**Lactational Events Related to Glucocorticoid Binding in Bovine Mammary Tissue**

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**ABSTRACT**

Various synthetic glucocorticoids reduced binding of tritiated cortisol and tritiated dexamethasone to 700 x g supernatant and precipitate fractions of mammary tissue slices from lactating cows. Unlabeled progesterone, testosterone, and 17β-estradiol had no effect on tritiated glucocorticoid binding in mammary tissue slices. Cortexolone, cortisol, triamcinolone, and dexamethasone inhibited [carbon-14]glucose incorporation into mammary tissue slices from lactating cows in a dose response relationship. Glucocorticoid binding to 700 x g supernatant and precipitate fractions of mammary tissue was correlated with the ability of glucocorticoids to reduce [carbon-14]glucose uptake into mammary tissue slices from lactating cows. In experiments designed to measure mammary uptake of glucocorticoids, the differences in concentration of total glucocorticoids between external pudic artery and mammary vein were greatest at 6 (5.8 ng/ml) and 12 (2.9 ng/ml) min after the start of milking. These intervals correspond to the times when glucocorticoid concentrations in serum were maximal after application of the milking stimulus. We conclude that glucocorticoid uptake and binding are associated with lactational events.

**INTRODUCTION**

Receptors for glucocorticoid hormone are in mammary tissue and cultured mammary cells. For example, mammary tissues of lactating rat (4, 6), mouse (13), vole (19), and cow (7, 8, 9) were capable of binding specifically either triamcinolone acetonide, dexamethasone, or cortisol. Mammary tumors of rat (5, 6) and mouse (14) and bovine mammary cells cultured in vitro (17) also bound glucocorticoids. Binding was specific because it was reduced by unlabeled glucocorticoids, and binding sites were saturated at physiological concentrations of hormone. The mechanism whereby glucocorticoids affect mammary tissue is unknown, but binding of hormone to receptor molecule(s) is probably the initial event. No reports relate specific mammary uptake and binding of glucocorticoids to lactational events. This paper describes our attempts to relate glucocorticoid binding and uptake by bovine mammary tissue to events associated with lactation.

**MATERIALS AND METHODS**

Triamcinolone and Cortexolone Competition

Experiments were to determine if the glucocorticoids, triamcinolone (9α-fluoro-11β, 16α, 17, 21-tetrahydroxy-pregna-1, 4-diene-3, 20 dione), and cortexolone (11-deoxy cortisol) would reduce the binding of [3H]cortisol and [3H]dexamethasone in tissue slices from lactating cows as for unlabeled cortisol and dexamethasone (7, 9). Cortexolone and triamcinolone were chosen because of reports (11, 18) that cortexolone was biologically inactive; i.e. it did not affect glucose uptake into thymus tissue, but it was an avid competitor for binding sites of triamcinolone or cortisol.

Preparation of mammary tissue before incubation with hormones was described (7). Briefly, mammary tissue slices from four lactating Holstein cows were incubated for 1 h at 37 C in...
Medium 199: Eagle’s Minimal Essential Medium containing either $2.7 \times 10^{-9}\text{M} [1,2,3\text{H}]$ cortisol (44 Ci/m mol, New England Nuclear) or $2.7 \times 10^{-9}\text{M} [1,2,4\text{H}]$ dexamethasone (5.2 Ci/m mol, Schwartz Mann) plus ethyl alcohol; or $2.7 \times 10^{-9}\text{M} [3\text{H}]$ cortisol or $[3\text{H}]$ dexamethasone plus $6.7 \times 10^{-8} (\text{final concentration})$ unlabeled triamcinolone, cortexolone, cortisol, dexamethasone, progesterone, or 17\beta-estradiol. Each steroid combination was tested in quadruplicate for each cow. After incubation, the tissues were washed, homogenized, separated into $700 \times g$ supernatant and precipitate fractions and data expressed as dpm/\mu g total DNA as in (7).

### Uptake of $[14\text{C}]$ Glucose

After it was established that triamcinolone and cortexolone reduced binding of $[3\text{H}]$ cortisol and $[\text{H}]$ dexamethasone in mammary tissue slices, we designed experiments to determine the effects of these glucocorticoids on a physiological event associated with lactation. The effect of glucocorticoids on mammary glucose uptake was chosen.

