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Inhibition of Suckling-Induced Prolactin Release by Estrogen in Ovariectomized Lactating Rats: Bioassay versus Radioimmunoassay[1] (41620)

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Abstract. Plasma levels of prolactin (PRL) were determined before and during suckling in intact and ovariectomized (OVX) lactating rats treated 5 days earlier with a single sc injection of 50 μg polyestradiol phosphate (PEP) or saline. Radioimmunoassay (RIA) and the Nb_2 lymphoma cell bioassay (BA) were used to measure plasma PRL. Pituitary PRL concentrations were also determined by the two assays. In addition, lactational performance was estimated by measuring litter weight gain before and after estrogen treatment. Lactation was inhibited by PEP in intact rats; however, PEP did not alter plasma or pituitary levels of PRL in this group. Lactation was not inhibited by estrogen in OVX rats, but plasma PRL levels were significantly reduced at 10, 30, and 45 min of suckling. In addition, there was no evidence of an estrogen-induced afternoon prolactin surge in either PEP-treated group. The lactational inhibition in the intact, PEP-treated rats was probably due to combined effects of estrogen and progesterone. Plasma progesterone concentration was 64 ± 8 ng/ml in the intact PEP-treated group compared to 3 ± 1 ng/ml in the OVX, PEP-treated rats. The exact mechanism for the supression or delay in suckling-induced plasma PRL in the OVX, PEP-treated females remains unresolved but it was not due to an increase in suckling-induced corticosterone levels. The qualitative changes in plasma PRL during suckling in the various groups detected by RIA were also detected by the BA. However, there were slight quantitative differences between the two assays. When plasma PRL was low (nonsuckled states) the BA/RIA ratio was less than that observed when the plasma PRL was high (during suckling), i.e., more bioactive PRL was apparently released by suckling. However, these differences between RIA and BA may have been due in part to the fact that very low or very high levels of prolactin approached the minimal and maximal assay limits of the RIA but not the BA indicating that care be exercised when making such assay comparisons.

The inhibition of lactation in rats and other mammals including man by high doses of estrogens was documented over 40 years ago [see (1) for review]. In the rat this inhibition requires the presence of the ovaries (2) and the ovarian contribution appears to be progesterone since this latter steroid permits suppression of lactation by estrogen in ovariectomized rats [see (2) for references]. The exact mechanism for estrogen suppression of lactation has not been completely resolved. Possible mechanisms include an estrogen-induced increase in cell proliferation (3) and reduction in PRL receptors in the mammary gland (4). Another possibility is an estrogen-induced reduction in PRL secretion. This latter mechanism has not been considered likely since early reports indicated that PRL secretion is enhanced by estrogen as evidenced by increased pituitary levels of PRL in estrogen-treated lactating females (1) and because the synthetic estrogen, diethylstibesterol, has been shown to stimulate basal secretion of PRL in postpartum women (5). However, to our knowledge no data have been published on the effects of estrogen on suckling-induced PRL secretion in any species. To do so in the rat was the objective of the present study. In addition, we set out to compare the levels of pituitary and plasma PRL in lactating rats using radioimmunoassay and the newly developed Nb_2 lymphoma cell bioassay.

Materials and Methods. *Animal procedures.* Twenty mature Sprague–Dawley female rats (Harlan–Sprague-Dawley, Inc., Indianapolis, Ind.) were co-habitated with male rats until they were determined to be pregnant by physical examination at which time they were housed in individual polycarbonate maturnity cages in an environmentally con-

