Effect of Presence of Calf on Milking-Induced Release of Prolactin and Oxytocin During Early Lactation of Dairy Cows

R. MICHAEL AKERS
Department of Dairy Science
Lactation Physiology Laboratory
Virginia Polytechnic Institute and State University
Blacksburg 24061

ALAN M. LEFCOURT
Milk Secretion and Mastitis Laboratory
SEA-AR, ASI, USDA
Beltsville 20705

ABSTRACT
The ability of dairy cows to release either prolactin or oxytocin in response to machine milking in a conventional milking parlor was reduced among cows maintained with their calves during the 1st wk postpartum. However, presence of the calf caused no prolonged inhibition; cows evaluated 9 or 23 days after calf removal released similar quantities of prolactin and oxytocin after milking compared to controls (cows whose calves were removed within 24 h after parturition) at the same stage of lactation. Ambient temperature on the day of sampling was positively associated with the premilking serum prolactin concentration among cows housed with or without their calves (correlation coefficients were .52 and .59), but neither ambient temperature nor premilking serum prolactin concentrations were closely associated with area under milking-induced prolactin response curves (correlation coefficients from -.15 to .18). Unlike basal serum prolactin concentration, ambient temperature on the day of sampling was not closely associated with premilking serum concentrations of oxytocin or quantities released to the milking stimulus (correlation coefficients from -.19 to -.12). These results confirm that the calf can inhibit maternal secretion of prolactin and oxytocin in the dairy cow. These data also indicate that the capacity of the cow to respond to the milking stimulus is independent of premilking serum concentrations of prolactin or oxytocin. Better understanding of factors that regulate the secretion of these hormones at milking may allow development of techniques to enhance hormonal response to milking stimuli and possibly enhance milk production.

INTRODUCTION
Prolactin (Prl) is necessary for initiation and maintenance of milk synthesis and secretion in laboratory animals (10). Recent reports have established that the periparturient secretion of Prl is crucial for both biochemical (3) and cytological (4) differentiation of milk synthesizing cells in the bovine udder. Furthermore, in vitro studies have confirmed that Prl markedly stimulates synthesis and secretion of the specific milk protein alpha-lactalbumin in bovine mammary tissue (13). Because differentiation of mammary epithelium continues into early lactation in cattle (1, 7, 21), factors regulating secretion of Prl during this period may influence mammary development and consequently milk production. Indeed, Koprowski and Tucker (18) found that the quantity of prolactin released at milking was positively correlated with milk yields. Also, Akers et al. (2) reported that increased clearance and secretion rates of Prl were positively associated with milk yields throughout lactation.

In addition to Prl, continued removal of
milk is crucial for maintenance of milk production. It seems likely that some species may have an absolute requirement for oxytocin release during suckling if the young are to obtain milk (20). In cattle and other dairy ruminants a portion of the milk in the udder can be obtained without evidence of an acute release of oxytocin during milking. However, release of oxytocin during machine milking in cattle is thought to facilitate milk removal and thus alter efficiency and completeness of milk removal (19). Consequently, greater understanding of the factors that influence secretion of Prl and oxytocin during early lactation in cattle could be of great practical importance to the dairy industry. In (5) we reported that cows maintained with their calves showed minimal Prl responses to either milking (via portable milker in the presence of the calf) or suckling compared with milking responses in cows whose calves were taken away soon after birth. Cows kept with their calves also released less oxytocin in response to milking or suckling. However, it was not determined whether inhibition of hormone release was observed only when cows were milked in the presence of their calves, or whether inhibition of hormone release continued into subsequent lactation. Because milk let-down can be modified by variation in milking routine within the parlor and in response to conditioning stimulation (20, 27), we reasoned that it was particularly important to determine if inhibition of oxytocin and Prl release was also evident when the cows were milked routinely in the milking parlor. We extend these observations to include 1) measurement of Prl and oxytocin responses when cows maintained either with or without their calves are milked in a milking parlor (in accord with modern dairy practice), 2) effects of presence of the calf (during the 1st wk postpartum) on hormonal response to machine milking during later stages of lactation, 3) relationship between basal hormone and capacity to respond to milking stimulation, and 4) association of ambient temperature with basal serum hormone concentrations and area under response curves of milking-induced hormone.

