior pituitary glands were weighed after removal, and were stored at −20°C until they were homogenized. Pituitary tissue was homogenized in phosphate-buffered saline (PBS), pH 7, by sonification. The pituitary homogenates were then diluted with PBS to concentrations suitable for RIA.

Prolactin was measured using the method of Niswender *et al.* (10). Serum prolactin concentrations were expressed in terms of NIAMDD rat PRL-RP-1. Prolactin values of EB treated rats were compared at each day against the respective control values by the Student's *t* test. Suckling vs nonsuckling was also compared at each day by *t* test. In the restraint and L-tryptophan experiments hormone levels were compared by one-way analysis of variance. When treatment mean squares were significant, means were compared by the least significant difference method (11).

Results. The effect of 15 min physical restraint on serum prolactin levels in lactating and nonlactating rats is shown in Fig. 1. Pretreatment of male rats with 1 mg ovine prolactin did not significantly reduce the release of prolactin by restraint stress. In the BSA-treated males prolactin levels were increased fivefold by restraint but were increased threefold after restraint in ovine prolactin treated rats ($P < 0.10$). In diestrous female rats restraint stress increased serum prolactin levels significantly ($P < 0.05$), but restraint stress did not release prolactin in lactating rats.

Figure 2 shows the effect of L-tryptophan injection on serum prolactin levels in lactating and nonlactating rats. Tryptophan injection increased serum prolactin in male rats pre-

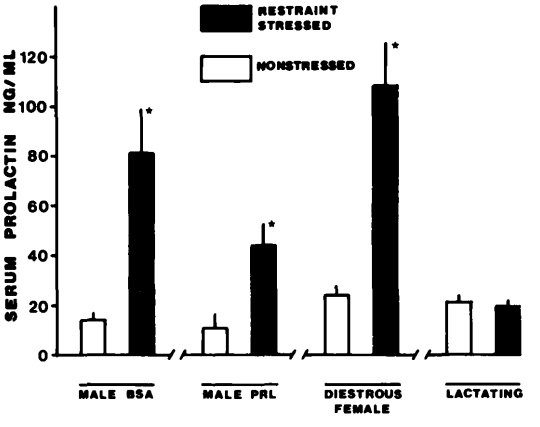

FIG. 1. Effect of 15 min restraint stress on serum prolactin levels in: male rats treated 4 hr prior to restraint with 1 mg ovine prolactin (PRL), or 1 mg bovine serum albumin (BSA), and PRL levels in diestrous female and lactating rats removed 4 hr from their litter (*denotes significant increase over the respective nonstressed controls, $P < 0.01$, $N = 10$, mean ± SEM).

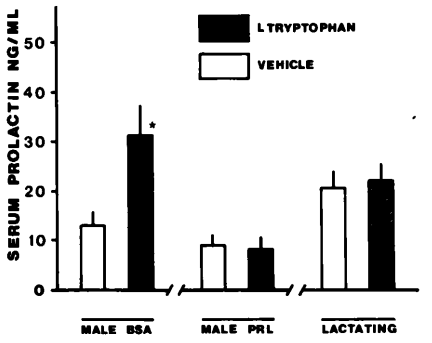

FIG. 2. Serum prolactin levels 30 min after injection of 100 mg L-tryptophan/kg body wt or vehicle in: male rats treated 4 hr prior to tryptophan injection with 1 mg ovine prolactin (PRL) or 1 mg bovine serum albumin (BSA), and lactating rats removed from their litters 4 hr before tryptophan injection (*denotes significant increase over controls, $P < 0.01$, $N = 10$, mean ± SEM).

treated with BSA ($P < 0.05$). In contrast L-tryptophan treatment had no effect on serum prolactin in male rats pretreated with ovine prolactin. In lactating rats separated from their litters for 4 hr, L-tryptophan treatment had no effect on serum prolactin.

The effects of EB treatment on serum prolactin levels in lactating and nonlactating rats are shown in Fig. 3. Panel A shows prolactin in ovariectomized nonlactating rats. Daily EB treatment resulted in a significant increase in serum prolactin on Days 3, 6, 10, and 14 ($P < 0.01$). In intact lactating rats (panel B) daily EB treatment had no effect on basal prolactin, litters removed 4 hr (squares). EB treatment of intact rats did not augment prolactin release induced by 30 min suckling (circles). As expected suckling resulted in a significant increase in serum prolactin ($P < 0.01$). In ovariectomized lactating rats, panel C, neither basal serum prolactin, squares, or serum prolactin levels after 30 min suckling, circles, were effected by daily EB treatment. Prolactin levels were increased significantly ($P < 0.01$) by 30 min of suckling. Table I shows litter weights, on the days before the nursing trials. As expected EB treatment of the intact rats reduced litter growth in the latter part of the study. In ovariectomized lactating rats EB treatment had no effect on litter growth. Table II shows that EB treatment of lactating rats for 14 days did result in a significant increase in pituitary weight and pituitary prolactin content. There was, however, no effect of EB treatment on serum prolactin levels when pups and dams were separated 4 hr.

