Serum Minerals, Leukocyte Profiles, and Plasma Corticoids in Dairy Heifers After an Injection of Corticotropin

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Abstract

Adrenal response to corticotropin administration was studied in six Holstein heifers injected intramuscularly once with 250 IU of corticotropin. Blood was sampled at 0, .25, .50, .75, 1, 2, 4, 8, and 24 hr after injection and was analyzed for plasma corticoids, total leukocytes, differential leukocytes, serum sodium, potassium, calcium, magnesium, and chloride. Plasma corticoids rose within 15 min and were associated with leukocytosis, lymphopenia, eosinopenia, hypernatremia, hyperchloremia, hypokalemia, hypomagnesemia, and hypocalcemia. Time-response and duration of these changes were compared as an index of adrenal activity.

Introduction

The experimental administration of corticotropin (ACTH), through its steroidogenic effect (10, 16), as well as a wide variety of “stressful” stimuli working through the hypothalamic-pituitary-adrenal system (16), alters several blood constituents of man and animals (2, 22, 26).

Changes in the number of circulating leukocytes, i.e., a lymphopenia (7), an eosinopenia (14), and an increase in the number of polymorphonuclear leukocytes (14), have been associated with an increase in the secretion of 11-oxygenated steroids (7, 22). Corticotropin induced secretion of adrenal glucocorticoids (10, 16) and mineralocorticoids (5, 11), caused hypernatremia and hypokalemia through renal retention of sodium and excretion of potassium as well as depression of serum calcium (3, 8) and magnesium (6). Prior to development of more sensitive techniques for measuring adrenal steroids and corticotropin, changes in the differential blood leukocyte count were bioassays for adrenal activity (22), ACTH (16) and as an index of “stress” (28).

This paper is concerned with quantifying the adrenal response to corticotropin administration in dairy cattle by comparing the time changes of plasma corticoids with changes in five serum minerals and blood leukocytes.

Methods

Six 18-month-old Holstein heifers were each given one intramuscular injection of 250 international units of porcine ACTH (corticotropin) (Sigma Chemical Company, St. Louis, Missouri). Blood samples were taken at 0 time (pre-injection), .25, .50, .75, 1, 2, 4, 8 and 24 hr post injection by jugular puncture with one heparinized and one non-heparinized “Vacutainer” (Becton-Dickinson Company, Rutherford, New Jersey 07070). Total leukocytes were counted within 30 min on the heparinized blood sample in duplicate on a double Neubauer, A. O. “Bright Line” hemacytometer. A differential leukocyte count was made on a Wrights-Giesma (Fisher Scientific Co.) stained blood smear. Two hundred leukocytes were counted from each slide. Plasma corticoids were determined by competitive protein binding method of Murphy (19) as modified by Abernethy and Stott (1). Serum sodium, potassium, calcium, and magnesium were determined on a Perkin-Elmer Model 290B atomic absorption spectrophotometer (Norwalk, Conn.) according to Analytical methods for atomic absorption spectrophotometry, 1970 (The Perkin-Elmer Corp.). Serum chloride was determined by the method of Schales and Schales (23).

Results

Injection of corticotropin increased (P<.01) plasma corticoids within 15 min (Table 1). Corticoids reached a peak by .5 hr and then showed no significant change through the 2-hr blood sample. They declined from the peak (P<.01) by 4 hr post-injection and reached pre-injection by 8 hr.

Leukocytosis was evident at 2 hr after injection and was statistically significant
### Table 1. Mean ± standard deviation of plasma corticoids and leukocytes by time period after corticotropin administration.