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trolled room (lights on 0600–2000 hr, temperature 23°C, relative humidity 50%). On the day after parturition (Day 2), the litters were adjusted to eight pups which were weighed and all animals were fitted with an indwelling right atrial catheter which was exteriorized at the back of the neck. One-half of the animals were also ovariectomized (OVX) on Day 2 of lactation. On Day 5 of lactation, half of the intact and OVX rats were injected sc with 50 μg polyestradiol phosphate (PEP, Estradurin, Ayerst Laboratories) in 0.9% NaCl, whereas the other intact and OVX rats were injected with 0.9% NaCl. Litters were also weighed on Day 5. The rats were left undisturbed until Day 10 of lactation when, at 0900 hr, the litters were removed and weighed and an extension was added to the exteriorized portion of the atrial catheters for blood sampling. At 1100 hr a 0.4-ml blood sample was withdrawn from each animal and an equivalent volume of warm 0.9% NaCl returned via the catheter. This and all subsequent blood samples were immediately mixed with an equal volume of phosphate-buffered saline (PBS) containing 10 U heparin/ml and the diluted blood sample stored in an ice bath until the plasma was separated by centrifugation and then frozen at −20°C until assayed. At 1500 hr a second 0.4-ml blood sample was withdrawn and the litter returned to the dam. Additional blood samples were withdrawn at 10, 30, 45, and 60 min after suckling was initiated. After withdrawal of each of these samples, 0.9% NaCl was returned to the animal as described above. At the completion of suckling experiment, the catheter extension was removed and the animals left undisturbed until Day 12 of lactation. At 0900 hr on Day 12 each litter was removed, weighed, and sacrificed. At 1200 hr each dam was sacrificed by decapitation following a 1-min exposure to ether and trunk blood was collected. This method has been shown not to alter pituitary PRL content (6). The anterior pituitary and uterus were removed and weighed and a vaginal smear was also taken. The anterior pituitary was bisected and each half was weighed and immediately homogenized in PBS containing 0.1% bovine serum albumin at either pH 7.6 or pH 10.6. The homogenates were centrifuged at 1500*g* for 20 min and the supernatants were collected and frozen at −20°C until assayed for PRL.

Assay procedures. All plasma samples and pituitary homogenates were assayed for prolactin (PRL) at two dilutions in duplicate by double antibody radioimmunoassay (RIA) (7) in which NIAMDD-RP-I_5, iodinated using a lactoperoxidase–glucose oxidase method (8), was used as tracer. The standard was NIAMDD-RP-1 (11 IU/mg). These same samples were also assayed for PRL by the rat Nb_2 lymphoma cell bioassay (BA) (9, 10) using NIAMDD-RP-1 as standard.

Plasma obtained from trunk blood at decapitation on Day 12 of lactation was assayed for progesterone at two dilutions in duplicate by the RIA method of Surve *et al.* (11). Plasma samples obtained from blood samples taken immediately before and at 10 and 30 min after suckling were assayed for corticosterone using a CBG-binding assay (12).

Lactational performance was estimated by measuring total litter weight gain during the 4 days prior to estrogen or vehicle treatment and during the 7-day period after administration of estrogen or vehicle.

Statistical analyses. Analysis of variance, Tukey's *hsd* multiple comparison test and paired *t* test (13) were used to assess statistical significance between treatment means. Differences were considered significant when $P \leq 0.05$.

Results. Litter weight gain (Table I) showed a significant decrease after estrogen treatment in only the sham OVX, PEP-treated group and then only after 10 days of exposure to PEP.

Anterior pituitary weights were not altered by the estrogen treatment in intact or ovariectomized females (Table II), however, uterine weights reflected the ovarian steroid status of each group. Vaginal smears obtained at decapitation also indicated the biological proportions of estrogen and progesterone. The latter steroid was measured and the results (Table II) showed the intact groups to be significantly higher than the respective OVX groups.