Mammary tissue slices from each of four lactating Holstein cows were used. One cow was used per day. Eight mammary slices (weighing a total of approximately 500 mg) were dispensed into each of 30 tubes containing 3 ml of Tris-EDTA buffer (.01 Trizma Base (Sigma), and .1 M ethylenediamine tetra acetic acid, adjusted to pH 7.4 with 6 N HCl). Ethyl alcohol (20 \mu l; 100%) was added to six tubes. Cortexolone, triamcinolone, dexamethasone, or progesterone, each at final concentrations of $10^{-8}$, $10^{-7}$, $10^{-6}$, $10^{-5}$, or $10^{-4}\text{M}$, were added to the remaining 24 tubes. Steroids were added in a total volume of 20 \mu l ethyl alcohol. Each set of 30 tubes was replicated four times for each cow with mammary tissue from a different area. Tissues were incubated with ethyl alcohol (control) or steroids for 1 h at 37 C. After 1 h of incubation .5 \mu Ci (.001mM) of $[U-14\text{C}]$ D-glucose (150 mCi/m mole, New England Nuclear) were added to each tube, and each tube was reincubated for 30 min at 37 C. Then tissues were washed three times at 4 C in Tris-EDTA buffer, homogenized in 2 ml of buffer, and centrifuged at 700 x g. The supernatant fractions were counted for radioactivity as described above. Data were expressed as percent inhibition of total $[14\text{C}]$ glucose uptake into 700 x g supernatants with ethyl alcohol controls at 0%.

### Glucocorticoid Competition and $[14\text{C}]$ Glucose Uptake

Natural and synthetic glucocorticoids reduced $[3\text{H}]$ cortisol binding in mammary tissue slices from lactating cows, and these glucocorticoids also reduced $[14\text{C}]$ glucose uptake into mammary slices from lactating cows. However, there was considerable variation among steroids in their ability to bind specifically to mammary tissue and in their ability to reduce glucose uptake. Therefore, the purpose of this experiment was to relate glucocorticoid binding activity of various steroids with quantity of $[14\text{C}]$ glucose uptake.

Mammary tissue slices were from four lactating Holstein cows. Binding activity was determined in the mammary slices as described in the first experiment. Thus, tissue slices (sets of eight weighing approximately 500 mg) were incubated for 1 h at 37 C with either $2.7 \times 10^{-9}\text{M} [3\text{H}]$ cortisol alone, or with $2.7 \times 10^{-9}\text{M} [3\text{H}]$ cortisol plus unlabeled progesterone, cortexolone, triamcinolone, cortisol, or dexamethasone at a final concentration of $1 \times 10^{-6}\text{M}$. Each combination of the steroids was tested on quadruplicate sets of tissue. Additional details of the methods were as described in the section on triamcinolone and cortexolone competition. Radioactivity was quantified in the 700 x g supernatant and precipitate fractions. Glucocorticoid binding activity was determined by subtracting the dpm of cortisol/\mu g DNA in each tissue slice set treated with $[3\text{H}]$ cortisol plus unlabeled steroids from the dpm cortisol/\mu g DNA in tissue slices treated with $[3\text{H}]$ cortisol plus ethyl alcohol.

In the second experiment quadruplicate sets of mammary tissue slices from each of the same cows in the first experiment in this section were incubated with $1 \times 10^{-6}\text{M}$ of progesterone, cortexolone, triamcinolone, cortisol, or dexamethasone. After 1 h of incubation, .5 \mu Ci of $[14\text{C}]$ glucose was added to the tubes containing tissue samples and steroids, and the mixture was reincubated for 30 min at 37 C. Controls were treated with ethyl alcohol and $[14\text{C}]$ glucose as described in the section on $[14\text{C}]$ glucose uptake. Tissue slices were washed three times at 4 C in Tris-EDTA buffer, homogenized in 2 ml of the buffer, and centrifuged at 700 x...
g. Supernatant fractions were counted for radioactivity.

**Mammary Uptake of Glucocorticoids**

Six lactating, nonpregnant Holstein cows were cannulated in the external pudic artery and in the subcutaneous abdominal mammary vein. Ten milliliters blood samples were collected during standing at the following intervals prior to and after milking: -30, -15, -6, 0, 6, 12, 16, 30, and 60 min. Arterial (A) and venous (V) blood samples were assayed for total glucocorticoids as described by Smith et al. (15).

