# Milking-Induced Release of Endogenous Prolactin In Cows Infused with Exogenous Prolactin[1] (36961)

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Chronic administration of exogenous prolactin or prolactin implanted into the anterior median eminence reduces the endogenous concentration of pituitary and serum prolactin in rats (1–5). Accordingly, an "auto" or "short-loop" feedback mechanism has been postulated as part of the control mechanism governing prolactin secretion in the rat. Grosvenor *et al.* (6) showed that two injections of ovine prolactin into rats shortly before nursing or stress failed to influence the normal decrease in pituitary prolactin concentration in response to these stimuli. It was of interest to us to reinvestigate this problem in another species using homologous prolactin. Thus, the objective of the present study was to determine if constant infusion of bovine prolactin alters the endogenous release mechanism for prolactin in response to milking stimuli (7, 8) in lactating cows.

*Materials and Methods.* Four Holstein cows lactating 2–4 months were injected on alternate days for 4 consecutive days at 1300 hr with 1 mg of bovine prolactin (NIH-$B_3$)[2] in 10 ml of 0.85% NaCl or with 10 ml of NaCl via a polyvinyl IV (Clay Adams, Inc.) cannula in a jugular vein, and immediately infused thereafter for 2.5 hr with bovine prolactin (3 mg/30 ml/hr) or NaCl (30 ml/hr), respectively. Two cows received prolactin and two cows received saline on the first day of the experiment. Similarly, two additional cows received for 4 consecutive days an initial dose of 3 mg bovine prolactin followed by infusing 9 mg/30 ml/hr or NaCl. After the second hour of infusion, the mammary glands were washed and a milking machine was attached to the teats for 6 min and then removed. Infusions were stopped 30 min after the milking procedure was initiated (22 min after the milking machine was removed).

Blood was collected from a cannula placed in the contralateral jugular vein. Samples were taken at hourly intervals between 900 and 1300 hr (infusion start), at 15-min intervals between 1300 and 1500 hr (milking start), at 2-min intervals until 1512 hr, at 1515, 1520, 1525, 1530 hr (infusion stopped), at 2-min intervals between 1530 and 1540 hr, at 5-min intervals between 1540 and 1550 and finally at 15-min intervals between 1600 and 1700 hr.

Blood was allowed to clot at room temperature for 2–4 hr, held at 5° for 15 hr, centrifuged at 6000*g* for 10 min, and the serum stored at −20° until assayed for prolactin as previously described (8, 9). Bovine prolactin (NIH $B_1$)[2] was used as the reference standard.

*Results.* Prolactin averaged about 6 ng/ml of serum in all cows between 900 and 1200 hr (Figs. 1 and 2). Serum prolactin concentration doubled between 1200 and 1300 hr in all groups of cows. Relative to the baseline established before 1300 hr serum prolactin concentrations in saline-infused cows remained elevated between 1300 and 1500 hr, averaging approximately 15 ng/ml.

Stable serum prolactin concentrations were achieved within 45 min after the infusions of exogenous prolactin were started. During this stable period and before milking was initiated (1345 to 1500 hr) serum prolactin averaged 59.1 and 144.0 ng/ml for cows infused with 3 or 9 mg prolactin/hr, respectively (Table I). Comparable samples from

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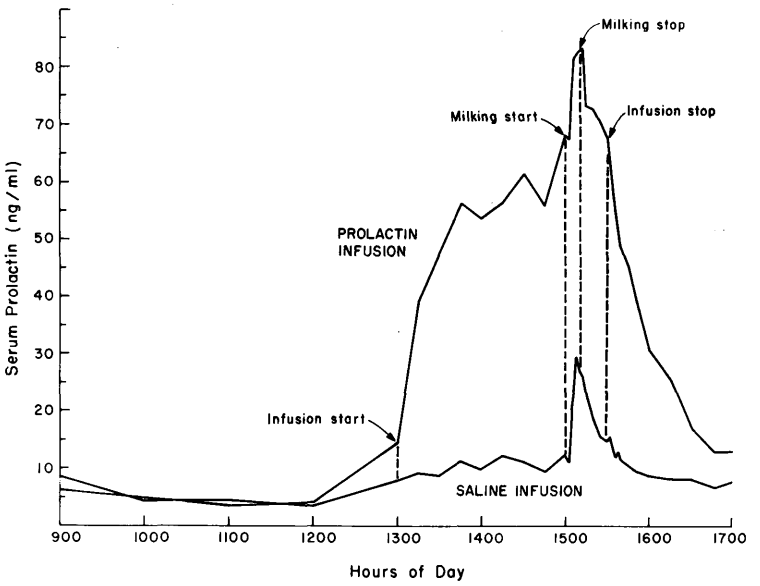


