Adrenocorticotropin Alteration of Bovine Peripheral Plasma Concentrations of Cortisol, Corticosterone, and Progesterone

F. C. GWAZDAUSKAS, W. W. THATCHER, and C. J. WILCOX
Department of Dairy Science, University of Florida, Gainesville 32601

Abstract

Seven Holstein cows, 60 to 90 days postpartum, received either 200 IU ACTH (n=4) or saline (n=3) intravenously with treatments reversed 2 days later. Blood samples (30 ml) were collected from indwelling jugular catheters at -60, 0 pre-injection, and 5, 15, 30, 45, 60, 120, 180, and 240 min post-injection. Solvent extraction, purification by Sephadex LH-20 column chromatography and competitive protein-binding analyses were used to isolate and quantify plasma cortisol, corticosterone, and progesterone. Mean cortisol (ng/ml) at the respective sampling times was 3.1 vs. 3.6 (saline vs. ACTH), 4.9 vs. 4.1, 5.9 vs. 33.7, 8.8 vs. 30.0, 4.8 vs. 45.8, 3.3 vs. 28.8, 3.2 vs. 31.3, 4.4 vs. 36.6, 2.6 vs. 26.8 and 5.2 vs. 34.7. Both the magnitude of the cortisol response and the interval to maximum response to ACTH varied among cows (P<.01). Adrenocorticotropin elicited a corticosterone increase (P<.01) concurrently with the cortisol increase, but it was of lower magnitude, reaching 7.6 ng/ml at 30 min. Mean progesterone at the respective times was 1.7 vs. 2.1, 2.4 vs. 2.0, 2.0 vs. 3.1, 2.0 vs. 4.5, 2.2 vs. 4.2, 2.5 vs. 4.2, 2.2 vs. 3.9, 2.3 vs. 2.4, 2.6 vs. 2.8 and 2.2 vs. 2.0 ng/ml, and the increase due to ACTH administration was significant (P<.025) between 15 to 60 min post-injection. Results suggest that the adrenal contributes a significant proportion of plasma progesterone.

Introduction

Estergreen and Venkataseshu (2) identified cortisol and corticosterone as the principal glucocorticoids in bovine jugular vein plasma. Progesterone concentration in adrenal venous blood of the bovine was 2 to 10% as much as glucocorticoid (1), although the physiological significance of this adrenal progesterone is uncertain. Several workers (8, 9, 10, 12) attempted to relate bovine peripheral plasma concentration of 17-hydroxycorticosteroids to various physiological states during lactation and pregnancy. Riegle and Nellor (7) found no changes in plasma cortisol or corticosterone with advancing age in bulls; however, they detected a decrease in adrenal responsiveness to adrenocorticotropic (ACTH). Wagner and Oxenreider (15) reported that plasma glucocorticoid reached 8.3 ng/ml plasma during milking and at 15 min after milking, compared to pre-milking of 5.4 ng/ml plasma in the cow. In response to 200 IU ACTH given intramuscularly, plasma cortisol concentrations were more than doubled at 1 hr and were still slightly elevated at 3 hr post-injection (14). Wiersma and Stott (16) suggested that increased progesterone secreted from the adrenal in response to a stressor caused an imbalance of hormones such that the uterus and embryo become incompatible.

The objectives of this study were to evaluate effects of an intravenous injection of ACTH on peripheral plasma concentrations of progesterone, cortisol, and corticosterone in lactating dairy cows.

Materials and Methods

Seven Holstein cows, 60 to 90 days postpartum, and less than 5 years of age were used in a single reversal trial. All cows had exhibited at least one estrus and were distributed: 4 between estrous cycle Days 1 to 7, 2 between Days 9 to 13 and 1 cow 34 to 36 Days of pregnancy. After 3 weeks of adaptation in which the animals were exposed to frequent handling in the stanchion barn, 15 cm of polyethylene tubing (Clay-Adams Intramedic PE 90/S12) were inserted into the jugular vein through a 14 gauge needle 24 hr prior to treatment. Each catheter was fitted with an
adapter (Clay-Adams plastic tubing adapter, size B), filled with a heparinized saline solution (15 U heparin/ml of .9% NaCl) and closed with a Leur-Lok cap (Clay-Adams). At initiation of the experiment (Day 1), four cows were given intravenously 200 IU ACTH (Adrenomone, Armour-Baldwin Laboratories) dissolved in 10 ml of .9% saline and 3 cows received 10 ml of .9% saline between 10 to 11 AM. On Day 3 of the experiment, treatments were reversed. Blood samples (30 ml) were taken via the jugular catheter at 60 min prior to injection, immediately prior to injection, and at 5, 15, 30, 45, 60, 120, 180, and 240 min post-injection without visible signs of stress to the animals. Blood samples were collected into heparinized syringes, immediately placed into an ice bath, centrifuged at 12,000 xg for 10 min at 4 C, and blood plasma was stored at -20 C until analysed for cortisol, corticosterone, and progesterone.