Pituitary PRL concentrations measured at Day 12 of lactation were not altered significantly by estrogen treatment, although at a homogenization pH of 10.6 there was consistently more PRL measured in PEP-treated

TABLE I. TOTAL WEIGHT GAIN OF EIGHT-PUP LITTERS BEFORE (DAYS 2–5) AND AFTER (DAYS 5–10, DAYS 10–12) ESTROGEN (PEP) TREATMENT OF INTACT (SHAM OVX) AND OVARIECTOMIZED (OVX) LACTATING RATS

Group	N	Total litter weight gain (g)		
		Days 2–5	Days 5–10	Days 10–12
Sham OVX + saline	4	33.5 ± 1.8[a]	66.3 ± 5.0[a]	24.3 ± 2.4[a,b]
Sham OVX + 50 μg PEP	4	33.5 ± 2.7[a]	62.8 ± 4.8[a]	14.8 ± 4.5[b]
OVX + saline	4	27.3 ± 2.9[a]	70.3 ± 6.2[a]	26.3 ± 2.4[a]
OVX + 50 μg PEP	5	27.2 ± 3.2[a]	64.6 ± 2.5[a]	26.6 ± 1.7[a]

[a,b] Among groups, means with different superscripts are statistically ($P = 0.05$) different from each other.

groups (Table III). In addition, there were no differences in pituitary PRL between radioimmunoassay (RIA) and bioassay (BA). Prolactin levels of plasma obtained at decapitation following a 3-hour isolation were low and the BA/RIA ratios were less than one. There were no differences between the plasma levels within each group as measured by BA and RIA ($P > 0.05$), however, analysis of data combined from all groups indicated significantly ($P < 0.01$) less PRL was detected in plasma by the BA than by RIA.

On Day 10 of lactation, when blood samples were obtained via indwelling atrial catheters, plasma levels of PRL, as measured by RIA, were low in animals not suckled for 2 (1100 hr) or 6 hr (1500 hr) (Fig. 1A). In addition, a comparison of PRL levels at 1100 and 1500 hr in nonsuckled rats showed no estrogen-induced afternoon surge in the intact or OVX rats treated with PEP. Continuous suckling initiated at 1500 hr induced a prominent release of PRL in all groups except the OVX, PEP-treated females which released significantly less PRL at 10, 30, and 45 min of suckling ($P < 0.001$) but not at 60 min of suckling. Similar results were obtained by BA (Fig. 1B) except that the absolute levels at 30, 45, and 60 min of suckling were 30–50% higher than those obtained by RIA. The BA/RIA ratios for PRL levels before and after suckling are shown in Table IV, as are the levels of significance from the paired t test comparison of the original PRL levels measured by RIA and BA. Prior to suckling ratios were less than one and during suckling, ratios were greater than one. When values in individual groups were analyzed, there was only one group that showed a significant difference between RIA and BA prior to suckling. After suckling, there was a significant difference between RIA and BA at one or more of the suckling intervals in each group. When the data from all animals were combined into before and after suckling groups, the BA detected significantly less PRL ($P < 0.01$) than RIA before suckling (BA/RIA = 0.57 ± 0.14) and significantly more ($P < 0.001$) than RIA after suckling (BA/RIA = 1.34 ± 0.04).

It has been reported that high plasma corticosterone suppressed PRL release (14). To determine if this could have caused the in-

TABLE II. ORGAN WEIGHTS, VAGINAL SMEARS, AND PLASMA PROGESTERONE LEVELS IN INTACT (SHAM OVX) AND OVARIECTOMIZED (OVX) LACTATING RATS TREATED 7 DAYS EARLIER WITH SALINE OR POLYESTRADIOL PHOSPHATE (PEP)

Group	N	Organ weight (mg/100 g body wt)		Vaginal smear	Plasma progesterone (ng/ml)
		Pituitary	Uterus		
Sham OVX + saline	4	3.9 ± 0.2[a]	93.3 ± 6.6[b]	Diestrus	61 ± 22[a]
Sham OVX + 50 μg PEP	4	4.2 ± 0.2[a]	186.3 ± 11.9[d]	Diestrus	64 ± 8[a]
OVX + saline	4	4.2 ± 0.1[a]	70.5 ± 3.4[a]	Diestrus	2 ± 0.1[b]
OVX + 50 μg PEP	5	4.2 ± 0.2[a]	148.6 ± 7.9[c]	Estrus	3 ± 1[b]

[a,b,c,d] Among groups, means with different superscripts are statistically ($P = 0.05$) different from each other.