Discussion. The present investigations indicate that during intervals between nursing, lactating rats are refractory to several stimuli which promote prolactin release in nonlactating rats. Restraint stress, L-tryptophan treatment, and EB treatment were ineffective in increasing serum prolactin concentrations in dams removed from their litters for 4 hr. This suggests that the prolactin released when the rats were nursing prevented additional prolactin release by the aforementioned stimuli through short loop feedback. These results suggest that this feedback may result from endogenously secreted prolactin.

Reductions of prolactin release in response to L-tryptophan treatment was observed in nonlactating rats given ovine prolactin. Advis *et al.* (3) have previously reported that pretreatment of male rats with 1 mg of ovine prolactin 4 hr prior to restraint stress, inhibits the stress-induced release of rat prolactin. In the present study, prolactin release in ovine prolactin-treated male rats subjected to restraint stress was not completely attenuated.

In the lactation studies the dams and pups were separated for 4 hr to match the ovine prolactin injection protocol (3) and to allow prolactin to decrease to basal levels. In this model the lactating rats were less responsive to stress and L-tryptophan than the nonlactating prolactin-treated rats. These observations and earlier reports of reduced prolactin release in stressed lactating rats (11, 12) suggests that apart from suckling responses prolactin release is attenuated between nursing periods.

In lactating rats removed from their litters or when suckling was allowed it was observed that serum prolactin levels were not increased by estradiol benzoate treatment. Estradiol treatment of lactating rats did increase pituitary gland weight and pituitary prolactin content as was reported by Meites and Turner (13). Welsch *et al.* (6) have reported that median eminence implants of prolactin could prevent increases in pituitary prolactin content by low doses of estrogen. However, high doses of estrogens were able to elicit increases in pituitary prolactin content. The descrepancy between this report (6) and the present data