METHODS

Seven pairs of multiparous Holstein cows due to calve within 1 wk of each other were scheduled for experimentation. One animal from each pair was assigned randomly to be maintained with her calf. All cows and calves were kept together 12 to 18 h postpartum so that the calves could obtain colostrum. Calves then were taken away from cows scheduled to be in the "cow alone" treatment group, and the cows were moved to an adjacent wing of the same barn. This was done so that cows in both treatment groups would be on the same milking and feeding schedules. Pens to which "cow alone" animals were assigned were identical to pens in which they calved except adjacent pens were used to house other cows only. Thus, cows maintained without their calves were exposed to neither their own nor other calves (except during the initial colostrum feeding period). Cows kept with their calves remained in the same pen in which they calved. On the afternoon of day 5 or morning of day 6 postpartum the cows were taken away from their calves and housed in pens in juxtaposition to the cows initially maintained without their calves. Cows in each treatment group were taken from their pens twice daily and milked in a milking parlor in accord with the usual routine with the remainder of the milking herd. Pairs of cows used in the experiment calved between March 10 and August 15, 1981.

A polyvinyl cannula was placed into a jugular vein of each animal on day 1 postpartum. On each sampling day cows were milked in a milking parlor with side-opening stalls to facilitate sample collection relative to milking. This experimental parlor was located immediately adjacent to the parlor in which the cows were milked routinely. The cows were sampled during the morning (0900 to 1100 h) on days 2, 4, 7, 14, and 28 postpartum. Blood samples were collected at 30, 20, 10, 5, and 1 min before milking and O, 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 25, 30, and 45 min during and after milking. Integrated samples were taken during consecutive 1-min intervals (20 ml/min) from 1 min before milking until 6 min after milking. Each cow's udder was washed for 30 s starting at sampling time 0; then the milking machine was attached for 4.5 min. Thus, milking stimulation consisted of 30 s of udder wash/massage and 4.5 min of machine milking. Blood was collected on ice and stored at 4°C overnight, sera were prepared by centrifugation the following morning. Sera samples were stored at
−20°C until assayed for Prl and oxytocin by double antibody radioimmunoassays. Antibody against bovine Prl was kindly supplied by H. A. Tucker (Department of Animal Science, Michigan State University; Koprowski and Tucker, 1971). Standard was NIH-B6 Prl and was iodinated as described by Bolt (9). Oxytocin was assayed as described by Gorewit (14). Antibody to oxytocin was purchased from Calbiochem (lot 860184); synthetic oxytocin (Calbiochem, lot 903825, 23.2 IU/mg) was used as standard and was iodinated according to Bolt (9). Aliquots of a pooled serum sample were measured in each assay for calculation of intra- and interassay coefficients of variation.

Average baseline concentrations of Prl or oxytocin measured before milking were calculated for each cow on each sampling day. This concentration then was subtracted within animal from hormone concentrations measured at each subsequent sampling time for individual cows. Areas under the resulting hormone response curves were calculated as in (11). Means of area under individual cow response curves at each sampling day postpartum were determined and differences between treatments evaluated by analysis of variance.

Concentrations of Prl in a serum pool averaged 18.1 ± 1.1 (± SD; n=6) ng/ml based on quadruplicate determinations of 20-μl samples in six assays or 18.5 ± 1.3 (± SD; n=6) based on quadruplicate determinations of 80 μl in each of six assays. Within assay coefficients of variation (based on quadruplicate measurements of either 20 or 80 μl) averaged 4.6 ± 2.0% (± SD; n=12; range 2.8 to 7.9%); between assay coefficients of variation were 6.3 and 7.1% for 20 and 80 μl estimates.

In a serum pool, oxytocin concentration averaged 291 ± 10 (± SD; n=4) pg/ml based on quadruplicate determinations of 300-μl samples in each of four assays. Coefficients of variation within assay averaged 5.9 ± 2.3% (± SD; n=4; range 2.7 to 8.1%) and coefficients of variation between assays averaged 3.4%.