TABLE III. PITUITARY (AP) AND PLASMA LEVELS OF PROLACTIN IN INTACT (SHAM OVX) AND OVARIECTOMIZED (OVX) LACTATING RATS TREATED 7 DAYS EARLIER WITH SALINE OR POLYESTRADIOL PHOSPHATE (PEP)[a]

		AP prolactin								
		pH 7.6[b]			pH 10.6[b]			Plasma prolactin		
Group	N	RIA	BA	BA/RIA[c]	RIA	BA	BA/RIA	RIA	BA	BA/RIA
		(μg/mg)			(μg/mg)			(ng/ml)		
Sham OVX + saline	4	4.8 ± 0.7	5.0 ± 0.8	1.04 ± 0.05	13.3 ± 1.1	13.0 ± 0.7	0.98 ± 0.05	18 ± 3	8 ± 2	0.50 ± 0.22
Sham OVX + 50 μg PEP	4	5.7 ± 0.6	7.3 ± 1.6	1.27 ± 0.16	17.7 ± 1.5	17.8 ± 1.9	1.01 ± 0.05	20 ± 4	8 ± 2	0.46 ± 0.14
OVX + saline	4	6.5 ± 1.5	8.2 ± 2.3	1.15 ± 0.10	17.2 ± 2.1	17.2 ± 2.1	1.01 ± 0.06	26 ± 3	16 ± 3	0.64 ± 0.23
OVX + 50 μg PEP	5	6.1 ± 1.3	6.7 ± 1.5	1.11 ± 0.04	18.2 ± 1.5	20.3 ± 1.4	1.12 ± 0.06	15 ± 3	9 ± 2	0.60 ± 0.12

[a] Rats were decapitated at 1200 hr on Day 12 of lactation after a 3-h isolation from their litters.

[b] Anterior pituitaries were bisected, weighed, and each half homogenized in 2.0 ml phosphate-buffered saline containing 0.1% bovine serum albumin. The pH of the homogenization buffers was adjusted to 7.6 or 10.6 just prior to use.

[c] Mean ± SEM of individual BA/RIA ratios.

hibition of PRL release to suckling in OVX, PEP-treated rats, plasma corticosterone levels were determined and the results shown in Fig. 2. Plasma corticosterone levels increased during suckling in each group and these increases were statistically significant in all groups except the OVX, PEP-treated animals.

