The Role of Glucocorticoids for the Maintenance of Plasma Glucose Levels in Sheep

Hisashi HAMADA*, Shinji MURAYAMA** and Yoshiyuki SASAKI

Department of Animal Science, Faculty of Agriculture, Kyoto University, Kyoto-shi 606

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Abstract The role of adrenocortical hormones for the maintenance of plasma glucose levels in ruminants was evaluated in adrenalectomized (ADX) sheep under both fed and fasted conditions. Plasma glucose levels were compared between ADX sheep with glucocorticoid therapy (GC-treated ADX group) and without therapy (untreated ADX group) at fed state in Expt. 1, and between these ADX groups and intact sheep (intact group) at fasted state in Expt. 2. The therapeutic effectiveness of glucocorticoids on hypoglycemic ADX sheep was examined at fasted state in Expt. 3. At the fed state, untreated ADX group maintained plasma glucose levels at 90% of those observed in GC-treated ADX group in Expt. 1 (P<0.001). In contrast, plasma glucose levels of untreated ADX group were lowered to less than 60% of the pre-fasting levels at the 3rd day of fasting. In GC-treated ADX group, plasma glucose levels remained at the highest of all groups until 15 days of fasting. The intact and GC-treated ADX sheep in Expt. 2 did not show any hypoglycemic sign during the fasted period, but all of untreated ADX sheep showed clear hypoglycemic signs. When glucocorticoid was administrated to the hypoglycemic sheep, the levels of plasma glucose increased and recovery from hypoglycemia was observed in Expt. 3. From these results, it was concluded that glucocorticoids were essential for the maintenance of plasma glucose levels under fasted conditions in ruminants, but not at fed state.


Key words: adrenalectomy, glucocorticoids, fasting, glucose, sheep

It is well-known fact that glucocorticoids play an essential role in the maintenance of plasma glucose levels in nonruminants under fasted conditions. Without exogenous glucocorticoids, adrenalectomy causes hypoglycemia and results in death in fasted nonruminants1–3).

It can be postulated that glucocorticoids have a similar role in ruminants, as well as nonruminants according to the following facts. Plasma glucose concentrations were increased by the administration of glucocorticoids in fed and fasted sheep4,5) and in fed adrenalectomized (ADX) sheep6,7). Plasma glucocorticoid levels in sheep increased within 10 days after removal of feeds8). Furthermore, intravenous injection of glucose into fasted sheep caused marked decreases in levels of plasma...
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glucocorticoids. It is still unknown, however, that glucocorticoids are essential or not for the maintenance of plasma glucose levels in ruminants. In this paper, the changes of plasma glucose levels were investigated in fed and fasted sheep with or without glucocorticoids.

Materials and Methods

Animals: Forty-four Japanese Corriedale wethers [body weight (BW): {41±1.6} kg, mean±S.E.] were used. Twenty-eight of them were adrenalectomized using the method of Hamada et al. The mineralocorticoid therapy in the ADX sheep were performed as described in the previous paper. The animals were fed twice a day with a concentrate ration (DCP, 16.4%; TDN, 60.8%) at 0.6% level and legume hay at 1% level of kg • BW in total amount, respectively. They were also offered water and salt blocks ad libitum.

Expt. 1: In this experiment, the sheep were used three months after the ADX operation in order to neglect the influence of operation. The effect of glucocorticoids on plasma glucose levels was examined under fed condition, comparing glucocorticoid treated (GC-treated) ADX sheep (5 animals) with untreated ADX sheep (5 animals). In the GC-treated ADX group, animals received 0.50 mg/kg • BW/day of cortisol acetate (Saline Suspension of Hydrocortone Acetate, Nippon Merck–Banyu Co. Ltd, Tokyo) for the first 2 ds. The administration of the 0.50 mg of cortisol was practiced in order to raise up plasma cortisol level in the GC-treated ADX sheep to the normal plasma level observed in intact sheep as rapidly as possible. Then, 0.25 mg/kg • BW/day of the steroid for 20 ds intramuscularly, which is equivalent to the amount estimated from adrenal secretion rate in a normal sheep.

Blood samples were taken just before the feeding time in the morning by the external jugular venipuncture into a heparinized syringe, because plasma glucose levels tends to change after feeding. Blood samples were obtained at d 15, 10 and 5 before the start of the cortisol therapy and d 0, 3, 8, 13, 18 and 23 after the steroid therapy.