RESULTS AND DISCUSSION

Areas under Prl response curves after milking are summarized in Table 1. Quantities of Prl released in response to the milking stimulus were lower (P<.05) among cows maintained with their calves on days 2, 4, and 7 postpartum. However, after calves were removed, inhibition on milking-induced Prl release was quickly abolished because cows in both treatment groups released nearly identical quantities of Prl after milking on days 14 and 28 postpartum (P>.6). These results confirm that presence of calf can inhibit milking-induced release of Prl in dairy cows (5, 12) and indicate that suppression of Prl release attributed to the presence of the calf causes no prolonged inhibition once the cow and calf are separated. The relative Prl response to milking among cows maintained with their calves (Table 2) was greater than in (5). This may indicate that inhibition attributed to the calf is greatest when the cow actually is milked in the presence of her calf. Nonetheless, the ability of the cow to release

<table>
<thead>
<tr>
<th>Day postpartum</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>14</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>X</td>
<td>SE</td>
<td>X</td>
<td>SE</td>
<td>X</td>
</tr>
<tr>
<td>Cow + calf</td>
<td>186</td>
<td>114b</td>
<td>286</td>
<td>219c</td>
<td>-109</td>
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<td>Cow alone</td>
<td>1,228</td>
<td>458</td>
<td>1,417</td>
<td>163</td>
<td>854</td>
</tr>
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</table>

aOnly 4 cows in each treatment group were evaluated on day 2 postpartum; all other days n=7 per treatment group. Given are means ± SE; area measurements are expressed as ng ml⁻¹ min.

bP<.05 different from cows maintained without their calves.
cP<.03 different from cows maintained without their calves.
dP<.001 different from cows maintained without their calves.

TABLE 1. Area under prolactin response curves after milking in cows maintained with or without their calves through day 5 postpartum.
Prl during milking was suppressed when cows and calves were housed together. Furthermore, quantities of Prl released after milking among cows kept without their calves or after calf removal were similar to those for cows during early lactation.

Mechanism(s) responsible for inhibition of Prl release at milking among cows housed with their calves is unknown. It could be intuitively suggested that reduced release of Prl occurred because of depletion of pituitary reserves as cows kept with their calves were suckled repeatedly in addition to being milked twice daily. However, Goodman et al. (12) reported that simply maintaining the cow in the presence of her calf (suckling not allowed) decreased basal-induced, milking-induced (cows milked in presence of the calf), and thyrotropin releasing hormone-induced release of Prl. Also, continuous infusion of thyrotropin releasing hormone for up to 9 h results in progressively decreased Prl release (although greater than prestimulation even after 9 h), but teat stimulation during infusion evoked an additional Prl release (25). Thus, it seems unlikely that reduced Prl release results because of depletion of pituitary reserves of Prl.

A second possibility is that Prl release is dependent upon quantity of milk removed from the udder at milking. Average (± SE) quantities of milk obtained on days 2 and 4 postpartum from cows housed with their calves were lower (3.8 ± .5 and 4.9 ± .6 kg/milking) than from controls (7.2 ± 1.1 and 9.4 ± 1 kg/milking). Mean (± SE) milk yields were similar among cows previously housed with their calves or among controls on other sampling days (9.5 ± .6, 9.9 ± .9, 10.6 ± .9 kg/milking on days 7, 14, and 21 postpartum among cows previously housed with their calves) and (9.9 ± 1.2, 10.7 ± .9, 11.6 ± .6 kg/milking among controls). However, simple relationships between Prl release and milk obtained at a given milking seem unlikely.

Hart (15) suggested that three types of stimuli may induce release of hormones from the pituitary at milking in ruminants: physical, tactile manipulation of the teats; conditioned stimuli associated with milking; and a metabolic stimulus, perhaps as a response to uptake of nutrients by the udder. Subsequent experiments (16) showed 1) that Prl was released during milking of intact mammary glands, but no Prl was released in response to milking of autotransplanted (denervated) glands in goats; 2) that similar quantities of Prl were released by goats milked under anesthesia or while conscious; and 3) that tactile stimulation of one teat produced less Prl release than stimulation of both teats for an equivalent time. These workers concluded that tactile stimulation was essential for Prl release during milking.

