Suppression of Proestrous and Suckling-Induced Increase in Serum Prolactin by Hypothalamic Implant of Prolactin[1] (37175)

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The hypothesis that high levels of circulating prolactin may inhibit prolactin secretion by the pituitary was first suggested in 1953 (1). Subsequently it was demonstrated that *in situ* pituitary prolactin stores were decreased in rats carrying prolactin-secreting pituitary tumors (2, 3) or normal rat pituitary grafts underneath the kidney capsule (4). Implantation of prolactin into the median eminence (ME) of intact or ovariectomized rats reduced pituitary prolactin content and increased prolactin-inhibiting factor (PIF) in the hypothalamus (5). Voogt and Meites (6) found that an ME implant of prolactin interrupted pseudopregnancy and blocked the increase in serum prolactin that normally occurs during estrus. Clemens, Sar and Meites (7) reported that an ME implant of prolactin during postpartum lactation in rats significantly reduced milk secretion as indicated by depressed litter weight gains. In the present study we wished to determine whether an ME implant of prolactin could (a) prevent the rise in serum prolactin normally observed after suckling in lactating rats, and (b) suppress the increase in serum prolactin regularly observed on the afternoon of proestrus in cycling rats.

Materials and Methods. Female Sprague-Dawley rats (Spartan Animal Farms, Inc., Haslett, MI) were housed in a temperature (75 ± 2°F) and light (lights on 7 AM to 9 PM) controlled room. All rats received a standard diet of Wayne Lab Blox (Allied Mills, Chicago, IL) and tap water *ad libitum*.

Implantation procedure. Prolactin (NIH-P-S8) supplied by the Endocrinology Study Section, NIH, was mixed with equal amounts of cocoa butter. The prolactin–cocoa butter mixture (approx 250 μg prolactin) or cocoa butter alone was tamped into one end of a 23-gauge glass tube, implanted into the ME with a Stoelting stereotaxic instrument and left *in situ*. Implants were fixed in place with dental cement and skull screws as described previously (5). Implantations were made under ether anesthesia in lactating mother rats on the fourth day postpartum and in cycling rats by 10 AM on the morning of proestrus. Only rats with definite ME implants as determined by examination of the hypothalamus under a dissecting microscope at the end of the experiments were used.

Blood (1 ml) was obtained by cardiac puncture under light ether anesthesia from all rats. In the lactating rats the litters were equalized to 8 pups each, removed for 3 hr and then returned for 1 hr of suckling. This was followed immediately by withdrawal of a blood sample. This procedure was followed on the fourth day of lactation prior to implantation of prolactin, and at 2, 4 and 6 days after implantation. The cycling rats implanted with prolactin on the morning of proestrus were bled 4 times hourly on that day, beginning at 4.30 PM.

Radioimmunoassay (RIA). Serum was separated from the 1 ml blood samples and kept frozen at —20° until assayed. Prolactin concentration in individual serum samples was determined at 2 or 3 dose levels by a

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double antibody radioimmunoassay for rat prolactin (8). Purified rat prolactin was used as a reference preparation (HIV-8-C, biological potency = 0.77 × NIH-P-B1). Student's t test was used to compare serum prolactin levels in cocoa butter vs prolactin implanted rats.

Results. On Day 0 (fourth day of lactation), prior to ME implantation, rats designed to receive prolactin implants had a mean serum prolactin of 275 ± 71 ng/ml in response to 1 hr of suckling (Fig. 1). Rats designated to receive control cocoa butter implants had a mean serum prolactin of 282 ± 48 ng/ml. Rats with an implant of prolactin in the ME had significantly lower serum prolactin values in response to 1 hr suckling, 2, 4 and 6 days later, than found in cocoa-butter-implanted controls. The litters of control lactating rats implanted with cocoa butter in the ME gained an average of 16.6 g/day during the 6 days, whereas litters from mother rats implanted with prolactin in the ME gained an average of only 7.9 g/day ($p < 0.02$).

On the day of proestrus, cocoa-butter-implanted control rats showed a mean serum prolactin of 186.7 ± 33.3 ng/ml at 6:30 PM compared to 18.0 ± 5.6 ng/ml in rats implanted with prolactin (Table I). All 4 blood samples from prolactin-implanted rats showed very low serum prolactin concentrations, about equal to those found in ovariectomized rats (9).

