Cortisol and Corticosterone in Bovine Plasma and the Effect of Adrenocorticotropic Hormone

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Abstract
Plasma levels of cortisol and corticosterone were determined in 18 lactating, non-pregnant dairy cows of three breeds and also at hourly intervals following a single injection of 200 IU ACTH to five cows and no treatment to one cow. The mean for all breeds was 7.3 μg cortisol per 100 ml plasma. The cortisol of Guernseys was 5.2 ± 2.4 (mean ± SD) μg per 100 ml plasma, which was significantly lower (P < 0.01) than in Holsteins (9.3 ± 2.4); whereas, cortisol levels in Jerseys (7.4 ± 1.5) were not significantly different from the two other breeds. The mean was 3.0 μg corticosterone per 100 ml plasma, with no significant differences between breeds. Administration of ACTH more than doubled the plasma concentration of cortisol in one hour, resulting in highly significant (P < 0.01) treatment effects between hours post-treatment, whereas only a 33% increase was found in the untreated cow. A significant (P < 0.05) linear downward trend in corticosterone level and a significant (P < 0.05) quadratic effect on the cortisol:corticosterone ratio were also found following ACTH treatment. This would appear to indicate that ACTH treatment preferentially stimulated the 17-hydroxylating system of the bovine adrenal.

Introduction
It has been well established that cortisol (11β, 17α, 21-trihydroxy-4-pregnene-3,20-dione) and corticosterone (11β, 21-dihydroxy-4-pregnene-3,20-dione) are the principal secretory products of the bovine adrenal cortex. In 1953, Bush (4) reported these two steroids were secreted in a characteristic 1:1 ratio by the bovine adrenal gland. In the same year, Balfour (1) reported that cortisol was the principal corticosteroid secreted by the adrenal of day-old calves and that corticosterone appeared in the adrenal effluent about ten days of age. He later reported that the cortisol:corticosterone ratio increased from 1.4:1 at 23 days of age to 3.9:1 at 334 days of age of dairy calves (2). More recently, Whipp et al. (18) have measured the secretory rate of corticosteroids from the adrenal vein of five calves after stimulation with ACTH. They found a mean cortisol:corticosterone secretory ratio of 5.8:1 under the conditions of their study.

Numerous investigators (9, 12, 14–16) have attempted to relate adrenocortical activity to various physiological states of cattle by measuring the 17-hydroxycorticosteroid levels in peripheral plasma, but this would not measure corticosterone, which lacks a 17-OH group. Brush (3) and Saba (13) have identified and measured cortisol in bovine jugular vein blood by paper chromatography, but neither could detect corticosterone by the methods used. In a recent abstract, Riegle and Nellor (11) reported that bovine plasma levels of cortisol and corticosterone did not change significantly with age from one month to five years, but gave no details of identification or method of analysis.

Positive identification of both corticosterone and cortisol in bovine jugular plasma has been reported by this laboratory, by solvent partition, chromatographic mobility on paper and thin-layer, as well as ultraviolet, sulfuric acid, and infrared spectrum analysis (5).

Our study was made to determine the amounts of cortisol and corticosterone in the blood plasma of lactating, nonpregnant dairy cows of three breeds and to determine the changes in concentration and ratio of these corticosteroids following administration of adrenocorticotropic hormone (ACTH). A preliminary report of part of this work was published in an abstract (17).

Experimental Procedures
Only one sample of one to two liters of blood was collected one to two months postpartum...
from the jugular vein of each of six Holsteins, six Guernseys, and six Jersey cows which were lactating and nonpregnant. All blood collections were made between 8 AM and 12 noon (six to ten hours post-milking) to minimize variation between animals from diurnal changes in corticosteroids. The blood was collected into bottles containing 10% potassium oxalate (20 ml/liter) and was immediately chilled in an ice bath and centrifuged within 30 minutes of collection. The plasma was deep-frozen and stored at -20°C until extracted, unless extracted immediately after collection.

In a second experiment, blood was collected immediately before and one, two, and three hours after a single intramuscular injection of 200 IU ACTH (Adrenomone, Armour-Baldwin Laboratories, Omaha, Nebraska) from each of two Holsteins, two Jerseys, and one Guernsey. Blood was collected at the same frequency from one untreated Holstein cow to determine the effect of frequent venipuncture on the plasma levels of cortisol and corticosterone. Four cows in this experiment were dry and nonpregnant and had been bilaterally or unilaterally ovarioctomized. One treated Holstein was lactating and pregnant, and the Guernsey cow was dry and in advanced pregnancy.

The corticosteroids were extracted from plasma with dichloromethane and purified by solvent partition and paper chromatography. Quantitative analysis was by isotope dilution and spectrophotometry. Details of the extraction, isolation, purification, and identification procedures and quantitative analysis have been published previously (5).

Results and Discussion

Plasma of untreated cows. Table 1 shows the means of cortisol and corticosterone found in jugular plasma of lactating, nonpregnant dairy cattle of three breeds. The means of cortisol within breeds ranged from 5.2 µg per 100 ml plasma in the Guernseys to 9.3 µg in the Holsteins sampled, with the Jerseys falling intermediate between these two breeds. There was a significant difference (P < 0.01) between the cortisol of Guernseys and Holsteins, but the amount in Jerseys was not significantly different from either of the other breeds.

The level of cortisol was higher than has been reported by Brush (0 to 3 µg/100 ml) (3) and Saba (0.5 µg/100 ml) (13) using different measurement techniques. Plasma 17-hydroxycorticosteroids in cattle, under a variety of physiological conditions, have also been reported by a number of authors (9, 12, 14-16) to range from 1.2 to 11.9 µg/100 ml. This compares favorably with the range of cortisol in this study.