<table>
<thead>
<tr>
<th>Day postpartum</th>
<th>2a</th>
<th>4</th>
<th>7</th>
<th>14</th>
<th>28</th>
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</thead>
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<td>Treatment</td>
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<td>SE</td>
<td>X</td>
<td>SE</td>
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<tr>
<td>Cow + calf</td>
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<td>81</td>
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<td>64</td>
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<td>Postmilking</td>
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<tr>
<td>Cow + calf</td>
<td>93</td>
<td>22</td>
<td>105</td>
<td>32</td>
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<td>Cow alone</td>
<td>134</td>
<td>34</td>
<td>122</td>
<td>21</td>
<td>97</td>
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*p<.05; lower than other means within a row.

*Only 4 cows in each treatment group were evaluated on day 2 postpartum; all other days n=7 per treatment group. Given are means ± SE.

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Reinhardt and Schams (23, 24) showed that Prl is released in response to teat stimulation regardless of milk removal in lactating cows and that degree of response is dependent upon duration and intensity of stimulation. Our work also showed that teat stimulation is an effective promoter of Prl release in nonlactating cattle (6).

Relative Prl release at milking declines as lactation advances (18), but this effect may depend upon physiological changes associated with advancing lactation other than milk yield. Koprowski and Tucker (18) found that correlations within stage of lactation between serum prolactin in samples collected immediately postmilking and milk yield were generally low and negative during early lactation but positive during later stages of lactation. If Prl response were dependent upon milk yield, it would be logical to expect that the relationship between milk yield and Prl release would be more consistent throughout lactation. Aono et al. (8) also reported positive relationship between amount of Prl released at suckling and quantity of milk produced in humans. However, observation that all women in their study showed similar increases of serum Prl when tested with the constant stimulus of a breast pump, suggests that differences in Prl responses were related to suckling intensity of their infants. These examples suggest that intensity and duration of stimulation are more important in controlling release of Prl at milking than volume of milk removed from the udder at a given stage of lactation.

Moreover, with regard to the current experiment, Goodman et al. (12) found that cows maintained immediately adjacent to their calves (suckling not permitted) exhibited decreased milking-induced release of Prl compared with cows whose calves were removed. Because suckling was not allowed, decreased Prl response at milking cannot be attributed to differences in udder content of milk at the time of sampling. In our experiment, calves were removed from their dams in the cow plus calf treatment group on the evening of day 5 or morning of day 6 postpartum. Measurements on day 7 still indicated marked inhibition of Prl release in response to milking. Because milk yields were similar in both groups, lack of Prl response among cows previously housed with their calves cannot be attributed to differences in milk yields. Similarly, because milking time and udder preparation time were constant for all animals, inhibition cannot be attributed to variation of degree of udder stimulation during milking. We hypothesize that lower milk yields among cows housed with their calves (days 2 and 4 postpartum) have little if any relation with quantities of Prl released in response to milking. Finally, areas under Prl response curves were little correlated with milk obtained during milking on days 2 or 4 postpartum among cows housed with (r = .23) or without their calves (r = .22).

It seems more likely that exteroceptive signals from the calf modify the capacity of the cow to respond to stimuli normally associated with Prl release at milking. This suggests that factors other than simple physical stimulation of the teats during milking are involved in release of Prl. The possibility that higher brain centers can modify the hypothalamic-pituitary axis and alter release of Prl normally dependent upon teat stimulation is not unlike conditioned release of oxytocin (19, 20, 27).

Mean premilking serum concentrations of Prl were nearly identical between treatment groups on each sampling day. Although in both treatment groups basal Prl was approximately 50% lower (P < .05) on days 14 and 28 than on days 2, 4, or 7 postpartum (Table 2). The basal serum Prl among cows is markedly greater than we reported for cows during the same physiological state (5). This difference likely is a consequence of varying environmental conditions. Both ambient temperature and photoperiod can markedly alter basal serum Prl concentrations in cattle. For example, long photoperiods and warm ambient temperatures are associated with increased Prl, whereas short photoperiods and cold ambient temperatures are associated with decreased Prl in blood (26). In contrast to seasonal modulation of basal Prl secretion, effects of ambient and photoperiod on induced Prl release apparently are highly dependent upon the stimulus used to elicit Prl secretion. For example, Peters et al. (22) found that neither photoperiod nor ambient temperature affected milking-induced release of Prl, but basal Prl and Prl released in response to thyrotrophin releasing hormone were 1.5 to 1.8 times as great in cows exposed to 16 h of light per day compared with cows exposed to 9 or 12 h of
natural light per day. We discussed physiological factors involved in teat stimulation-induced release of Prl (6). In the present study, the ambient temperature on the day of sampling was positively correlated with the premilking serum Prl concentrations among cows housed with (r=.59, P<.01) or without (r=.52, P<.01) their calves. In contrast, ambient temperature prior to sampling was not correlated with quantity of Prl released after milking (response area) among cows kept with their calves (r=−.05) or cows housed in the absence of their calves (r=.06). In addition, premilking serum concentrations of Prl were little correlated with quantities of Prl released after milking among cows housed either with (r=−.15) or without (r=.18) their calves. These results support our earlier suggestion that the Prl response to milking is largely independent of basal concentrations of Prl in cows (5) and confirm the results of (22).