Discussion. The present study confirms very

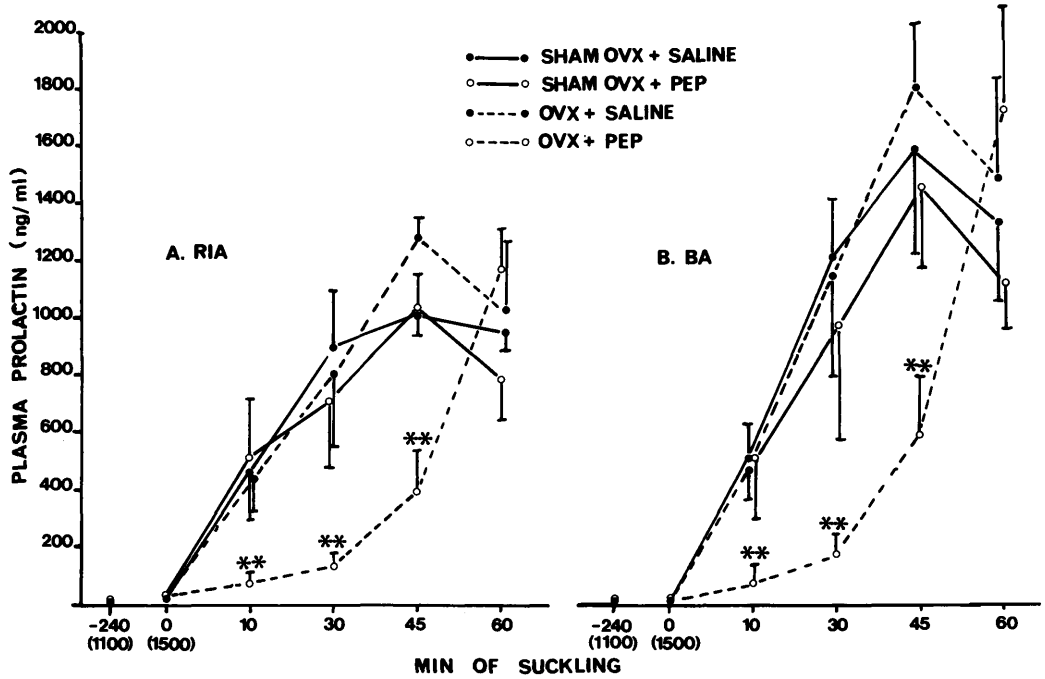

FIG. 1. Plasma levels of prolactin (mean ± SEM) measured by (A) radioimmunoassay (RIA) or (B) Nb_2 lymphoma cell bioassay (BA) before and after suckling in intact (Sham OVX) and ovariectomized (OVX) rats on Day 10 of lactation. Litters were standardized to eight pups on Day 2 and were isolated from their dams for 6 hr prior to suckling. Blood samples were withdrawn via indwelling intraatrial catheters in freely moving rats. PEP = 50 μg polyestradiol phosphate administered sc on Day 5 of lactation. $^{**}P = 0.01$ among groups within each time period.

TABLE IV. BIOASSAY (BA) AND THE RADIOIMMUNOASSAY (RIA) RATIOS (BA/RIA) OF PLASMA PROLACTIN BEFORE AND AFTER SUCKLING IN INTACT (SHAM OVX) AND OVARIECTOMIZED (OVX) LACTATING RATS TREATED 5 DAYS EARLIER WITH SALINE OR POLYESTRADIOL PHOSPHATE (PEP)

		Minutes of suckling					
Group	*N*	−240	0	10	30	45	60
Sham OVX + saline	4	0.45 ± 0.29[a] (NS)[c]	0.25 ± 0.14 (P = 0.05)[b]	1.26 ± 0.19 (NS)	1.67 ± 0.31 (P = 0.05)	1.51 ± 0.25 (NS)	1.39 ± 0.27 (NS)
Sham OVX + 50 μg PEP	4	0.85 ± 0.50 (NS)	0.87 ± 0.54 (NS)	1.03 ± 0.15 (NS)	1.25 ± 0.14 (NS)	1.39 ± 0.13 (NS)	1.45 ± 0.07 (P = 0.01)
OVX + saline	4	0.53 ± 0.36 (NS)	0.80 ± 0.56 (NS)	1.18 ± 0.16 (NS)	1.42 ± 0.06 (P = 0.05)	1.42 ± 0.17 (NS)	1.35 ± 0.13 (NS)
OVX + 50 μg PEP	5	0.31 ± 0.31 (NS)	0.55 ± 0.55 (NS)	0.86 ± 0.22 (NS)	1.36 ± 0.07 (NS)	1.45 ± 0.18 (P = 0.05)	1.44 ± 0.16 (P = 0.05)
Combined groups		0.57 ± 0.14 (P = 0.01)			1.34 ± 0.04 (P = 0.001)		

[a] Mean ± SEM of individual BA/RIA ratios.
[b] Significance levels determined by paired t test of original prolactin levels measured by RIA and BA are in parentheses.
[c] NS = not significant, $P > 0.05$.

early reports that estrogen treatment of intact lactating rats suppressed lactation as evidenced by decreased litter size (1, 3). We have also confirmed that ovariectomy precludes the inhibition of lactation (2). Our report extends the earlier studies in that we have shown that estrogen has no detrimental effects on suckling-induced PRL release in rats with suppressed lactation. Neither were there any alterations in pituitary PRL concentration, pituitary weight, or suckling-induced corticosterone release in rats with suppressed lac-