The mean plasma corticosterone ranged from 2.4 to 3.5 µg per 100 ml in these same animals with no significant difference between breeds for this steroid. Corticosterone has only recently been identified in bovine peripheral plasma (5), and no other data are available for comparison.

The mean ratio of cortisol:corticosterone, in the cattle sampled, ranged from 1.5:1 in the Guernseys to 4.0:1 in the Holsteins. This was a significant difference (P < 0.01) in ratio between the cattle of these two breeds. The Jerseys had a hormone ratio which was intermediate and not significantly different from either of the other breeds.

In 1953, Bush (4) stated that the ratio of cortisol:corticosterone secreted by the adrenal gland was relatively constant within adults of most species. The characteristic ratios found by him were 1:1 for the bovine, 6:1 for the dog, 15 to 20:1 for sheep, and greater than 20:1 for the monkey. Balfour, Comline, and Short (2) later reported a slow but steady increase with age in the ratio of cortisol to corticosterone in the adrenal venous blood of calves sampled from 23 to 334 days of age.

The ratio of corticosteroids secreted by the adrenal gland may not necessarily be the same

<p>| Table 1. Mean plasma corticosteroids of nonpregnant, lactating dairy cows of three breeds. |</p>
<table>
<thead>
<tr>
<th>Breed</th>
<th>Number</th>
<th>Cortisol (µg/100 ml ± sd)</th>
<th>Corticosterone (µg/100 ml ± sd)</th>
<th>Cortisol:Corticosterone (Ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guernsey</td>
<td>6</td>
<td>5.2 ± 2.4a</td>
<td>3.5 ± 1.1a</td>
<td>1.5 ± 0.6a</td>
</tr>
<tr>
<td>Jersey</td>
<td>6</td>
<td>7.4 ± 1.5a,b</td>
<td>3.0 ± 1.3a</td>
<td>2.4 ± 1.2a,b</td>
</tr>
<tr>
<td>Holstein</td>
<td>6</td>
<td>9.3 ± 2.4b</td>
<td>2.4 ± 0.5a</td>
<td>4.0 ± 1.2b</td>
</tr>
<tr>
<td>All cows</td>
<td>18</td>
<td>7.3</td>
<td>3.0</td>
<td>2.8</td>
</tr>
</tbody>
</table>

a,b Means in a column with the same letter are not significantly different (P < 0.01) (Duncan's new multiple range test).

c Corticosterone is 1.0 in the ratio.
as the ratio found in peripheral blood. The levels found in the peripheral circulation would depend upon rates of distribution into body pools, utilization by tissues, and removal from the circulation by degradation and elimination. These rates may differ for the two hormones and, thus, change the ratio of the hormones after they are secreted by the adrenal gland. Thus, it is not known whether the breed differences in steroid ratio in this study represent differences in the secretion rate of these two hormones between breeds or differences in distribution, utilization, and degradation.

**ACTH administration.** Figure 1 compares plasma after administration of ACTH to cows with plasma collected from one cow not receiving ACTH. The administration of this level of ACTH more than doubled (4.1 to 8.9 μg/100 ml) the concentration of cortisol by one hour after im administration. In the cow not given ACTH the concentration increased by only one-third in the same time interval, probably due to endogenous ACTH released owing to the stress of blood collection. In both cases the cortisol declined by the second hour at approximately the same rate. In the ACTH-treated cows the cortisol was still 127% of pre-injection levels at three hours post-injection, whereas in the same interval the cortisol level had dropped to 57% of the initial level in the cow not receiving ACTH.

On the other hand, corticosterone levels held nearly constant during the first hour for both treatments and then declined to lows of 52% at two hours post-injection for the ACTH-treated animals and 61% at three hours in the nontreated animal. The ratio of cortisol to corticosterone ranged from 1:1 before to 3:1 at two hours after administration of ACTH, whereas in the untreated animal the ratio varied from 1.1 to 1.5:1 in the four blood samples taken at hourly intervals.

Analysis of variance of the plasma steroid levels in the ACTH-treated animals revealed a highly significant difference in cortisol concentration between hours post-treatment. This was illustrated by highly significant (P < 0.01) nonlinear (quadratic and cubic) effects on the cortisol level following administration of ACTH. A significant (P < 0.05) linear downward trend in corticosterone level and a significant (P < 0.05) quadratic effect on the cortisol:corticosterone ratio in bovine jugular plasma were also found following ACTH treatment. A rapid rise in cortisol (3) or 17-hydroxycorticosteroids (12, 15) following ACTH administration has also been reported by others.

The level of corticosterone in plasma was not elevated following ACTH treatment as was cortisol; in fact, it declined at two hours post-treatment. This would seem to indicate that the 17-hydroxylating system of the adrenal gland is preferentially stimulated by ACTH and leads to the synthesis of cortisol while decreasing substrate available for corticosterone, as suggested by Kass et al. (8) and reinforced by Fevold (6, 7) in experiments with rabbits. This concept is further supported by the previous finding that the cortisol:corticosterone ratio increased from 1:1 to 3:1 after perfusion of bovine adrenals with ACTH (10) and the report of a corticosteroid ratio of 5.8:1 in adrenal venous plasma of calves during intravenous infusion of ACTH (18).

The slight elevation of cortisol in the animal not treated with ACTH probably indicates a small release of endogenous ACTH due to the stress associated with blood sampling. Shaw and Nichols (15) did not find any elevation of 17-hydroxycorticosteroids in cows or calves sampled at 30-minute to one-hour intervals. However, sampling at ten-minute intervals for two hours caused an elevation of 17-hydroxycorticosteroids in 30 to 110 minutes, which could be further increased by administration of ACTH (16).
References