FIG. 1. Serum prolactin response to milking in cows infused with 3 mg of NIH-$B_3$ prolactin per hour or infused with 0.85% NaCl. Standard errors of means ($n = 8$ at each point) for prolactin-infused cows ranged from 0.5 to 10.6 ng/ml serum and for saline-infused cows, they ranged from 0.5 to 5.0 ng/ml serum. Standard errors were proportional to the mean.

cows infused with saline averaged 11.0 and 18.4 ng prolactin/ml serum. Assuming the average serum prolactin concentration of saline-infused cows between 1345 and 1500 hr represented endogenous hormone concentration, the increase during this interval due to exogenous prolactin can be calculated by the difference between serum prolactin in prolactin-infused and saline-infused cows. These values were 48.1 and 125.6 ng/ml for cows infused with 3 or 9 mg prolactin/hr, respectively. The 2.6-fold difference between the two doses of infused prolactin which was measured in the serum represents a recovery of 87% (2.6/3.0) of the true dose administered.

Average values at each sampling after milking are shown in Figs. 1 and 2, but these averages do not represent maximal responses because the prolactin response to milking was asynchronous with respect to time. For example, among individual cows, the range in time required to achieve maximal prolactin concentrations was 2 to 12 min after the start of milking with an average across all cows of 7.9 min. Thus, the maximum concentration of prolactin in the serum attained during the 12 min after the start of milking was taken to represent the response of the hormone to milking. Baseline values were established as the average for samples collected between 1345 and 1500 hr. Using these criteria, serum prolactin in cows infused with 3 mg prolactin per hr increased an average of 31.5 ng/ml above baseline ($p < 0.01$); and in the control cows infused with saline, serum prolactin was increased 20.4 ng/ml

TABLE I. Effect of Exogenous Prolactin Infusions on Release of Endogenous Prolactin.

| Infusion treatment | Serum prolactin | | |
|---|---|---|---|
| | Baseline[a] | Post-milking peak[b] | Δ |
| | ng/ml | | |
| Saline | 11.0 | 31.4 | 20.4 |
| Prolactin 1 mg; 3 mg[c] | 59.1 | 90.6 | 31.5 |
| Saline | 18.4 | 77.6 | 59.2 |
| Prolactin 3 mg; 9 mg[c] | 144.0 | 179.4 | 35.4 |

[a] Average serum prolactin concentration 1345–1500 hr.

[b] Maximum serum prolactin concentration 2–12 min post-milking.

[c] Cows were injected with 1 or 3 mg then infused continuously with 3 or 9 mg prolactin per hr for 2.5 hr.

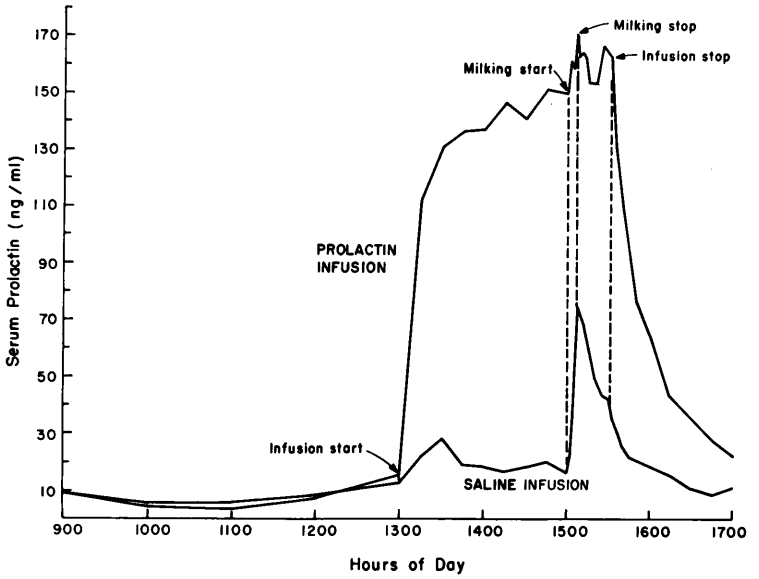


FIG. 2. Serum prolactin response to milking in cows infused with 9 mg of NIH-$B_3$ prolactin per hour or infused with 0.85% NaCl. Standard errors of mean ($n = 4$ at each point) for prolactin-infused cows ranged from 0.4 to 38.5 ng/ml serum and for saline-infused cows, they ranged from 1.5 to 14.2 ng/ml serum. Standard errors were proportional to the mean.