For analysis of steroids, 4,500 dpm of corticosterone 1, 2, 3H (Amersham/Searle, 41.5Ci/mM), 4,500 dpm cortisol 1, 2, 3H (Amersham/Searle, 36.7Ci/mM) and 4,500 dpm progesterone 1, 2, 3H (Amersham/Searle, 41 Ci/mM) were added to 2 to 4 ml of plasma, and the plasma was extracted vigorously with five volumes of freshly distilled 2, 2, 4-trimethylpentane (iso-octane). The remaining plasma residue was extracted with five volumes of freshly distilled methylene chloride. Both progesterone, in the iso-octane extract, and the corticoids, in the methylene chloride extract, were isolated by chromatography on 2 X 27 cm Sephadex LH 20 columns. Progesterone was separated from any 20β-hydroxy-pregnen-4-en-3-one, that might have been co-extracted if present, with a heptane:chloroform:ethanol:water (200:200:1: sat) solvent elution system (Fig. 1). Corticosterone and cortisol were isolated by a chloroform:ethanol (91:9) solvent elution system (Fig. 2). Fractions (5.0 ml) were collected from the column, pooled, and aliquots of isolated steroids were quantified by competitive protein binding assay (5, 6). Procedural losses of specific steroids were calculated from recovery of the radioactive steroids added before extraction.

Analysis of variance and multiple regression analyses were used to evaluate effects of treatment (ACTH), cow and time, and their various interactions, on steroid concentrations.

**Results and Discussion**

Aliquots of column eluant blanks, equal to steroid elution volumes, did not alter (P > .05) the competitive protein binding standard curves for cortisol or corticosterone. Regression equations for cortisol standard curves with and without the addition of column eluant blanks were Y (% binding) = 100.8 - 52.5X + 13.7X^2 - 1.2X^3 and Y = 101.9 - 59.9X + 17.2X^2 - 1.6X^3, (X = 0 to 5 ng). The corresponding corticosterone standard curves with and without the addition of column eluant blanks were Y = 100.5 - 47.8X + 14.2X^2 - 1.4X^3 and Y = 99.6 - 47.1X + 13.6X^2 - 1.3X^3. In contrast, our laboratory has conclusively shown that solvent eluant from blank areas of thin layer chromatographic plates drastically distorted the standard displacement curves of the competitive protein binding assay (3). Therefore, thin-layer chromatography was not used for isolation of steroids. When 2, 5, and 10 ng of corticosterone and cortisol were added together to 1 ml plasma samples, the amounts recovered (X ± SE) after extraction, purification, and quantification were 1.7
ACTH CHANGES OF PERIPHERAL PLASMA

TABLE 1. Mean bovine plasma cortisol, corticosterone and progesterone prior to and after intravenous injection of 200 IU adrenocorticotropic or saline.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Cortisol (ng/ml plasma)</th>
<th>Corticosterone (ng/ml plasma)</th>
<th>Progesterone (ng/ml plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-60</td>
<td>Saline: 3.1 ± .6*</td>
<td>ACTH: 3.6 ± .7</td>
<td>Saline: 1.7 ± 1.3</td>
</tr>
<tr>
<td>0</td>
<td>Saline: 4.9 ± 1.4</td>
<td>ACTH: 4.1 ± .8</td>
<td>Saline: 2.4 ± .9</td>
</tr>
<tr>
<td>+5</td>
<td>Saline: 5.9 ± 1.4</td>
<td>ACTH: 33.4 ± 4.1</td>
<td>Saline: 2.0 ± .8</td>
</tr>
<tr>
<td>+15</td>
<td>Saline: 8.8 ± 2.6</td>
<td>ACTH: 30.0 ± 4.1</td>
<td>Saline: 2.0 ± .8</td>
</tr>
<tr>
<td>+30</td>
<td>Saline: 4.8 ± 1.1</td>
<td>ACTH: 45.8 ± 8.6</td>
<td>Saline: 2.2 ± 1.2</td>
</tr>
<tr>
<td>+45</td>
<td>Saline: 3.3 ± 1.0</td>
<td>ACTH: 28.8 ± 5.8</td>
<td>Saline: 2.5 ± .8</td>
</tr>
<tr>
<td>+60</td>
<td>Saline: 3.2 ± .7</td>
<td>ACTH: 31.3 ± 4.3</td>
<td>Saline: 2.2 ± .7</td>
</tr>
<tr>
<td>+120</td>
<td>Saline: 4.4 ± 1.0</td>
<td>ACTH: 36.6 ± 5.6</td>
<td>Saline: 2.3 ± .8</td>
</tr>
<tr>
<td>+180</td>
<td>Saline: 2.6 ± .4</td>
<td>ACTH: 26.8 ± 5.2</td>
<td>Saline: 2.6 ± .9</td>
</tr>
<tr>
<td>+240</td>
<td>Saline: 5.2 ± 1.5</td>
<td>ACTH: 34.7 ± 6.9</td>
<td>Saline: 2.2 ± .8</td>
</tr>
</tbody>
</table>

