High Cortisol Concentrations and Mediation of the Hypogalactia During Endotoxin-Induced Mastitis

ABSTRACT

A series of experiments was conducted to define the role of cortisol in the hypogalactia during endotoxin-induced mastitis. In the first experiment, three of six nonmastitic cows were given a continuous infusion of trilostane, a 3β-hydroxysteroid dehydrogenase inhibitor that blocks enhanced cortisol synthesis. Trilostane had no effect in these cows. In the second experiment, six midlactation cows were given 10 pg of endotoxin in each of two homolateral quarters to induce mastitis. Three of these cows also received trilostane. Increased serum cortisol following endotoxin infusion was blocked by trilostane treatment, whereas serum glucose and rectal temperatures were unaffected. Preventing the cortisol increase failed to reduce hypogalactia in endotoxin-infused or uninfused quarters. Decreases in milk production and increases in measures of mammary inflammation were greater in trilostane-treated cows, indicating that endogenous cortisol may moderate the cow’s inflammatory response. In the third experiment, three of six nonmastitic cows were injected intramuscularly with 150 IU of ACTH. Serum cortisol concentration exceeded 70 ng/ml for at least 3 h in cows receiving ACTH. This cortisol concentration, comparable with concentrations found during endotoxin mastitis, did not inhibit milk production. Together, these data demonstrate that the acute cortisol increase does not mediate the hypogalactia associated with endotoxin-induced mastitis. (Key words: cortisol, milk production, mastitis)

INTRODUCTION

Hypogalactia is a major cause of economic loss during mastitis. Earlier experiments using intramammary endotoxin infusion as a model of coliform mastitis demonstrated that mastitic hypogalactia is inhibited by local and systemic mechanisms (16, 17, 18, 19). The systemic inhibition of milk production is illustrated by hypogalactia in all quarters of cows given intramammary infusions of endotoxin, including the uninfused quarters, which did not become inflamed. Among systemic physiological responses to intramammary endotoxin treatment is a pronounced increase in serum cortisol; such concentrations often exceeded 50 ng/ml for 2 to 4 h in treated cows (6, 12).

A number of studies have investigated the effects of corticosteroids on milk production in lactating cows. Corticosteroids are necessary for the maintenance of ruminant lactation (21). However, several studies have demonstrated that various high or long-acting doses of ACTH or synthetic glucocorticoids reduced milk synthesis. Milk production was inhibited 10 to 30% for up to 5 d following 100- to 300-IU injections of ACTH (2, 4, 20). Cows injected four times daily with 200 IU of ACTH for 42 h had peak serum corticosteroid concentrations of 90 ng/ml (22). Milk volume and protein yields were inhibited by 35% in these cows, but fat yields were little affected because of a compensatory increase in the fat composi-
tion of milk. Interestingly, a compensatory increase in fat composition also occurs during endotoxin mastitis (17). Milk production by cows given 9α-fluoroprednisolone acetate or dexamethasone was markedly reduced (3, 5). In contrast, cows treated with moderate or very high doses of cortisol continued to produce normal volumes of milk (9, 11). Hence, effects of elevated serum glucocorticoid concentrations on milk production remain unclear, but data indicate that suppression of milk production by elevated serum cortisol concentrations during mastitis is a possibility worthy of investigation. The objective of this study was to investigate the effects of the corticosteroid response during endotoxin mastitis on lactational performance. This objective was accomplished in three separate experiments. In the first experiment, the effects of the cortisol synthesis inhibitor, trilostane, was studied in nonmastitic cows. In the second experiment, trilostane was used to block the cortisol response during mastitis. In the final experiment, serum cortisol concentration was increased by ACTH administration to nonmastitic cows.

MATERIALS AND METHODS

Cattle

Healthy Holstein and Jersey cattle, which were at least 1 mo postpartum, had SCC below 500 x 10³ cells/ml in all quarters, and were producing 15 to 30 kg of milk/d, were used for this experiment. Cows received a total mixed ration consisting of alfalfa and corn silages plus concentrate and mineral supplement. In addition, 1.5 kg of a pelleted concentrate were offered prior to each milking. Cows were milked twice daily at 0600 and 1800 h with a quarter milking machine. To allow cows to adjust to experimental conditions, all cows were housed in tie stalls and milked with the quarter milker for 3 d before start of data collection.

Sampling Procedures

Coccygeal blood samples and rectal temperatures were taken just prior to milking and several times during the day following endotoxin or ACTH treatment. Blood samples were refrigerated (4°C) until the serum was harvested. Two bulk milk samples were taken from each quarter reservoir of the milking machine. In Experiments 1 and 3, milk samples and production data were collected from two homolateral quarters of each cow. In Experiment 2, all quarters of each cow were sampled. One milk sample was preserved with potassium dichromate and refrigerated for subsequent milk composition and SCC analyses. The other sample was stored (4°C) for NA/Gase analysis and whey sample preparation. Whey samples were prepared by acidification within 15 h of milk collection as previously described (17).