<table>
<thead>
<tr>
<th>Hours after injection</th>
<th>0^a</th>
<th>.25</th>
<th>.50</th>
<th>.75</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma corticoids (ng/ml)</td>
<td>7.9±4.7</td>
<td>43.9±4.5</td>
<td>57.4±7.0</td>
<td>63.0±7.6</td>
<td>60.0±8.7</td>
<td>57.4±8.9</td>
<td>22.3±5.6</td>
<td>7.4±1.7</td>
<td>12.1±5.7</td>
</tr>
<tr>
<td>WBC's cells/mm³X10^6</td>
<td>8.4±3.6^b</td>
<td>8.3±2.8</td>
<td>9.1±3.3</td>
<td>9.0±3.3</td>
<td>8.9±2.5</td>
<td>10.6±2.5</td>
<td>11.4±2.1</td>
<td>12.0±2.1</td>
<td>9.1±2.6</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>55±16</td>
<td>53±8</td>
<td>59±12</td>
<td>55±5</td>
<td>55±15</td>
<td>47±14</td>
<td>39±13</td>
<td>43±14</td>
<td>48±10</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>33±15</td>
<td>32±13</td>
<td>32±12</td>
<td>33±14</td>
<td>28±10</td>
<td>44±16</td>
<td>55±16</td>
<td>54±15</td>
<td>44±13</td>
</tr>
<tr>
<td>Lymphocyte/PMNL</td>
<td>1.7</td>
<td>1.7</td>
<td>1.8</td>
<td>1.7</td>
<td>2.0</td>
<td>1.1</td>
<td>.7</td>
<td>.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>12±5</td>
<td>12±5</td>
<td>8±4</td>
<td>10±9</td>
<td>12±8</td>
<td>8±4</td>
<td>5±4</td>
<td>3±2</td>
<td>7±4</td>
</tr>
</tbody>
</table>

^a Injected 250 IU of porcine ACTH intramuscularly.
^b Mean ± SD.

### Table 2. Mean ± standard deviation of serum minerals by time period after corticotropin administration.

<table>
<thead>
<tr>
<th>Minerals</th>
<th>0^a</th>
<th>.25</th>
<th>.50</th>
<th>.75</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (meq/liter)</td>
<td>137±2^b</td>
<td>136±2</td>
<td>140±4</td>
<td>144±8</td>
<td>142±4</td>
<td>141±2</td>
<td>143±3</td>
<td>145±6</td>
<td>133±4</td>
</tr>
<tr>
<td>Chloride</td>
<td>102±1</td>
<td>100±2</td>
<td>101±3</td>
<td>101±3</td>
<td>108±2</td>
<td>109±4</td>
<td>112±3</td>
<td>110±2</td>
<td>105±2</td>
</tr>
<tr>
<td>Potassium</td>
<td>6.59±.92</td>
<td>6.65±.71</td>
<td>4.63±.50</td>
<td>4.70±.21</td>
<td>4.84±.28</td>
<td>4.99±.40</td>
<td>4.60±.24</td>
<td>5.19±.43</td>
<td>5.20±.24</td>
</tr>
<tr>
<td>Calcium</td>
<td>5.67±.56</td>
<td>5.27±.32</td>
<td>5.38±.52</td>
<td>5.31±.40</td>
<td>4.40±.14</td>
<td>4.04±.20</td>
<td>4.10±.20</td>
<td>4.15±.20</td>
<td>3.94±.10</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.37±.06</td>
<td>1.42±.13</td>
<td>1.35±.05</td>
<td>1.31±.09</td>
<td>1.21±.07</td>
<td>1.22±.04</td>
<td>1.28±.11</td>
<td>1.21±.07</td>
<td>1.12±.03</td>
</tr>
</tbody>
</table>

^a Injected 250 IU of porcine ACTH intramuscularly.
^b Mean ± SD.
The differential leukocyte count showed lymphopenia and eosinopenia \((P < .05)\) in the 4-hr blood sample. The decline in these two leukocyte types was accompanied by an increased \((P < .05)\) percentage of circulating polymorphonuclear leukocytes in the peripheral blood. The ratio of lymphocytes to neutrophils (polymorphonuclear leukocyte) reflected the change in percentage of these two cell types (Table 1). The 24-hr blood sample still showed an altered lymphocyte to neutrophil ratio but was not statistically different from the pre-injection percentages of these two cells. Percentage of eosinophils remained low \((P < .05)\) through the 24-hr blood sample.

The serum mineral profile showed a reciprocal relationship between sodium and potassium. Sodium increased and potassium decreased \((P < .05)\) in the .5 hr post injection blood sample (Table 2). Sodium remained high \((P < .05)\) through the 8-hr sample, and returned to normal by 24 hr post injection. Potassium remained low \((P < .05)\) throughout the 24-hr test. Serum potassium increased \((P < .05)\) in the 8 and 24-hr samples relative to low levels at 1 and 2-hr, but it had not reached pre-injection by 24 hr \((P < .05)\).