The oxytocin response to milking among cows maintained with their calves was lower (P<.05) on days 4 and 7 postpartum compared with cows maintained in the absence of their calves (Table 3). This confirms our observation that the calf can suppress maternal secretion of oxytocin (5). Like the Prl response to milking, inhibitory effects were dependent upon continued presence of the calf as both groups of cows released similar quantities of oxytocin after milking on day 14 and 28 postpartum. In contrast to Prl responses, cows in both treatment groups released greater (P<.05) quantities of oxytocin after milking on days 14 and 28 postpartum than during the 1st wk postpartum (Table 3). Mechanisms responsible for increased oxytocin release are unknown. However, relative increases of oxytocin after milking and premilking serum concentrations (Table 4) are similar to concentrations in (5). This suggests that, unlike Prl, oxytocin secretion in cows is relatively unaffected by environmental factors associated with season of the year. For example, ambient temperature on the day of sampling was not closely associated with premilking serum concentrations of oxytocin (r=−.19) nor with quantities of oxytocin released in response to milking (r=−.12). Nor were basal serum oxytocin concentrations closely associated with area under oxytocin response curves (r=.28).

These data confirm that housing the dairy cow with her calf following parturition can modify maternal release of Prl and oxytocin to machine milking and indicate that the calf need not be present during the actual milking to inhibit response. Furthermore, inhibition of hormone release after milking is dependent upon continued presence of the calf with the dam as inhibitory effects are lost within 10 days after calf removal. It was not determined if inhibition of milking-induced hormone release is calf-specific (associated only with the cow and her own calf) nor if the effect depends upon maternal-offspring interaction soon after birth. If enhanced milking-induced release of oxytocin and Prl stimulate continued differentiation of mammary cells and promote milk synthesis during the immediate postpartum.

<table>
<thead>
<tr>
<th>Table 3. Area under oxytocin response curves after milking in cows maintained with or without their calves through day 5 postpartum.</th>
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</thead>
<tbody>
<tr>
<td>Day postpartum</td>
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<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Cow + calf</td>
</tr>
<tr>
<td>Cow alone</td>
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</tbody>
</table>

ap<.05 greater than other means within a row without superscript a.
bOnly 4 cows in each treatment group were evaluated on day 2 postpartum; all other days n=7 per treatment group. Given are means ± SE; area measurements are expressed as pg ml⁻¹ min⁻¹.
cP<.05 different from cows maintained without their calves.
dP<.02 different from cows maintained without their calves.
TABLE 4. Premilking and maximal postmilking serum concentrations of oxytocin in cows maintained with or without their calves through day 5 postpartum.

<table>
<thead>
<tr>
<th>Day postpartum</th>
<th>Treatment</th>
<th>2a</th>
<th>4</th>
<th>7</th>
<th>14</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Premilking oxytocin (pg/ml)</td>
<td>Cow + calf</td>
<td>200</td>
<td>25</td>
<td>205</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>Cow alone</td>
<td>233</td>
<td>15</td>
<td>168</td>
<td>188</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>Postmilking oxytocin (pg/ml)</td>
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<td>254</td>
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<td>Cow alone</td>
<td>281</td>
<td>20</td>
<td>263</td>
<td>357</td>
<td>303</td>
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</tbody>
</table>

*Only 4 cows in each treatment group were evaluated on day 2 postpartum, all other days n=7 per treatment group. Given are means ± SE.*

period, then the common management scheme of removing dairy calves from their dams soon after birth is appropriate.

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specific stimuli causing the release of prolactin and growth hormone at milking in the goat. J. Endocrinol. 72:163.