Treatments

Endotoxin, a trichloracetic acid extract from Escherichia coli 055:B5 (Sigma Chemical Co., St. Louis, MO), was dissolved in pyrogen-free Hanks balanced salt solution (HBSS) and filter (0.2 μm)-sterilized. Treated cows were infused with 10 μg of endotoxin in 10 ml of HBSS in each of two homolateral quarters 1 h after the fourth preliminary period milking (milking 0).

Trilostane (The Upjohn Company, Kalamazoo, MI), a competitive inhibitor of 3β-hydroxysteroid dehydrogenase, which blocks basal—but not cortisol synthesis (13), was administered as a continuous infusion. Approximately 10 h prior to the start of infusion, trilostane cows were fitted with jugular vein catheters. Within 10 h of the start of infusion, 640 mg of trilostane were dissolved in 27 ml of N,N-dimethylacetamide. After complete dissolution, 54 ml of propylene glycol were added. The solution was thoroughly mixed and dispensed into syringes. At 3 h after the third preliminary period milking (milking –1), i.e., 10 h before endotoxin administration, trilostane infusion was started. The infusion pump was adjusted to give a 2.5 ml/h (20 mg of trilostane/h) flow rate from the syringe through silastic tubing into the jugular catheter. Trilostane infusion was continued until 11 h after endotoxin administration (milking 1) at which time catheters were removed. Cows showed no clinical symptoms in response to trilostane infusion other than mild discomfort from the jugular catheter.

Porcine ACTH (150 IU) in 16% gelatin (Organics/LaGrange, Chicago, IL) was given
intramuscularly 2 h after the morning milking (milking 0).

Assays

Analyses for milk composition, SCC, NAGase, bovine serum albumin (BSA), lactoferrin, and serum cortisol and glucose were conducted according to routine methods as described in detail previously (15, 19).

Statistical Analysis

Baseline values for all parameters were determined during a 2-d preliminary period prior to treatment (milking -3, -2, -1, and 0). To correct data for initial differences among cows and quarters, all data were converted to percentages by dividing all values by arithmetic preliminary period means for the corresponding cow and quarter. Data for SCC, NAGase,
BSA, lactoferrin, and cortisol were logarithmically transformed. All treatment comparisons were made by \( t \) test (14). An \( F \) test of variance equality was made at \( P = 0.05 \), and the appropriate \( t \) test was used for treatment comparisons at each milking. Milk parameter data for all quarters receiving the same treatment within each cow were averaged after conversion to percentages and logarithmic transformation. These data were then used for between cow treatment comparisons. For all tests, \( P = 0.05 \) was used to determine statistical significance.

RESULTS

Experiment 1

In the first experiment, effects of trilostane infusion to three normal lactating cows were studied to identify possible adverse effects of the trilostane, the diluent, or the stress of the continuous infusion, which may interfere with any beneficial effects of trilostane during endotoxin mastitis. The responses in these cows were compared with those in three untreated control cows. This experiment was conducted in the same manner with respect to infusion time and duration as if all cows received intramammary endotoxin treatment (see Experiment 2).

Overall, trilostane infusion had only mild, detrimental effects in nonmastitic cows. Milk production, milk fat, and protein composition were lower at some milkings in trilostane cows; these differences were significant (\( P < 0.05 \)) for milk production and protein composition (Figure 1). The lactose content of milk and parameters of mammary inflammation were unaffected (data not shown). Trilostane also had no effect on basal temperature and serum cortisol concentration, which were roughly 38.7°C and 4 ng/ml in all cows throughout this experiment.

![Figure 3](image3.png)  
Figure 3. Milk production by endotoxin-infused and uninfused quarters of control and trilostane cows. All cows received 10 \( \mu \)g of endotoxin in two homolateral quarters 1 h after milking 0 (arrow).

![Figure 4](image4.png)  
Figure 4. Fat, protein, and lactose composition of milk from infused quarters of control and trilostane cows. All cows received 10 \( \mu \)g of endotoxin in two homolateral quarters 1 h after milking 0 (arrow).
Experiment 2

In the second experiment, effects of trilostane infusion on lactational, inflammatory, and systemic responses to intramammary endotoxin treatment were determined in three cows. Three control cows received endotoxin, but they were not catheterized and did not receive trilostane. Trilostane infusion was begun 10 h before endotoxin treatment and continued until 11 h after treatment. This infusion protocol was designed to achieve effective concentrations of trilostane at the time of endotoxin treatment and maintain those concentrations for at least 11 h after treatment. The acute response, which includes the cortisol response, is complete within roughly 11 h following intramammary endotoxin treatment (6, 17).