Expt. 2: The effect of glucocorticoids on plasma glucose levels in fasted sheep was compared among 13 untreated ADX sheep, 5 GC-treated ADX sheep and 16 intact sheep. During the fasted period, all sheep were free access to water and salt blocks, but were not fed any feed. In the GC-treated ADX group, animals were treated with im injection of cortisol at the level of 0.25 mg/kg • BW/day during 7 ds prior to the fasting and continuous 16 ds of the fasted period. By the end of the days in this experiment, all untreated ADX sheep showed one by one hypoglycemic signs categorized by Reid. When the untreated ADX sheep showed hypoglycemic sign, the animal was removed from this fasted experiment. Therefore, the relative numbers of untreated ADX sheep to the GC-treated ADX and intact sheep were reduced gradually during the experimental period.

Blood samples were obtained at one-d or two-d intervals.

Expt. 3: Therapeutic effectiveness of glucocorticoids on hypoglycemia in untreated ADX sheep was examined at the fasted state. Four untreated ADX sheep which
showed hypoglycemic signs in Expt. 2 were used in this experiment. The animals were treated with iv injection of about 7.5 g glucose solution [Daiich toueki (20 W/V%), Daiichi Seiyaku Co. Ltd, Tokyo] and (or) ip injection of about 65 mg cortisol acetate.

Blood samples were obtained at one-h or two-h intervals after glucose injection and ad libitum after the steroid administration observing the conditions of each sheep, respectively.

In these Expts., the blood plasma was separated by centrifugation immediately after bleeding and stored at -20°C.

Plasma glucose level was determined by the O-toluidine method\textsuperscript{14).} Plasma cortisol level was measured using the radioimmunoassay method described by Murayama et al.\textsuperscript{15).}

Statistical Analysis: Data were analyzed by Student’s t test or analysis of variance\textsuperscript{16).} For multiple mean comparison, Duncan’s new multiple range test was used\textsuperscript{17).} The calculations were done using the program of the Statistical Analysis System (SAS)\textsuperscript{18).}

Results

Expt. 1: The results of Expt. 1 are shown in Fig. 1. At the fed state, plasma cortisol levels in the untreated ADX sheep were extremely low (0.5 to 0.8 ng/ml), but

![Figure 1](image-url)
plasma glucose levels were maintained within the almost normal range, and their means with S.E. were 50±1 mg/100 ml. Since the GC-treatment started, plasma cortisol levels significantly increased (P<0.001) and reached a plateau level, i.e., 11.8 ng/ml, which seemed to be comparable to the normal level observed in intact sheep19). At the same time, plasma glucose levels were elevated by the GC-treatment (59±1 mg/100 ml). The increase was highly significant (P<0.001), while the difference was small. The untreated ADX sheep maintained plasma glucose level under fed condition at 90% of that observed in the GC-treated ADX sheep.

Expt. 2: The changes of plasma glucose levels in Expt. 2 are shown in Fig. 2. Plasma glucose levels in the untreated ADX sheep decreased dramatically after deprivation of feed and severe hypoglycemic signs were observed within 3 ds after fasting except for three animals. Although the decrease of plasma glucose levels in the remaining 3 untreated ADX sheep was moderate, one of them showed mild hypoglycemic signs at 5 ds after fasting, and the other 2 sheep showed severe hypoglycemia at 15 ds after fasting. One of the latter 2 died of severe hypoglycemia. None of the animals in both the GC-treated ADX and intact groups suffered from hypoglycemia through the fasting period (Fig. 2). Hypoglycemic signs observed in the untreated ADX sheep are shown in Table 1. There was individual variation in the day of fasting when the signs appeared and in the degree of hypoglycemia. Furthermore, the changes in plasma glucose during the first three days of fasting, and in plasma cortisol levels before fasting and at hypoglycemic signs in the untreated ADX group are shown in Table 2. The mean plasma glucose level in the untreated ADX group was 52±2 mg/100 ml before the start of fasting. The value was significantly lower than that observed in the GC-treated ADX group (62±3 mg/100 ml) or the intact group.

![Fig. 2. Changes of plasma glucose levels in 13 individual untreated ADX sheep (・), ADX-GC group mean (△) and intact group mean (□) under fasting conditions in Expt. 2. The number of animals is shown in parenthesis.](image-url)

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(60±2 mg/100 ml). At the 3rd day of fasting, plasma glucose level in the untreated ADX group (31±4 mg/100 ml) was 60% of the value observed before fasting. On the other hand, plasma glucose levels at the same day of fasting in both the GC-treated ADX group (55±3 mg/100 ml) and the intact group (49±3 mg/100 ml) were maintained at about 90% of those observed before fasting. Plasma cortisol levels at both before and after fasting in the untreated ADX group were markedly low and the levels were not altered by fasting. On the other hand, in the intact and GC-treated ADX groups plasma cortisol levels tended to be higher during the fasting period than those observed prior to the fasting period, although the difference was not significant.