Discussion. These results demonstrate that an implant of prolactin into the ME interferes with the release of prolactin from the pituitary in response to suckling, and prevents the rise in serum prolactin normally observed during the late afternoon of proestrus. Thus, an implant of prolactin in the ME interfered with an external stimulus to prolactin release, suckling, and an internal stimulus to prolactin release during proestrus, estradiol.

Prolactin is essential for maintenance of milk secretion in the rat (10), and the suckling stimulus is necessary to evoke release of prolactin from the pituitary (9, 10). The present work shows that an implant of prolactin in the ME results in decreased lactation as indicated by lower litter weight gains,

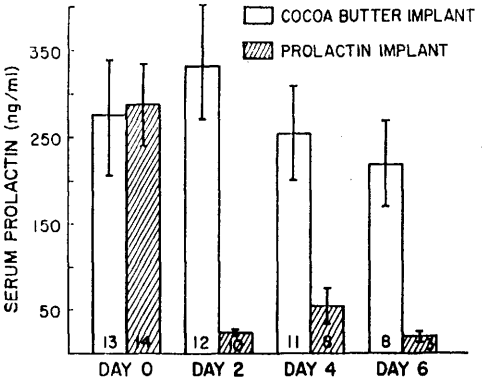

FIG. 1. Serum prolactin concentration in lactating rats. On Day 0 (fourth day of lactation) all rats were bled after 1 hr suckling and then received median eminence implants of either prolactin–cocoa butter or cocoa butter alone. Two, 4 and 6 days later they were bled after 1 hr suckling. The vertical bars indicate the standard error of the mean, and the number within each bar the number of rats/group.

and that this is associated with reduced serum prolactin values. These results are in agreement with the previous report from our laboratory that an implant of prolactin into the ME of lactating rats inhibited milk secretion, reduced mammary gland weight and decreased pituitary prolactin concentration (5).

A spontaneous discharge of prolactin normally occurs on the late afternoon of proestrus in the rat during each cycle (11–13). Observations by Neill, Freeman and Tillson (14) and our laboratory (Clark and Meites, unpublished data) indicated that estrogen secretion during the last day of diestrus and on the morning of proestrus elicits the release of prolactin on the afternoon of proestrus. The present results indicate that prolactin implanted into the ME of proestrous rats before 10 AM can inhibit this stimulation by estrogen. This agrees with a recent report that an ME implant of prolactin can prevent an increase in pituitary prolactin by injections of estrogen (15).

The present observations support the concept that high circulating levels of prolactin may act to inhibit pituitary prolactin release (1–7). After an initial rapid rise in prolactin release produced by the suckling stimulus or by administration of certain prolactin stimu-

TABLE I. Effect of Prolactin Implant in Median Eminence on Serum Prolactin During Proestrus.

Type of implant	No. of rats	Serum prolactin (ng/ml)[a]			
		4:30 PM	5:30 PM	6:30 PM	7:30 PM
Cocoa Butter	8	71.6 ± 19.4	114.0 ± 22.1	186.7 ± 33.3	100.9 ± 14.9
Prolactin	8	14.1 ± 2.5[b]	11.0 ± 1.7[c]	18.0 ± 5.6[d]	11.6 ± 1.8[e]

[a] Mean ± SE of mean.
[b] $p < .02$.
[c] $p < .001$.
[d] $p < .0002$.
[e] $p < .0001$.

lating drugs (16), the subsequent fall in serum prolactin levels may be due in part to the inhibitory feedback exerted by circulating prolactin. That this action in mediated via the hypothalamus is supported by observations that high serum levels of prolactin or an implant of prolactin in the ME increase PIF activity (3, 5) and dopamine turnover (17) in the hypothalamus. An increase in hypothalamic catecholamines has been shown to produce enhanced release of PIF and decreased release of prolactin (16).

Summary. The effect of an implant of prolactin in the median eminence (ME) on serum prolactin concentration was assessed in postpartum lactating rats and on the day of proestrus. An implant of prolactin in the ME of mother rats on the fourth postpartum day completely suppressed any rise in serum prolactin by the suckling stimulus from rat pups, and resulted in significantly lower litter weight gains than in similar non-prolactin-implanted control rats. An ME implant of prolactin by 10 AM on the day of proestrus completely prevented the surge in serum prolactin normally observed on the late afternoon of proestrus. These results provide further evidence that high circulating levels of prolactin may serve to suppress pituitary prolactin secretion.

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