INSULIN RESPONSIVENESS TO GLUCOSE AND TISSUE RESPONSIVENESS TO INSULIN OVER THE FEEDING CYCLE IN SHEEP

H. Sano, S. Matsunobu, M. Nakagawa and Y. Terashima

Kitasato University, Towada-shi 034, Japan

ABSTRACT

Insulin responsiveness to glucose and tissue responsiveness to insulin, using the hyperglycemic clamp and the hyperinsulinemic euglycemic clamp techniques, were measured before, during and after feeding in sheep fed an alfalfa hay and commercial concentrate diet. Glucose infusion rate and the plasma insulin increment in the hyperglycemic clamp experiment were higher during the feeding period (0 to 1 h after initiating feeding) than during the pre- and post-feeding periods. The ratio of plasma insulin increment to glucose infusion rate remained unchanged over the feeding cycle. Only a slight increase (P > .05) in the glucose infusion rate was observed during feeding in the hyperinsulinemic euglycemic clamp experiment. These results suggest that insulin responsiveness to glucose tends to be enhanced during the feeding period but that tissue responsiveness to insulin is not changed over the feeding cycle in sheep.

(Key Words: Glucose, Insulin, Feeding, Sheep.)

Introduction

Many of the daily patterns in the concentrations of hormones and metabolites in plasma of ruminants are related closely to feeding (Trenkle, 1978). Plasma insulin rises after feeding in ruminants and nonruminants (Basset, 1972; Sasaki et al., 1984a). This insulin secretory response to glucose injection is influenced by cold exposure (Sasaki and Takahashi, 1980), obesity (McCann et al., 1986), stage of lactation (Sartin et al., 1985; Denbow et al., 1986) and feeding (Sasaki et al., 1984b) in ruminants. However, it is not clear whether insulin responsiveness to glucose and tissue responsiveness to insulin vary according to the feeding cycle in ruminants. Recently, a number of studies using the glucose clamp technique to assess insulin responsiveness and sensitivity in vivo have been published (DeFronzo et al., 1979; Bergman et al., 1985), though the publications concerning ruminant animals are limited (Weekes et al., 1983; Hay et al., 1984; Janes et al., 1985; Debras et al., 1989).

The objective of this experiment was to study insulin responsiveness to glucose and tissue responsiveness to insulin over the feeding cycle using the hyperglycemic clamp and the hyperinsulinemic euglycemic clamp techniques in sheep.

Materials and Methods

Animals

Four shorn Suffolk rams aged 1 to 2 yr and weighing 41 to 69 kg were used. They were surgically prepared with a skin loop enclosing the left carotid artery. Animals were kept in metabolic cages in a controlled environment chamber at an air temperature of 20 ± 1°C for more than 1 mo. They were fed 2% BW of alfalfa hay (12.9% moisture, 8.6% ash, 14.6% CP, 1.7% ether extract, 23.2% crude fiber and 39.0% nitrogen-free extract) and .5% BW of a commercial concentrate (11.6% moisture,
POSTPRANDIAL INSULIN RESPONSIVENESS IN SHEEP

7.6% ash, 16.7% CP, 3.0% ether extract, 5.9% crude fiber and 55.2% nitrogen-free extract) daily at 1300. This diet was calculated to contain 2.4 Mcal ME/kg and 11.0% digestible CP. All sheep normally consumed their diet within 60 min after feeding. Water was available ad libitum. At least 2 d before the experiment started, a polyethylene catheter for glucose and insulin infusions was placed in a femoral vein under local anesthesia. Catheters were flushed and filled with 3.8% sterile solution of trisodium citrate once daily.