Table 1. The time and signs of hypoglycemia observed in the fasted untreated ADX sheep in Expt. 2

<table>
<thead>
<tr>
<th>Sheep No.</th>
<th>Day of fasting fallen into hypoglycemia</th>
<th>Signs of hypoglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>Convulsion, dyspnoeic.</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Coma, salivation.</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>Inability to stand, involvement of the neck.</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>Convulsion, dyspnoeic.</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>Disturbance of consciousness.</td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>Convulsion, dyspnoeic.</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
<td>Involvement of the neck.</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>Coma, salivation.</td>
</tr>
<tr>
<td>23</td>
<td>2</td>
<td>Spasms of the neck, convulsion</td>
</tr>
<tr>
<td>27</td>
<td>5</td>
<td>Lassitude.</td>
</tr>
<tr>
<td>29</td>
<td>1</td>
<td>Convulsion, dyspnoeic.</td>
</tr>
<tr>
<td>30</td>
<td>15</td>
<td>Disturbance of consciousness.</td>
</tr>
<tr>
<td>31</td>
<td>2</td>
<td>Lassitude.</td>
</tr>
</tbody>
</table>

Table 2. Mean±S.E. of plasma glucose and cortisol levels by the three treatment and fasted day in Expt. 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mg/100 ml)</th>
<th>Cortisol (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before 1) 1 day 2 days 3 days</td>
<td>Before 1) After 2)</td>
</tr>
<tr>
<td>Untreated ADX</td>
<td>52±2(13) A 31±3(10) Bb 30±3(10) Bb 31±4(7) Bb</td>
<td>0.2±1.2(13) B 0.1±0.1(13) A</td>
</tr>
<tr>
<td>GC-treated ADX</td>
<td>62±3(5) Aa 57±4(5) Ab 54±4(5) Ab 55±3(4) Ab</td>
<td>10.4±0.7(5) A 13.9±1.1(5) A</td>
</tr>
<tr>
<td>Intact</td>
<td>60±2(16) Aa 53±1(12) Ab 62±2(11) Ab 49±3(9) Ab</td>
<td>10.0±1.1(16) A 13.5±1.1(16) A</td>
</tr>
</tbody>
</table>

1) The fasting period corresponded 16 hours after the last feeding. Data obtained at this time were included in “Before” division.
2) The fasted period varied from 1 to 15 days after fasting. All data in this period were gathered in “After” division. The number of animals is shown in parenthesis. Means in the same column with unlike superscripts are significantly different between groups (A and B; P<0.001, by Student's t test)
Means in the same row with unlike superscripts are significantly different between days: (a and b; P<0.05, by Duncan's new multiple range test).
Expt. 3: The effect of glucose and (or) glucocorticoid administration on plasma glucose levels in the fasted and hypoglycemic ADX sheep is presented in Fig. 3. Glucose treatment resulted in a rapid and clear alleviation of hypoglycemic signs, but the signs appeared again at 6 hs after the treatment in 3 of them and at 20 hs after the treatment in one of them (Fig. 3). The administration of both glucocorticoid and glucose was associated with a sustained elevation of plasma glucose level and recovery from hypoglycemia.

Discussion

The ADX sheep was under a deficient condition in both adrenocortical hormones and adrenomedullary hormones. Since no adrenomedullary hormones were administered in the present study, it appeared that these hormones were not essential for the maintenance of euglycemia in both the fed and fasted sheep, and also were not essential for recovery from hypoglycemia in the fasted sheep (Fig. 1 to 3).

Glucocorticoids also did not appear to be essential for the maintenance of euglycemia in the fed sheep, in spite of their ability to promote hyperglycemia\(^{6,7}\) (Fig. 1).

At fed state, the major gluconeogenic precursors at fed state in ruminants are propionate absorbed from the rumen and glucogenic amino acids absorbed from the small intestine\(^{20}\). Glucocorticoids do not stimulate gluconeogenesis from propionate in sheep\(^{21,22}\). Therefore, availability of precursors is probably more important than the hormonal intervention for the maintenance of euglycemia in fed ruminants.