**Statistical Analyses**

Statistical analyses of hormone competition experiments were two-way analyses of variance with individual animals as blocks and hormones as treatments. Dunnett’s “t” test (3) was used to compare control means ([3 H] cortisol or [3 H] dexamethasone plus ethyl alcohol) with individual treatment means ([3 H] cortisol or [3 H] dexamethasone plus unlabeled hormones). Glucocorticoid binding and [14 C] glucose uptake were related by linear regression. The variable X represented the difference for each treatment in competition experiments. This value was obtained by subtracting the dpm/μg total DNA of treatments from that of the ethyl alcohol control. The variable Y represented the difference for each treatment in [14 C] glucose experiments. Again, this value was obtained by subtracting the dpm glucose/μg total DNA in tissues treated with steroids from dpm glucose/μg DNA in tissues treated with ethyl alcohol.

The significance of treatments (i.e. milking time) in A-V difference experiments was examined by applying Students “t” statistic on the null hypothesis that the A-V difference was 0 at each interval of milking.

**RESULTS**

**Triamcinolone and Cortexelone Competition**

As compared with ethyl alcohol controls, unlabeled triamcinolone, cortexelone, cortisol, and dexamethasone added to mammary slices reduced (P<.01) the binding of [3 H] cortisol in 700 × g supernatant fractions by 32.1, 31.1, 34.9, and 37.7%, and by 50.0, 44.4, 53.6, and 61.1% in 700 × g precipitates (Table 1). However, progesterone and 17β-estradiol did not reduce binding of [3 H] cortisol in either fraction (Table 1).

When [3 H] dexamethasone was used in place of [3 H] cortisol and the various unlabeled

<table>
<thead>
<tr>
<th>Unlabelled hormone</th>
<th>[3 H] cortisol dpm/μg total DNA</th>
<th>[3 H] dexamethasone dpm/μg total DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>700 × g supernatant</td>
<td>700 × g precipitate</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>72c</td>
<td>.9c</td>
</tr>
<tr>
<td>Cortexelone</td>
<td>73c</td>
<td>1.0c</td>
</tr>
<tr>
<td>Cortisol</td>
<td>69c</td>
<td>.8c</td>
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<tr>
<td>Dexamethasone</td>
<td>66c</td>
<td>.7c</td>
</tr>
<tr>
<td>Progesterone</td>
<td>100</td>
<td>1.6</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>105</td>
<td>1.9</td>
</tr>
<tr>
<td>Ethyl alcohol control</td>
<td>106</td>
<td>1.8</td>
</tr>
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</table>

aConcentration of unlabeled steroids was 6.7 × 10⁻⁸ M in ethyl alcohol while the concentration of [3 H] cortisol and [3 H] dexamethasone was 2.7 × 10⁻⁸ M in ethyl alcohol.

bLess than ethyl alcohol control (P<.05).

cLess than ethyl alcohol control (P<.01).

dTotal DNA was measured in 700 × g precipitate after ethyl alcohol extraction of [3 H] glucocorticoids and was used to adjust data in 700 × g supernatant and precipitate fractions.
hormones were added to mammary slices, triamcinolone, cortexolone, cortisol, and dexamethasone reduced the binding of $[^{3}H]$ dexamethasone in $700 \times g$ supernatants by 8.0, 6.7, 9.3, and 14.0%, and by 42.3, 36.5, 51.9, and 76.9% in $700 \times g$ precipitates (Table 1). Progesterone and 17β-estradiol did not affect $[^{3}H]$ dexamethasone binding in $700 \times g$ supernatant or precipitate fractions.

Uptake of $[^{14}C]$ Glucose

Cortexolone, cortisol, triamcinolone, and dexamethasone reduced $[^{14}C]$ glucose in mammary slices from lactating cows (Fig. 1). A reduction in mammary slice $[^{14}C]$ was apparent with all glucocorticoids. Progesterone did not inhibit $[^{14}C]$ glucose uptake. As the concentration of glucocorticoid increased, inhibition of $[^{14}C]$ glucose uptake increased in a dose response relationship (Fig. 1). At concentrations of $10^{-4}$, cortisol and dexamethasone reduced uptake of $[^{14}C]$ glucose into mammary tissue from lactating cows (Fig. 1). A reduction in mammary slice $[^{14}C]$ was apparent with all glucocorticoids. Progesterone did not inhibit $[^{14}C]$ glucose uptake. As the concentration of glucocorticoid increased, inhibition of $[^{14}C]$ glucose uptake increased in a dose response relationship (Fig. 1). At concentrations of $10^{-4}$, cortisol and dexamethasone reduced uptake of $[^{14}C]$ glucose into mammary tissue by approximately 55% (2.3 × $10^{-9}$ moles $[^{14}C]$ glucose/µg DNA) as compared with ethyl alcohol controls (5.0 × $10^{-9}$ moles $[^{14}C]$ glucose/µg DNA). Triamcinolone and cortexolone reduced glucose uptake 48% (2.6 × $10^{-9}$ moles $[^{14}C]$ glucose/µg DNA), and 30% (3.5 × $10^{-9}$ moles $[^{14}C]$ glucose/µg DNA) at concentrations of $10^{-4}$ M as compared with ethyl alcohol controls (Fig. 1). Approximately 5% of the applied $[^{14}C]$ glucose disappeared during the 30 min incubation of controls, which suggested that glucocorticoids were inhibiting uptake of $[^{14}C]$ glucose into mammary cells. Additional studies are required to determine the extent of $[^{14}C]$ glucose uptake which is manifested in tissue CO$_2$ production and incorporation of glucose into milk components.