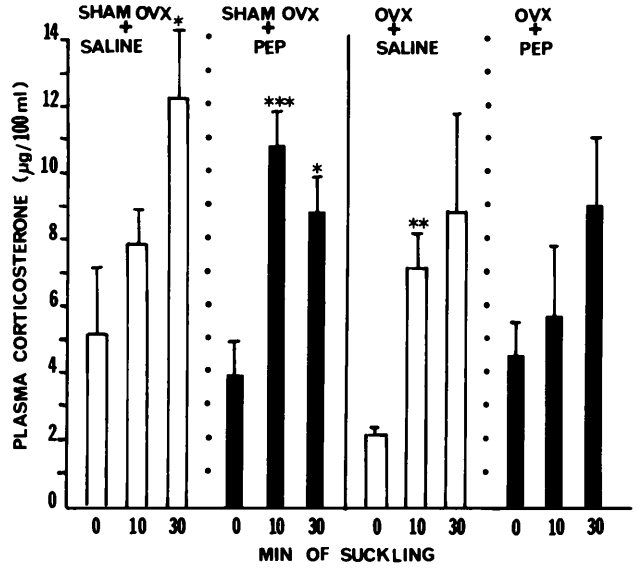

FIG. 2. Plasma levels of corticosterone (mean ± SEM) measured by competitive protein binding assay before and after suckling in intact (Sham OVX) and ovariectomized (OVX) rats on Day 10 of lactation. Samples are the same as those measured for prolactin at 0, 10, and 30 min in Fig. 1. *P = 0.05, **P = 0.025, ***P = 0.01; 10- or 30-min vs 0-min levels within each group.

tation. Thus, it does not appear that decreased PRL secretion is the cause of the lactation inhibition. Perhaps, direct effects of estrogen and progesterone on the mammary gland such as increased proliferation of mammary cells (3) or decreased PRL receptors (4) account for the inhibition.

An unexpected finding, that of suppressed or delayed suckling-induced PRL release in OVX rats treated with PEP, was most interesting since this is the first time to our knowledge that PRL release has been shown to be reduced or delayed by estrogen. Whatever the mechanism is for the estrogen-induced inhibition, it does not occur in intact lactating rats. Perhaps the high plasma progesterone levels found in the intact animals (Table II), which were similar to those reported by Smith and Neill (15), were responsible for antagonizing the action of estrogen. The suppression of PRL by estrogen in OVX lactating rats does not appear to be due to an augmented increase in the suckling-induced release of corticosterone documented by Voogt *et al.* (16) since corticosterone levels were not significantly increased above nonsuckled levels in this group at the time when prolactin release was suppressed.

In addition, the OVX, PEP-treated lactating rat apparently does not possess the neural mechanisms necessary for the induction of an afternoon PRL surge as do nonlactating, OVX, PEP-treated rats (17–20). Studies in our laboratories (18, 20) show that nonlactating animals have surges that are nearly maximal by 1500 hr, whereas, in the present study there was no significant difference between 1100- and 1500-hr values in lactating rats (Fig. 1).

Comparisons of plasma PRL levels in lactating rats measured by RIA and the newly developed Nb_2 cell lymphoma BA showed some subtle differences. When plasma PRL was low, the BA estimates were less than those by RIA. On the other hand, when plasma levels of PRL were high such as after suckling, the BA estimates exceeded those made by RIA. One logical interpretation of these findings is that suckling releases more bioactive PRL than is present during basal, nonsuckled, conditions. However, since we previously observed that high PRL levels is nonlactating rats also correlated with more biologically active PRL (10), release of more bioactive PRL may not be specifically related to suckling. In addition, these diverging responses with RIA and BA occur at levels that approach the minimal and maximal limits of the RIA standard curve but not the BA standard curve and great care should be exercised in studies that make use of any BA–RIA comparisons.

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