Experimental Procedures

Experiment 1. The hyperglycemic clamp technique described by DeFronzo et al. (1979) was utilized for determination of insulin responsiveness to exogenous glucose infusion during four periods of the feeding cycle. A catheter for blood sampling had been inserted into the carotid artery of the skin loop at least 2 h before the experiment commenced. The feeding cycle from 2 h prefeeding to 4 h after initiation of feeding was divided into four 2-h periods termed B (2 h prefeeding), A2 (0 to 2 h after initiation of feeding), A4 (2 to 4 h after initiation of feeding) and D, the 2-h period from 1 h prefeeding to 1 h after initiation of feeding. The hyperglycemic clamp (steady-state high blood glucose designed to minimize gluconeogenesis) was established during the 1st of periods B, A2, A4 and D. The glucose infusion rate (GIR, the rate required to maintain the desired blood glucose) and mean plasma insulin increment (MPII, the increase in plasma insulin over the basal level determined at three consecutive 10-min intervals immediately before glucose infusion was started) were determined during the 2nd h of periods B, A2, A4 and D. Hence, these measurement periods were 1 h before feeding (B), during a 1-h feeding period (D), 1 h after the end of feeding (A2), and between 2 and 3 h after the end of feeding (A4). These four periods and four rams were arranged with a minimum of 4 d between infusion periods.

Glucose solution (20% dextrose in distilled water) was infused by a multichannel peristaltic pump through the femoral catheter for 2 h in each period. The glucose infusion rate was initially designed to be 9.0 mg·kg⁻¹·min⁻¹ and plasma glucose concentration was maintained at an initial level (~60 mg/100 ml) plus 100 mg/100 ml by adjusting the glucose infusion rate at 10-min intervals during the latter experimental period. Blood samples (4 ml) were taken from the carotid catheter at 10-min intervals during the experiment. An aliquot of blood (1 ml) was immediately centrifuged and plasma glucose concentration was determined within 2 min after the blood sampling. Residual blood samples were stored in ice-water until the centrifugation (10,000 × g, 10 min, 4°C). The glucose infusion rate was monitored every 10 min throughout the experimental period. Glucose excretion into urine, which was collected for 4 h from immediately before glucose infusion to 2 h after the end of the glucose infusion, was measured. Because little glucose was detected in the urine, urinary loss of glucose was not used to correct the glucose infusion rate.

Experiment 2. The hyperinsulinemic euglycemic clamp technique (Weeke et al., 1983) was applied to the determination of responsiveness of peripheral tissues to insulin over the feeding cycle. Insulin⁴ (400 U/liter) dissolved in 9% sodium chloride solution was administered by the peristaltic pump through the femoral catheter as a primed continuous infusion at a rate of 6.0 mU·kg⁻¹·min⁻¹ for the same 2-h periods described for Exp. 1. Ninety mU/kg BW of insulin was infused during the initial 10 min in a logarithmically decreasing manner as a priming dose (Weeke et al., 1983). The insulin solution contained 2.5% potassium chloride to prevent hypokalemia. Glucose (20%) was infused by the other peristaltic pump through the same catheter beginning about 12 min after the start of the insulin infusion. The infusion rate was adjusted at 10-min intervals in order to maintain the initial level of plasma glucose. Blood samples (4 ml) were taken at 10-min intervals during the experimental period. Plasma glucose concentration was determined as described in Exp. 1.

Analyses

Glucose concentrations in plasma and urine were determined using an automated glucose analyzer⁵. Plasma samples were stored −20°C until insulin assay. Plasma insulin was assayed

---

³Model AC-2120, Atto Co. Ltd., Tokyo, Japan.
⁴Actrapid monocomponent porcine insulin, Novo Indust. Denmark.
by a RIA kit based on the double antibody RIA method. Anti-insulin guinea pig serum was the antiserum and anti-guinea pig-IgG goat serum was the second antibody. Intra- and interassay CV were 5 and 12%, respectively.

**Calculations**

Results are expressed as mean ± SD of four animals. The difference was evaluated by Student's paired t-test to compare values in the preinfusion period with values obtained after the start of the glucose or insulin infusion. The differences between experimental periods B, D, A2 and A4 of the feeding cycle were analyzed by one-way ANOVA. When the F-test was significant, differences between means were determined by Tukey's test.

**Results**

**Hyperglycemic Clamp Technique (Exp. 1)**

The new plateau in glucose concentration was achieved within 40 min after the start of the glucose infusion (Figure 1). During the second half of the glucose infusion period, plasma glucose concentration was clamped at 100 mg/100 ml above the initial level that was 99 ± 4% of the desired goal.