**Fig. 3.** Effects of glucose and (or) glucocorticoid on plasma glucose levels in the ADX sheep which showed the hypoglycemic signs under fasting conditions in Expt. 3. G and GC show the times when glucose and glucocorticoid were injected, respectively. Broken line indicates that no record was obtained during the experimental period. Each marker indicates an individual animal.
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At fasted state, however, dietary precursors are unavailable. Then, the gluconeogenic precursors must be derived from the extrahepatic tissues in the form of glycerol from the adipose tissue, glucogenic amino acids from body protein and lactate from glycogen or glucose origin in ruminants\(^{20,23}\). Therefore, at this state the gluconeogenic precursors in ruminants are the same as those in nonruminants. Glucocorticoids may affect the mobilization of these gluconeogenic precursors at fasted state. This fact may account for positive effects of glucocorticoid therapy to the fasted ADX sheep (Fig. 3).

It has been generally considered that glucocorticoids affect glucose metabolism by \(^{24-26}\) : (1) decreasing utilization of glucose in the peripheral tissues; (2) increasing gluconeogenic substrate release from the peripheral tissues; (3) direct effect on hepatic gluconeogenesis by increasing synthesis or activation of gluconeogenic enzymes; (4) increasing glycogen accumulation in the liver; (5) permissive effects of glucocorticoids on gluconeogenesis in the liver by glucagon and epinephrine. Glucocorticoids in ruminants probably have some similar effects. REILLY and BLACK\(^6\) and RANAWEERA et al.\(^{27}\) showed that glucocorticoids inhibited peripheral utilization of glucose in sheep. BAIRD and HEITZMAN\(^{28}\) studied on ketotic cow and showed that intermediates of citric acid cycle (citrate, oxaloacetate, 2-oxoglutarate and malate), and also of alanine, glutamate, histidine and glycogen in the liver were increased by administration of glucocorticoid. The increase of these intermediates consisted with the increased hepatic gluconeogenesis from amino acids in sheep\(^{29}\). The steroids may involve effect on hepatic phosphoenolpyruvate carboxykinase in ruminants\(^{30-33}\), although this effect has not been confirmed.

Our results obtained in the fasted ADX sheep without glucocorticoid therapy suggested that the mentioned glucose metabolism was abnormal due to absence of glucocorticoids. Thus, glucocorticoids are essential for the maintenance of plasma glucose levels under fasted conditions in ruminants, but not at the fed state.

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References

ヒッジの血糖維持に対するグルコルチコイドの役割

浜田 久・村山真治*・佐々木義之

京都大学農学部, 京都市 606
* オールインワン K.K., 大阪市 530

反芻動物の血糖維持機構におけるグルコルチコイド（GC）の役割を明らかにするために、副腎摘出（ADX）ヒッジを用いて飼料給与条件下および絶食条件下での実験を行なった。まず、実験 1 ではADXによる術後の影響を除くために、ADX後3か月以上経過した個体を用い、GCを投与したGC処理ADX群と無処理ADX群に分け、血糖値を経日に調べてみた。つぎに、実験 2 では絶食条件下において正常群、GC処理ADX群および無処理ADX群の3群間で血糖値を比較した。さらに、実験 3 では絶食条件下で低血糖症状を示したADXヒッジに対するグルコースおよびGC投与の効果について検討した。1）飼料給与条件下では、無処理ADX群の血糖値はGC処理ADX群の90%の値を維持した。2）絶食条件下では無処理ADX群の血糖値は絶食3日目に絶食前値の半分近くの水準である31±4mg/100mlに低下した。一方、GC処理ADX群および正常群では、絶食3日目の血糖値がそれぞれ55±3mg/100mlおよび49±3mg/100mlであり、両群とも絶食前値の80%以上の水準を維持した。13頭の無処理ADX群は絶食15日目までにすべて低血糖症状を示し、そのうち1頭は低血糖による昏睡死をした。一方、GC処理ADX群および正常群では絶食期間中、低血糖症状を示す個体は観察されなかった。3）絶食条件下で低血糖症状を示したADXヒッジでは、グルコース補給によって急激かつ明瞭に低血糖症状からの回復が認められたが、その後再び低血糖症状を示した。一方、これらADXヒッジへのGC投与は、血糖値の持続的上昇と低血糖症状からの回復に効果があった。以上の結果から、グルコルチコイドは絶食条件下におけるヒッジの血糖維持に必須の要因であることが明らかになった。

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