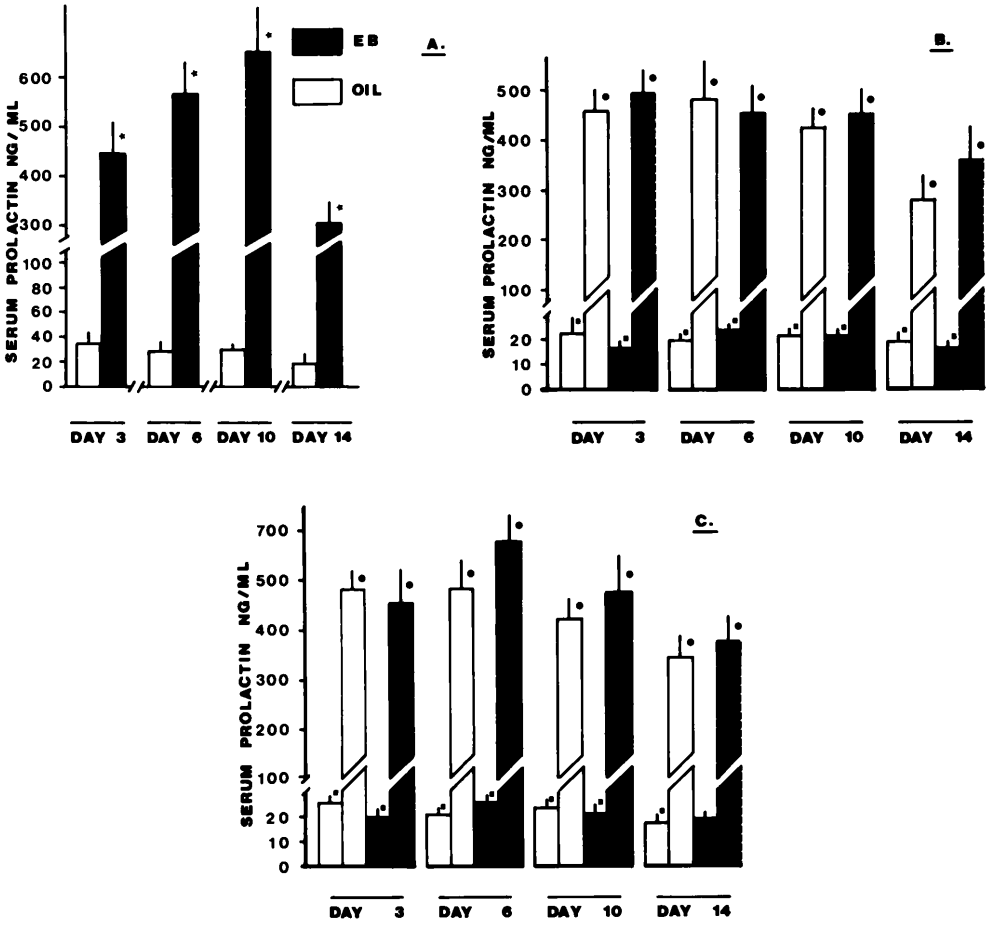

FIG. 3. Effects of daily estradiol benzoate treatment (EB 1 μg/day) in: (A) ovariectomized nonlactating rats, in (B) intact lactating rats, and in (C) ovariectomized lactating rats. Samples were collected 4 hr after litter removal, solid squares, and after 30 min suckling, solid circles (*denotes significant difference from corn oil control, and solid circles denote significant increase after suckling $P < 0.01$, $N = 8$, mean ± SEM).

suggests prolactin implants cause a more permanent activation of the short loop feedback system than prolactin release by suckling. This conclusion is supported by reports indicating that prolactin implants in rat median eminence terminate lactation (5) and pseudo-

TABLE I. LITTER WEIGHTS (SIX PUPS PER LITTER) OF INTACT AND OVARIECTOMIZED LACTATING RATS TREATED DAILY WITH OIL VEHICLE OR 1 μg ESTRADIOL BENZOATE (EB)

Treatment	Days of lactation			
	2	5	8	13
Intact oil	50 ± 2.0[a]	77 ± 4.6	108 ± 4.9	165 ± 6.7
Intact EB	48 ± 2.0	77 ± 2.9	98 ± 2.7	118 ± 6.7*
Ovariectomized oil	46 ± 2.5	72 ± 1.9	106 ± 3.8	176 ± 3.1
Ovariectomized EB	46 ± 1.0	66 ± 2.9	102 ± 3.6	162 ± 4.1

[a] Mean ± standard error of mean (N = eight per group).
* Significant difference from intact control and ovariectomized rats ($P < 0.05$).

TABLE II. PITUITARY AND SERUM PROLACTIN LEVELS OF LACTATING RATS TREATED DAILY WITH OIL VEHICLE OR 1 μg ESTRADIOL BENZOATE (EB) FOR 14 DAYS[a]

Treatment	N	Prolactin (μg/mg AP)	Prolactin (μg/AP)	Prolactin (ng/ml of sera)
1 μg EB	16	3.9 ± 0.31**	48.1 ± 6.8**	12.6 ± 1.9
Vehicle	13	2.6 ± 0.22	23.8 ± 2.2	11.5 ± 3.8

[a] Samples were collected from decapitated animals removed from their litters 4 hr.
** Significant difference from controls, $P < 0.01$.

pregnancy (4). Though the inhibition of lactation by median eminence prolactin implants is probably a result of short loop feedback, the operation of the short loop feedback system during lactation is not excluded by these reports.

It is generally believed that prolactin acts through the tuberoinfundibular dopamine system to exert short loop feedback. Though factors yet unidentified, besides prolactin, may increase tuberoinfundibular dopamine activity, many reports suggest that tuberoinfundibular dopamine activity is directly related to prolactin release. For example, some drugs and hormones that increase prolactin secretion increase the activity of the tuberoinfundibular dopamine system subsequent to the release of prolactin (15, 16). Since the effect of these agents on dopamine turnover can be prevented by hypophysectomy the turnover effects may be mediated by prolactin (16, 17). Furthermore, Hökfelt and Fuxe *et al.* (17) have reported that hypophysectomy can reduce tuberoinfundibular dopamine activity.

To explain the present observations it is suggested that the increased release of prolactin in lactating rats increases inhibitory short loop feedback activity. This suggestion of increased inhibitory short loop feedback activity is made on the basis of the following reports. (i) Tuberoinfundibular dopamine system activity is increased in lactating rats (1). (ii) Prolactin release in response to suckling may stimulate tuberoinfundibular dopamine activity during lactation (18).

The increased short loop feedback of prolactin in lactating rats may prevent prolactin release in response to stressful stimuli and may prevent prolactin release in intervals between nursing. Short loop feedback by endogenous prolactin does not prevent suckling-induced prolactin release. Prolactin can be administered to lactating rats and short loop feedback can be augmented to reduce suckling-induced prolactin release (13), or inhibit lactation (5). The present observations indicate that increased short loop feedback activity reduces the responsiveness of the lactating rats to estrogens and L-tryptophan. If stimulus strength were increased, these agents would probably evoke prolactin release in the lactating rats. Such a stimulus strength-response relationship has been reported in the case of placental lactogen inhibition of pituitary prolactin release (8) and when pituitary prolactin response to estrogen is inhibited by median eminence prolactin implants (6).

These data and prior reports suggest that activity of the inhibitory short loop feedback system regulate the extent of tonic inhibition of prolactin release.

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