The glucose infusion rate, which ranged initially from 8.5 to 9.4 mg.kg⁻¹.min⁻¹, decreased gradually thereafter and was relatively constant during the second half of each period except the D period. The glucose infusion rate for the D period increased between 80 and 100 min after the start of the infusion, corresponding to 20 to 40 min after the initiation of feeding. The GIR was higher (P < .05) for the D period and was lower (P < .05) for the A4 period than for other three periods (Table 1).

The basal insulin concentration was higher (P < .05) for the A4 period than for the other three periods. Plasma insulin concentration was increased (P < .01) by glucose infusion in all periods. The MPII was greater (P < .05) for the D and A2 periods than for the A4 period. However, the MPII/GIR ratio was not significantly different between the periods.

**Hyperinsulinemic Euglycemic Clamp Technique (Exp. 2)**

Because the glucose infusion commenced about 12 min after the initiation of the insulin infusion, the concentration of plasma glucose at 10 min after the start of the insulin infusion decreased in every period (Figure 2). During the second half of the insulin infusion, plasma glucose concentration was clamped at the initial level at 98 ± 7% of the desired goal. The GIR of the D period was elevated slightly but did not differ significantly from the other periods (Table 2). The basal insulin concentration of the A4 period was higher (P < .05) than those of the B and A2 periods. The MPII and the GIR/MPII ratio also did not differ significantly among periods.

**Discussion**

Results of the hyperglycemic clamp experiment revealed that insulin responsiveness (MPII) to glucose was greater during feeding than 3 h after feeding, but MPII did not differ significantly from the value before feeding. This does not agree with many reports that secretory responses of hormones and utilization rates of metabolites as well as their circulating levels change during the feeding cycle (Bassett, 1975; Forbes, 1980; Sasaki et al., 1984b; Trenkle, 1989). This discrepancy may be due to the small scale of the present experiment (n = 4). Sasaki et al. (1984b) reported that the insulin response to i.v. glucose injection increased during a meal in sheep fed orchardgrass hay. Our decrease in MPII between 3 and 4 h after initiation of feeding may be related to an increase in hepatic gluconeogenesis (Katz and Bergman, 1969).

In both the hyperglycemic clamp and the hyperinsulinemic euglycemic clamp techniques of the present experiment, the GIR was altered instead of being steady-state (Weekes et al., 1983) because the glucose infusion rate of the D period fluctuated compared with those of other periods. Glucose infusion depresses but may not entirely stop endogenous glucose production in ruminants (Judson and Leng, 1973). Therefore, the GIR in the hyperglycemic clamp experiment does not provide an absolute measure of glucose turnover rate, but it should be a good indicator of glucose utilization rate. Nevertheless, the enhanced
POSTPRANDIAL INSULIN RESPONSIVENESS IN SHEEP

Figure 1. Plasma glucose and insulin concentrations and glucose infusion rate before and during the hyperglycemic clamp technique over the feeding cycle in sheep. B, D, A2 and A4 represent the periods of 2 h prefeeding, from 1 h prefeeding to 1 h after initiation of feeding, 0 to 2 h after initiation of feeding and 2 to 4 h after initiation of feeding, respectively (Exp. 1).

GIR of the D period of Exp. 1 suggests that hepatic glucose production from absorbed VFA was reported to increase 3 h after feeding in sheep (Katz and Bergman, 1969). This result agrees with Sasaki et al. (1984b), who reported in sheep that blood glucose turnover rate increased during feeding. Hepatic glucose production from absorbed VFA was reported to increase 3 h after feeding in sheep (Katz and Bergman, 1969).