Glucocorticoid Competition and $[^{14}C]$ Glucose Uptake

Least squares regression analysis revealed that steroid reduction of $[^{3}H]$ cortisol binding into $700 \times g$ supernatant fractions of mammary tissue from lactating cows was related linearly ($r^2 = .99$) to steroid reduction of $[^{14}C]$ glucose uptake (Fig. 2). Progesterone was least effective in reducing mammary $[^{3}H]$ cortisol binding and $[^{14}C]$ glucose uptake while dexamethasone was most effective (Fig. 2).

Reduction of $[^{3}H]$ cortisol binding in $700 \times g$ precipitates was also linearly related ($r^2 = .91$) to steroid inhibition of $[^{14}C]$ glucose uptake into $700 \times g$ supernatant fractions of tissue slices (Fig. 3). Again progesterone was least...
FIG. 3. Relationship of glucocorticoid binding in 700 × g mammary precipitate and [14C]glucose uptake in mammary tissue from lactating cows. $\Delta_2 = [{}^{3}\text{H}]$ dpm/μg total DNA in 700 × g precipitate of ethyl alcohol control minus [{}^{3}\text{H}] dpm/μg total DNA of radioactive plus unlabeled steroid in 700 × g precipitate fraction. $\Delta_3 = [{}^{14}\text{C}]$ dpm/μg total DNA of ethyl alcohol control minus [{}^{14}\text{C}] dpm/μg total DNA of radioactive glucose plus unlabeled steroid. Each point represents the mean of four cows.

Effective and dexamethasone most effective in reducing both [{}^{3}\text{H}] cortisol binding and [{}^{14}\text{C}] glucose uptake in mammary tissue.

**Mammary Uptake of Glucocorticoids**

Serum glucocorticoids from external pudic artery samples averaged 6.7 ± 1.3 ng/ml (baseline ± SE) prior to the initiation of milking (Table 2). During milking, arterial glucocorticoids increased to 13.1 ± 1.1 ng/ml and continued to increase until 12 min after the initiation of milking to 17.3 ± 1.0 ng/ml. After this interval, glucocorticoids decreased to 3.6 ± 1.0 ng/ml 60 min after the initiation of milking.

Serum glucocorticoids from mammary vein samples averaged 6.6 ± 1.2 ng/ml prior to the initiation of milking (Table 2). At the start of milking, venous glucocorticoids increased to 14.8 ± 1.4 ng/ml, dropped slightly at 6 min, then increased again at 12 min after the initiation of milking to 14.5 ± 1.1 ng/ml. After this interval, venous glucocorticoids decreased to 3.6 ± 1.0 ng/ml 60 min after the initiation of milking. Differences (A-V) in glucocorticoids were greatest at 6 and 12 min after the initiation of milking. The mean A-V differences at these intervals were 5.8 ng/ml ± 2.4 and 2.9 ng/ml ± 2.4 (Table 2).

<table>
<thead>
<tr>
<th>Blood serum sample</th>
<th>Time relative to milking</th>
<th>(Total glucocorticoids, ng/ml)</th>
<th>Arterial-venous difference</th>
<th>Arterial serum difference</th>
</tr>
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<tbody>
<tr>
<td>-30</td>
<td>0a</td>
<td>13.1</td>
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<td>0a</td>
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</tr>
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<td>90</td>
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<td>1.9</td>
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<tr>
<td>180</td>
<td>0a</td>
<td>5.0</td>
<td>2.1</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*Indicates difference was greater than zero (P < .10).

aStandard error was ± 0.5 ng/ml.

bGreater than zero (P < .10).

cWors than zero (P < .10).

