Effects of Intramammary Endotoxin Infusion on Milking-Induced Oxytocin Release

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ABSTRACT

One overt sign of clinical coliform mastitis in dairy cows is the failure to eject milk normally or to "milk out" the udder. The effect, if any, of coliform mastitis on oxytocin release is unknown. Therefore, the objective of this study was to determine the effect of endotoxin mastitis on milking-induced release of oxytocin in lactating cows. Fifteen multiparous pregnant lactating Holstein cows were divided into three groups of 5 cows each. Cows in group 1 served as controls and received an intramammary infusion of sterile physiological saline. Cows in groups 2 and 3 received intramammary infusions of 12.5 and 25 μg of Escherichia coli endotoxin, respectively. Serum concentrations of oxytocin were measured by radioimmunoassay before, during, and after milkings commencing at 6 and 12 h after treatment. Rectal temperatures and milk SCC were monitored to follow the course of inflammation and to verify the biological activity of infused endotoxin. Endotoxin resulted in a 1.5- to 2-fold increase in milking-induced oxytocin release compared with that of control treatments. The effect was most prominent during the first 6 h after infusion and coincided with the peak pyretic response. This study shows that endotoxin-induced mastitis potentiates, rather than inhibits, milking-induced oxytocin release.

(Key words: endotoxin, oxytocin, milking)

Abbreviation key: OT = oxytocin.

INTRODUCTION

Clinical coliform mastitis results in rapid swelling and hardness of the infected quarter. Onset of the inflammatory response is rapid, and inflammation usually reduces milk yield significantly. One overt sign of clinical coliform mastitis is the failure of the quarter to eject milk normally or to "milk out" the udder. In addition to antibiotic treatment, multiple milkings, machine stripping, and oxytocin (OT) injections are among recommended therapies for acute or peracute cases (11). Clearing the infected quarter of bacteria and toxins is essential to ensure more effective treatment. Oxytocin is released from the posterior pituitary in response to a variety of milking-related stimuli (4, 5, 6) and is responsible for contracting myoepithelial cells of the udder and moving milk from the alveoli to the milk ducts and cisterns. An effective milk ejection reflex, resulting in the release of OT, is essential for lactational maintenance (5).

Several workers (5, 6, 9) have attempted to inhibit or to reduce OT release during milking with catecholamines and stressful stimuli. These experiments did not show an inhibitory response. In addition to local tissue effects, acute or peracute coliform mastitis results in a systemic change in body temperature, shift in blood leukocyte profile, and release of monokines (interleukin-1 and tumor necrosis factor-α), histamine, and prostaglandins (7, 8, 10). Clinical coliform mastitis can therefore be considered to be a form of stress. Certain types of stress inhibit the milk ejection reflex, either by interfering with the synthesis and release of OT or by its action at the mammary myoepithelium (5, 9).

At present, no information exists concerning the effect of coliform mastitis or endotoxin-associated inflammation on the milk ejection reflex or the milking-induced release of OT. With this in mind, the objective of the
present study was to determine whether intramammary infusion of endotoxin influences milking-induced OT release.

MATERIALS AND METHODS

Cows

Fifteen multiparous, pregnant, lactating Holstein cows were used from the Cornell University dairy herd. Cows ranged from 100 to 150 d in lactation and had SSC of less than 200,000 cells/ml of milk prior to experimentation.

General Procedures

Quarter foremilk samples were analyzed for SCC using a Fossomatic cell counter (Foss Food Technology, Eden Prairie, MN), and rectal temperatures were taken using a standard glass mercury rectal thermometer to document the temporal response to endotoxin-induced mammary inflammation. Endotoxin (Escherichia coli 055:B5; Sigma Chemical Co., St. Louis, MO) was dissolved in sterile pyrogen-free physiological saline (.85%) and stored frozen (-20°C) in aliquots. Prior to use, each aliquot was thawed and passed through a .2-µm sterile syringe filter.