Serum chloride increased \((P < .05)\) in the 1-hr sample and remained high through 8-hr and declined \((P < .05)\) in the 24-hr sample. However, the 24-hr sample was still significantly higher than at pre-injection.

Calcium and magnesium declined \((P < .05)\) in the 1-hr sample and continued to decrease \((P < .05)\) through the 24-hr blood sample (Table 2).

**Discussion**

Within .25 hr after the injection of corticotropin, plasma corticoids increased significantly, and within 2 hr after the injection, or at a time when plasma corticoids were beginning to decline, there was evidence of a leukocyte response to adrenal corticoids. This leukocyte response has been characterized by a decrease in the number of circulating lymphocytes and eosinophils and an increase in the circulating numbers of polymorphonuclear leukocytes \((4, 22, 26)\). In animals where lymphocytes rather than neutrophils are the predominant leukocyte (cattle, pigs, sheep), lymphopenia does not result from treatment with adrenal corticoids \((22)\). The results of this experiment confirm this finding. When the relative percentage of leukocytes (Table 1) is multiplied by total number of circulating leukocytes in the same time period, a graph such as Figure 1 is generated. Figure 1 shows that the number of circulating lympho-

![Fig. 1. Effect of corticotropin administration on leukocytes.](image-url)
cytes does not change measurably following injection of corticotropin in cattle. However, the number of circulating neutrophils doubled 4 to 8 hr after injection. This dramatic increase in neutrophils (Fig. 1) parallels the rise in total leukocytes, accounts for most of the leukocytosis, and is the primary factor in altering the lymphocyte-leukocyte ratio (Table 1).

Smith and Merrill (24) reported this leukocyte response in dairy cattle at parturition and after ACTH administration and termed it a "relative lymphopenia" as compared to the lymphopenia reported in humans (22) where there is an actual decline in number of circulating lymphocytes. Figure 1 also shows that heifers injected with corticotropin undergo a true decline in circulating eosinophils.

Corticotropin releases aldosterone as well as glucocorticoids from the adrenal cortex (18) although this is not the primary mechanism for control of aldosterone release (18). The chief biological action of aldosterone is on the kidney to stimulate renal absorption of sodium and excretion of potassium, hydrogen, ammonium, and magnesium ions (18, 26).

Figure 2 shows that within 30 min after administration of corticotropin or within 15 min after the rise of corticoids in the blood, there was a significant increase in serum sodium and a dramatic decline in serum potassium. These results indicate that blood content of these minerals responds rapidly to changes in adrenal cortical activity and are consistent with an aldosterone effect (25). While the initial changes of sodium and potassium closely paralleled those of plasma corticoids (Fig. 2), the altered serum amounts of these two minerals persisted for 4 to 20 hr after corticoids returned to normal. This could be the result of a persistent renal absorption mechanism possibly involving de novo synthesis of enzyme systems (9).

Increase in serum chloride and decline in calcium and magnesium lagged about 30 min compared to the time-response of corticoids, sodium, and potassium. In addition, calcium and magnesium remained low in the 24-hr blood sample whereas the other minerals were approaching normal (Table 2). This apparent relationship indicates that hypomagnesemia and hypocalcemia may be indirect effects, perhaps a competitive ion effect, on the kidney.

![Fig. 2. Effect of corticotropin administration of serum potassium, sodium, and corticoids.](image-url)
Vestermarck (27), who showed an increase in serum calcium of milk fever cows 30 min after treatment with ACTH. His discussion implies possible adrenal exhaustion and malaise (27) in cows with prolonged milk fever. An explanation of this discrepancy is offered by Levin and Cade (15), who found that aldosterone infused into diseased humans sharply decreased excretion of calcium and magnesium compared to the normal subject. These differences between the diseased and normal subjects could result from a blunted or depressed adrenal response or may also result from an alteration in the renal tubular handling of these ions.

Generally these results agree with reports (22) for man and other animals which show an adrenal steroidogenic effect by corticotropin resulting in a leukocytosis, eosinopenia, lymphopenia, hypematremia, hypokalemia, hypocalcemia, and hypomagnesemia.

References