dMilking was stopped and continued for approximately 5 min.
DISCUSSION

Synthetic glucocorticoids, triamcinolone, and cortexolone were capable of reducing the uptake of \[^{3}H\] cortisol and \[^{3}H\] dexamethasone into mammary tissue slices from lactating cattle. The percent reduction in uptake of radioactive glucocorticoid was similar for both synthetic steroids in 700 x g supernatant and precipitate fractions of mammary tissues (Table 1). However, the percent reduction in uptake of radioactive glucocorticoids, triamcinolone, and cortexolone was less as compared with the natural glucocorticoid, cortisol, or the fluorinated-synthetic glucocorticoid, dexamethasone. Thus, these findings extend to cortexolone and triamcinolone our previous reports of the efficacy of unlabeled dexamethasone and cortisol to reduce binding of \[^{3}H\] cortisol and \[^{3}H\] dexamethasone in mammary tissue slices from cattle in a variety of physiological states (7, 9).

The present data also extend to lactating cattle the findings of others in lactating rats (5), mice (13), and voles (19) that unlabeled glucocorticoids reduce binding of triamcinolone acetone and dexamethasone in mammary tissues. However, other nonglucocorticoid hormones also reduced uptake of glucocorticoids in the laboratory species. Progesterone, for example, reduced binding of glucocorticoids in rat (5), mouse (13), and vole (19) mammary tissues, and progesterone or 17α-hydroxy progesterone reduced binding of cortisol in cultured bovine mammary cells (17). In this study, progesterone failed to reduce \[^{3}H\] cortisol and \[^{3}H\] dexamethasone binding, confirming our previous reports (7, 9).

Cameron et al. (1) demonstrated that glucocorticoids alone with other mammotrophic hormones regulate uptake of \[^{14}C\] glucose into mammary explants of midpregnant mice. Furthermore, Turnell et al. (18) demonstrated that glucocorticoids inhibited \[^{14}C\] glucose incorporation into thymocytes. Hartmann and Kronfeld (10) showed that dexamethasone decreased mammary A-V differences in glucose concentrations and decreased milk yield. Our study (Fig. 1) is the first evidence that glucocorticoids may inhibit glucose uptake directly at the mammary tissue. Since glucose is an important substrate in the synthesis of milk lactose and is thought to be regulated by a complex of mammotrophic hormones including glucocorticoids (2), at least partially, the data may explain why elevated glucocorticoids usually inhibit milk production (16).

This study further suggests that glucocorticoid binding may be related to regulation of glucose metabolism in mammary tissue slices from lactating cows. For example, reduction of glucocorticoid uptake and binding into 700 x g supernatant or precipitate fractions of mammary tissue from lactating cows by unlabeled steroids was related to steroid reduction of \[^{14}C\] glucose uptake into 700 x g supernatant fluids (Fig. 1). Progesterone was least effective and dexamethasone most effective in reducing both \[^{3}H\] cortisol and \[^{14}C\] glucose uptake, further suggesting a strong relationship between glucose uptake and glucocorticoid binding. Serum glucocorticoids normally fluctuate in the physiological range of \(10^{-8}\) to \(10^{-7}\) M, and within this range \[^{14}C\] glucose was reduced 4 to 15% by the glucocorticoids with greatest biological activity. The biologically inactive cortexolone (11, 18) was effective at doses above physiological concentrations.

In vivo experiments designed to measure differences in A-V concentration of glucocorticoid across the mammary gland suggested that glucocorticoids were taken up by the mammary gland shortly after onset of milking. In these experiments blood in the subcutaneous abdominal vein may have been diluted with blood from the external pudic vein. Furthermore, it was not possible to prove that all of the conditions proposed by Zierler (20) were met to obtain valid estimates of the arteriovenous differences in serum glucocorticoids; thus, these data should be interpreted with caution. Nonetheless, the mean A-V differences at 6 and 12 min after the start of milking (5.8 ng/ml and 2.9 ng/ml) were comparable to A-V differences in glucocorticoids observed by Patterson and Linzell (12). Whether the uptake was specific to the mammary gland and represented utilization of glucocorticoids for milk secretion or was merely nonspecific and a result of increased arterial glucocorticoid concentrations remains to elucidated. The rise in arterial and venous concentrations of glucocorticoids at 15 and 6 min before milking was believed to be a result of exteroceptive stimuli induced by the milker or milking unit. Greatest concentrations of serum glucocorticoids occurred at 12 min after milking was initiated. Similar results have been
reported by Smith et al. (15), who showed that the milking stimulus per se and exteroceptive stimuli may cause increased serum glucocorticoids in cows.

Binding and uptake of glucocorticoids appear to be related to glucose uptake and milking and form a basis for further studies to determine the specific role glucocorticoids play in lactation.

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REFERENCES