($p < 0.01$) above baseline (Table I). Similarly, serum prolactin in cows infused with 9 mg prolactin/hr rose an average 35.4 ng/ml above baseline ($p \simeq 0.10$), whereas saline-infused cows increased 59.2 ng/ml above baseline ($p < 0.01$) in response to milking. There was no significant difference ($p > 0.05$) between the maximal serum prolactin responses to the milking stimulus in the 3-mg prolactin per hr infused and saline control cows. But the milking-induced response in cows receiving 9 mg prolactin per hr was less ($p < 0.05$) than that in respective control cows.

The time required for the exogenous serum prolactin concentrations to decrease to one-half of maximal values was calculated from the differences between serum prolactin of prolactin-infused cows and that of saline-infused cows after the infusions were stopped. The disappearance rates averaged 25 and 23 min for cows infused with 3 or 9 mg prolactin/hr, respectively (Fig. 3). The reduced disappearance rates after 60 min may reflect additional discharges of endogenous prolactin which did not occur in saline-infused cows.

*Discussion.* The data presented strongly suggest that increasing the serum concentration of prolactin approximately 5- or 8-fold for 2 hr did not prevent the endogenous release of the hormone in response to milking. Our results in cattle agree with those of Grosvenor *et al.* (6) who reported that exogenous ovine prolactin did not prevent the decrease in pituitary prolactin concentration in rats which normally occurs after nursing or stress. We suggest that the inhibitory effects of exogenous prolactin previously reported (1–5) on prolactin secretion must be exerted primarily on mechanisms associated with synthesis rather than release or they may require chronic exposure to increased levels of the hormone. The magnitude of the prolactin release in response to milking is in agreement with those previously reported (7–9) for lactating cows.

It is not clear if the increase in serum prolactin during the infusion of saline represents a response to the saline or reflects a rising concentration of the hormone associated with circadian periodicity. We have recently presented evidence (10) suggesting that serum prolactin rises in untreated cows during the portion of the day when these infusions were conducted.

The calculated disappearance rates for

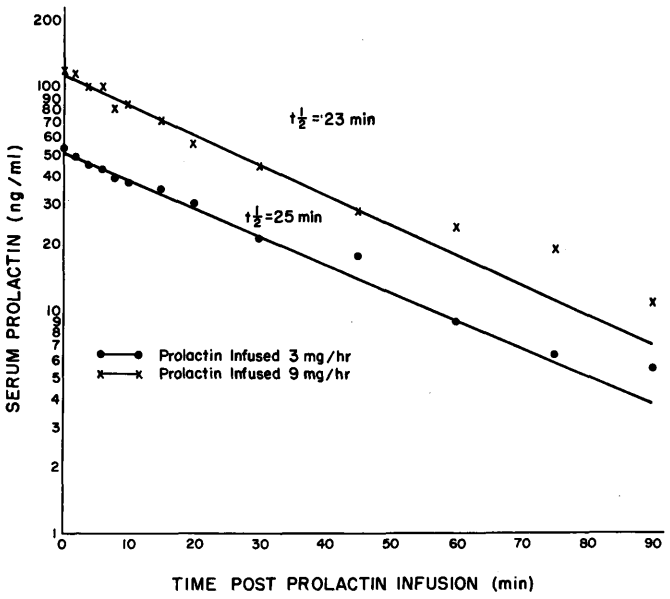


FIG. 3. Disappearance of prolactin from serum of cows after infusion of 3 or 9 mg NIH-$B_3$ prolactin per hour.

serum prolactin following the cessation of the continuous infusion of the hormone are in good agreement with other published data (11) using a single injection of NIH-$B_2$ prolactin.

*Summary.* Exogenous prolactin (3 or 9 mg NIH-$B_3$/hr) or saline was infused for 2.5 hr into 6 cows. The prolactin or saline infusions were repeated on alternate days for a total of 4 days. Approximately 45 min was required to achieve a stable baseline after the prolactin infusion was started. The exogenous prolactin, which increased serum prolactin approximately 5- and 8-fold above baseline endogenous concentrations, did not prevent the release of endogenous prolactin in response to milking. When the infusion was stopped serum prolactin disappeared with a 1/2 time of 23–25 min. We conclude that the inhibitory effects of exogenous prolactin previously reported on endogenous prolactin secretion must be exerted primarily on synthesis rather than release mechanisms, or the animals may require chronic exposure to high concentrations of prolactin.

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