* Mean and standard error for seven cows; see text for results of statistical analyses.

The absolute responses of corticosterone to ACTH were of a lower magnitude than cortisol responses (Table 1). Plasma corticosterone increased within 5 min, reached 7.6 ng/ml plasma at 30 min, and had not returned to basal levels by 240 min. Saline injection did not alter plasma cortisol concentrations.

The concurrent increase of cortisol and corticosterone is in contrast to observations of Venkataseshu and Estergreen (14), who reported a decline in corticosterone associated with increased cortisol in response to ACTH. The positive cortisol and corticosterone systemic responses indicate increased adrenal synthesis or release of both steroids after ACTH stimulation.

The absolute responses of cortisol to ACTH were of a lower magnitude than cortisol responses (Table 1). Plasma cortisol increased within 5 min, reached 7.6 ng/ml plasma at 30 min, and had not returned to basal levels by 240 min. Saline injection did not alter plasma cortisol concentrations.

The overall ratio of cortisol:corticosterone was 7.2:1. Ranges in ratios for ACTH and saline treatment groups were 1.6 to 35.9:1 and .87 to 15.9:1. In general, the two glucocorticoids tended to vary together in response to both treatment and time. The positive response of both cortisol and corticosterone to ACTH, the relatively stable cortisol: corticosterone ratios, and the low corticosterone suggested that separation of these two glucocorticoids may not be necessary in evaluating adrenal glucocorticoid secretion in the bovine. In addition, the relatively stable cortisol and corticosterone of cows receiving saline indicates catheterization procedures provide a means to obtain basal glucocorticoid measurements.

Plasma progesterone increased (P<.05) 2.5 ng/ml plasma between 15 to 30 min after injection of ACTH (Table 1). At 2 hr post-injection, the ACTH-stimulated progesterone response, unlike that of cortisol and corticosterone, had returned to basal levels. The
magnitude of response varied significantly (P<.05) among cows. Increased progesterone, due to ACTH, occurred during the time of early corpus luteum development (Days 1, 3, and 5), during luteal phases of the estrous cycle (Days 7, 11, and 13) and during pregnancy (Day 34). However, the present experiment was not designed to evaluate differences in adrenal responsiveness associated with stage of ovarian cycle or pregnancy. Additional studies are needed to determine if differences among cows in the positive progesterone response to ACTH are related to reproductive status.

The major glucocorticoid hormones produced by adrenal are cortisol and corticosterone. The adrenal contribution of progestational, estrogenic, and androgenic compounds is small compared to contributions of other steroid producing endocrine glands (11). In adrenal, progesterone primarily is regarded as a precursor steroid for synthesis of gluco- and mineralocorticoids. The 2.5 ng/ml plasma progesterone increase following ACTH injection in our experiment was considerably less than the cortisol and corticosterone increases. Synthesis of progesterone probably could not keep pace with its fast conversion into C21-hydroxylated steroids during the 2 to 4-hr sampling period. Consequently, corticoid was still elevated at 4 hr post-injection, whereas progesterone had returned to basal levels. Our study did not differentiate source of progesterone. However, 200 IU of ACTH increased systemic plasma progesterone concentrations, and the progesterone may be of adrenal origin since the primary target tissue of ACTH is the adrenal. The ACTH preparation was presumed free of any luteinizing hormone activity since chemical extraction procedures, including heat inactivation, precluded any luteinizing hormone activity (Armour-Baldwin Laboratories, personal communication).

The significance of elevations of progesterone due to ACTH or stress has not been evaluated in cows. The acute increase in the present study is comparable to the increase in progesterone expected between Days 1 to 5 of the estrous cycle (13). Whether an increase in progesterone of this magnitude due to stress may influence reproductive function remains to be tested.

Acknowledgment

The authors are grateful to Dr. H. H. Head for technical assistance with catheterizations.

References