Blood Collection and Hormone Assay

Cows were fitted with indwelling jugular cannulas 2 d before the experiment. Blood samples were taken at 60, 30, 15, 10, 5, and 0 min before milking stimulation. Milking stimulation consisted of washing and drying of the udders for 30 s followed by a 30-s waiting period. Blood sampling continued at this point (+1 min), at every minute thereafter for 15 min, and at 20, 30, and 60 min after the milking procedure commenced. Blood sera were harvested at 4°C by centrifugation at 1000 × g and frozen at -20°C until they were assayed for OT.

Concentrations of OT were assayed by radioimmunoassay procedures described by Gorewit (4) and Wachs et al. (14). Intraassay and interassay variations of five assays averaged 1.3 and 6.7%, respectively. The area under each response curve for OT release during milking (after adjustment for basal concentrations) was integrated and used to compare the total amounts of hormone released at milking before, during, and after endotoxin infusion. The peak concentration and time of peak were determined for OT before, during, and after saline or endotoxin infusion.

Treatments

Fifteen cows were separated into three groups of 5 each. The left rear and right fore-quarters were treated with 5 ml of physiological saline (control treatment, n = 5), 12.5 µg of endotoxin (n = 5), or 25 µg of endotoxin (n = 5). Endotoxin infusions were given in 5 ml of sterile pyrogen-free saline. All 15 cows were milked prior to infusions (milking 1). Directly after milking 1, cows were infused with their appropriate treatments. All cows were milked again 6 h after infusion (milking 2). This interval was chosen for milking 2 because preliminary experiments conducted at Cornell and work published by Jackson et al. (7) and Jain (8) showed that the maximum pyretic or systemic reactions to intramammary endotoxin infusion occurred at 5 to 6 h posttreatment. Milking and sampling resumed again at 12 h postinfusion (milking 3).

Statistical Analysis

Significance of treatments on rectal temperatures, milk SCC, and OT release were tested using ANOVA procedures (13). The statistical model included cow, treatment, milking number (1, 2, or 3), endotoxin dose, and their interactions. Peak concentrations of OT attained in response to milking were taken to be the highest concentration measured in any of the 15 samples taken immediately following the commencement of milking. Amounts of OT released were estimated by calculating the area under the curve of OT concentration from 10 min before until 20 min after the start of milking using an electronic digitizing planimeter. Means for these areas were compared for significant differences using a Student's t test (2, 5, 14).

RESULTS AND DISCUSSION

Rectal Temperature

Rectal temperatures for saline-infused cows ranged from 37.8 to 39.1°C and showed no
significant response to intramammary infusion (Figure 1). However, intramammary infusion of endotoxin caused significant ($P < .05$) elevations in rectal temperature. Rectal temperature was elevated by $3$ h of infusion for those cows treated with $12.5$ µg of endotoxin. The peak in pyretic response occurred at $6$ h. Rectal temperature remained elevated up to $12$ h postinfusion of $12.5$ µg of endotoxin. In those cows treated with $25$ µg of endotoxin, rectal temperature became elevated within $2$ h. Peak rectal temperature was at $7$ h postinfusion. Rectal temperatures of cows treated with the higher dose of endotoxin were higher during the infusion period compared with those of the cows receiving the lower dose. These data are in agreement with results from other workers (7, 8, 11), and they substantiated the biological activity and potency of the infused endotoxin.

**Somatic Cells**

Figure 2 illustrates the SSC in quarter foremilk samples for control and endotoxin-infused cows at milking. Each dose of endotoxin significantly ($P < .05$) increased milk SCC compared with SCC of controls. Peak numbers of somatic cells corresponded to the times when rectal temperatures were elevated (Figure 1), and effects of endotoxin infusion on OT release (see next section) in response to milking were maximum.