TABLE 1. GLUCOSE INFUSION RATE, PLASMA INSULIN LEVELS AND THE RATIO OF PLASMA INSULIN INCREMENT TO GLUCOSE INFUSION RATE IN HYPERGLYCEMIC CLAMP TECHNIQUE OVER THE FEEDING CYCLE IN SHEEP (EXP. 1)

<table>
<thead>
<tr>
<th>Feeding cycle</th>
<th>B</th>
<th>D</th>
<th>A2</th>
<th>A4</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIR, mg/(kg·min)</td>
<td>3.4e</td>
<td>4.5d</td>
<td>3.5e</td>
<td>2.4f</td>
<td>.8</td>
</tr>
<tr>
<td>Basal insulin, μU/ml</td>
<td>11e</td>
<td>16e</td>
<td>14e</td>
<td>28d</td>
<td>14</td>
</tr>
<tr>
<td>MPlI/GIR, μU/(ml·(mg/kg·min))</td>
<td>111de</td>
<td>170d</td>
<td>151d</td>
<td>68e</td>
<td>50</td>
</tr>
<tr>
<td>Mean plasma insulin increment during the 2nd h of each period.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| d,e,f,Means in rows without common superscripts differ (P < .05).
suppressed during the euglycemic clamp technique in sheep (Weekes et al., 1983; Brockman, 1985). The GIR in the hyperinsulinemic euglycemic clamp experiment should represent the sum of the insulin-induced suppression of gluconeogenesis and insulin-induced increase in glucose utilization (Weekes et al., 1983). The insulin infusion rate in our experiment was about 50 times greater than the basal secretory rate of insulin (Brockman and Bergman, 1975). Plasma insulin concentration during the insulin infusion did not differ between periods; it was considerably higher than the concentration that was required to reduce endogenous glucose output to half basal (Weekes et al., 1983). Therefore, the data obtained from the present experiment are considered indicative of the responsiveness of peripheral tissues to insulin during different periods of the feeding cycle. The GIR in the present experiment was similar to the value obtained by Janes et al. (1985). The GIR did not differ significantly between periods, though the GIR of the D period tended to be greater than those of other three periods. Insulin presumably increases glucose uptake by adipose tissue and skeletal muscle (Trenkle, 1981). Hay et al. (1984) reported that insulin

### Table 2. Glucose Infusion Rate, Plasma Insulin Levels and the Ratio of Glucose Infusion Rate to Plasma Insulin Increment in the Hyperinsulinemic Euglycemic Clamp Technique Over the Feeding Cycle in Sheep (Exp. 2)

<table>
<thead>
<tr>
<th>Feeding cycle</th>
<th>B</th>
<th>D</th>
<th>A2</th>
<th>A4</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIR, mg/(kg·min)</td>
<td>2.9</td>
<td>3.2</td>
<td>2.2</td>
<td>2.3</td>
<td>6.0</td>
</tr>
<tr>
<td>Basal insulin, μU/ml</td>
<td>18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.0</td>
</tr>
<tr>
<td>MPP, μU/ml</td>
<td>798</td>
<td>1,113</td>
<td>1,000</td>
<td>771</td>
<td>344</td>
</tr>
<tr>
<td>GIR/MPP×10&lt;sup&gt;3&lt;/sup&gt;, mg/(kg·min)/μU/ml</td>
<td>4.8</td>
<td>3.0</td>
<td>2.7</td>
<td>4.3</td>
<td>2.6</td>
</tr>
</tbody>
</table>

<sup>*B</sup> = 2 h prefeeding; <sup>†</sup>D = from 1 h prefeeding to 1 h after initiation of feeding; A2 = 0 to 2 h after initiation of feeding; A4 = 2 to 4 h after initiation of feeding.

<sup>1</sup>GIR = glucose infusion rate during the 2nd h of each period.

<sup>c</sup>MPPI = mean plasma insulin increment during the 2nd h of each period.

<sup>d</sup>Means in rows without common superscripts differ (P < .05).
produced a 4.9-fold increase in glucose extraction across the hindlimb of pregnant sheep. Hauguel et al. (1987) reported in pregnant and nonpregnant rabbits that changes in the overall glucose utilization during the hyperinsulinemic euglycemic clamp technique were reflected in the pattern of glucose uptake by the hindlimb muscles.

Implications

The insulin secretory response to glucose tended to increase and glucose utilization rate increased during feeding. Nevertheless, insulin's effect on peripheral tissues remained unchanged over the feeding cycle in sheep fed an alfalfa hay and commercial concentrate diet.

Literature Cited