All cows showed the usual swelling and tenderness in infused quarters in response to the intramammary endotoxin infusion. Trilostane effectively blocked the cortisol response to endotoxin (Figure 2). Only a small increase in serum cortisol was detected in trilostane cows compared with a marked response in the controls \( P < .05 \), but temperature responses did not differ \( P > .05 \). Trilostane cows had nonsignificantly \( P > .05 \) higher serum glucose concentrations following endotoxin treatment. Trilostane failed to prevent the decline in milk production, and milk production in either infused or uninfused quarters did not differ \( P > .05 \) between trilostane and control cows (Figure 3). In fact, the decline was slightly greater in trilostane cows. Fat, protein, and lactose composition of milk from infused (Figure 4) and uninfused (Figure 5) quarters did not differ \( P > .05 \) between trilostane and control cows, but those parameters tended to be lower in trilostane cows. Although differences were not significant \( P > .05 \), increases in SCC, NAGase, and lactoferrin in milk of infused quarters were 50 to 100% greater in trilostane cows (Figures 6 and 7). In contrast, milk BSA increased simi-
larly in control and trilostane cows. Inflammatory responses were not apparent in uninfused quarters of all cows, and little difference was apparent between trilostane and control cows (data not shown).

**Experiment 3**

Failure to reduce mastitic hypogalactia by blocking the elevated serum cortisol concentration suggested that cortisol does not mediate inhibition of milk production during endotoxin mastitis. To study this possibility more completely, a cortisol response similar to that during endotoxin mastitis was induced in three lactating cows by a single intramuscular injection of 150 IU of ACTH 2 h after the fourth preliminary period milking (milking 0). Three control cows remained untreated.

The ACTH injection immediately induced a significant \((P < .05)\) increase in serum cortisol concentration, which was similar in magnitude and duration to that during endotoxin mastitis (Figures 1 and 8). Milk production (Figure 9) did not differ between ACTH and control cows \((P > .05)\). For unknown reasons, a delayed decrease \((P < .05)\) in the fat content of milk occurred in ACTH-treated cows. The ACTH had no effect on protein or lactose composition of milk (data not shown). Average SCC remained below \(100 \times 10^3\) cells/ml throughout the experiment with no significant difference between treatment groups (also not shown).

**DISCUSSION**

The specificity of trilostane for inhibiting the cortisol response is indicated by its failure to affect the pyrexia. In addition, inhibition of endogenous glucocorticoid secretion did not lead to hypoglycemia, which could have adversely affected milk production. These experiments clearly demonstrate that elevated serum cortisol concentration is not necessary for the suppression of milk production during endotoxin mastitis and that a cortisol response similar to that during endotoxin mastitis is not sufficient for the suppression of milk production. Therefore, other mediators or physiologic changes must be involved in the hypogalactia associated with endotoxin-induced mastitis and probably with infectious mastitis as well.

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**Figure 7.** Bovine serum albumin (BSA) and lactoferrin concentration of milk from infused quarters of control and trilostane cows. All cows received 10 μg of endotoxin in two homolateral quarters 1 h after milking 0 (arrow).

**Figure 8.** Serum concentrations of cortisol in control and ACTH-treated cows before and .5, 1, 2, 4, and 9 h after injection of 150 IU of ACTH (arrow).
ACTH seemed to cause a mastitic episode as milk SCC increased, making it difficult to delineate the hypogalactic effects of ACTH from those of mastitis (20). The ACTH treatment may have aggravated a subclinical mammary infection to cause a more severe mastitis and a subsequent decline in milk production. Cows harboring chronic *Listeria monocytogenes* intramammary infections developed severe mastitis following dexamethasone injection (23). In that study, bacterial and leukocyte shedding in milk increased, but milk production declined. The severe mastitic episode presumably resulted from glucocorticoid-induced inhibition of mammary defenses. These studies suggest that the hypogalactic effect of ACTH and glucocorticoids could be mediated indirectly via inflammation. Milk SCC did not increase following ACTH treatment in our experiment, which may partially explain why milk production was unaffected. Furthermore, in many of the ACTH studies, multiple doses of ACTH were administered. In one study, it was shown that single injections of 100 or 200 IU of ACTH failed to affect milk production (2), which agrees with our findings. However, higher doses or multiple injections of ACTH did suppress milk production in the same study (2).

**CONCLUSIONS**

The cortisol response during endotoxin mastitis is neither a necessary nor a sufficient stimulus to mediate the hypogalactia during endotoxin-induced mastitis, and, therefore, other pathophysiological mechanisms must be involved. The cortisol response may moderate the mastitic response to intramammary endotoxin infusion and thereby reduce hypogalactia.

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REFERENCES