**Serum OT Response to Endotoxin**

Figure 3 shows serum concentrations of OT at $6$ and $12$ h after saline infusion. Milking stimuli resulted in the release of OT. Concentrations of OT at rest ranged from $5$ to $8$ pg/ml. Serum OT concentrations were elevated within $1$ min of stimulation. Peak concentrations were reached at $1$ to $2$ h after stimulation. Resting concentrations were apparent by $30$ min poststimulation. The amounts of OT released at the $6$ h versus the $12$ h after saline infusion milkings were not significant ($P > .05$). These data demonstrated that cows are able to release adequate concentrations of OT at milking intervals of less than $12$ h, which is in agreement with the report of Gorewit (4) and Gorewit et al. (6), who showed that cows do not exhaust their reserves of OT with repetitive milkings.

Changes in serum OT concentrations before, during, and after milking at $6$ and $12$ h after intramammary infusion of either $12.5$ or $25$ µg of endotoxin are illustrated in Figures 4 and 5, respectively. Intramammary infusion of endotoxin had a significant ($P < .05$) effect on milking-induced release of OT. Concentrations of OT averaged $10$ pg/ml prior to milking number $2$ at $6$ h after endotoxin infusion ($12.5$ µg, Figure 4a). Oxytocin concentrations were significantly elevated within $2$ min of stimula-
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Figure 3. Serum concentrations of oxytocin prior to, during, and after milking in cows 6 h (a) and 12 h (b) after they received intramammary infusions of sterile physiological saline. Data are means plus or minus standard errors.

Figure 4. Serum concentrations of oxytocin prior to, during, and after milking in cows 6 h (a) and 12 h (b) after they received intramammary infusions of 12.5 μg of Escherichia coli endotoxin. Data are means plus or minus standard errors.

Peak concentrations were at 2 min after stimulation and averaged 74 pg/ml, which were nearly 2-fold higher than saline-infused controls during this period. The total amount of OT released in response to milking was significantly (P < .05) greater than OT of saline-infused controls. Concentrations of OT decreased over milking and reached baseline by 15 min after machine attachment.

Milking-induced release of OT was still significantly amplified by 12.5 μg of endotoxin 12 h after infusion. Peak concentrations of OT occurred at 3 min poststimulation (Figure 4b). Resting concentrations occurred by 30 min poststimulation. The total amount of OT released in response to milking was significantly (P < .05) greater than that of saline controls.

Figure 5 shows the effects of 25 μg of endotoxin on the milking-induced release of OT at 6 and 12 h posttreatment. Endotoxin significantly (P < .05) increased milking-induced OT release compared with that of saline controls. Peak serum concentrations of OT were achieved at 2 min poststimulation during the 6-h postinfusion milking (Figure 5a). Peak concentrations of OT were approximately 2.5 times greater than that of control cows milked 6 h after saline infusion. Resting concentrations of OT appeared to be elevated over those of saline controls. Resting concentrations of OT were achieved by 30 min poststimulation.

At 12 h postinfusion, OT concentrations were significantly (P < .05) elevated compared with those of saline controls once again (Figure 5b). At that sampling time, peak concentrations were reached at 2 min poststimulation. They were still nearly twice those peak concentrations in saline controls. There was no
release of OT. Histamine and prostaglandins are released during inflammation, and they, in turn, may influence hormonal release (3, 10). Besedovsky et al. (1) described the immunoregulatory feedback between interleukin-1 and glucocorticoids. Jackson et al. (7) showed that *E. coli* endotoxin intramammally infused into cows increased concentrations of serum prolactin and cortisol. Furthermore, Peter et al. (12) found that intrauterine infusions of endotoxin blocked preovulatory luteinizing hormone surges in heifers.

**CONCLUSIONS**

Intramammary infusion of *E. coli* endotoxin potentiates, rather than inhibits, the release of OT during milking of lactating Holstein cows. The effect followed the pyretic or systemic response. Further work is necessary to determine the exact mechanism or mechanisms responsible for potentiation.

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**REFERENCES**

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