Plasma Insulin and Glucagon Responses to Propionate Infusion into Femoral and Mesenteric Veins in Sheep

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ABSTRACT: Propionate (1, 2, 4, 8, 16, 32, and 64 μmol·kg BW⁻¹·min⁻¹ for 30 min) was infused into the femoral and mesenteric veins of adult sheep to investigate the physiological significance of propionate in regulating plasma insulin and glucagon concentrations. The increments in arterial blood propionate concentrations during propionate infusion increased (P < .001) with increasing infusion rates for both infusion sites, and they were smaller (P < .001) for the mesenteric vein infusion than for the femoral vein infusion. Plasma insulin concentrations during propionate infusion increased (P < .0001) from preinfusion values with infusion rates of 2 to 8 μmol·kg BW⁻¹·min⁻¹ for both infusion sites. The response areas of plasma insulin concentration above basal tended to be smaller (P < .0121) for the mesenteric vein infusion than for the femoral vein infusion. Plasma glucagon concentrations during propionate infusion increased (P < .05) from preinfusion values with infusion rates of ≥ 8 and 64 μmol·kg BW⁻¹·min⁻¹ for the femoral and mesenteric vein infusions, respectively. The response areas of plasma glucagon concentration above basal were smaller (P < .011) for the mesenteric vein infusion than for the femoral vein infusion. We conclude that in sheep propionate absorbed from the alimentary tract has a physiological role in regulating circulating concentrations of insulin and glucagon.

Key Words: Insulin, Glucagon, Propionate, Sheep

Introduction
In ruminants, VFA can stimulate insulin and glucagon release (Manns and Boda, 1967; Horino et al., 1968; Ambo et al., 1973; De Jong, 1982), but it is not clear that VFA produced in the rumen have a physiological role in insulin and glucagon release. De Jong (1982) suggested that normal physiological variations in propionate and n-butyrate could control basal plasma insulin secretion in goats. Propionate is considered to be one of the most likely VFA to stimulate insulin and glucagon release in ruminants (Bines and Hart, 1984; Istasse et al., 1987; Harmon, 1992). Physiological concentrations of propionate infusion into a femoral vein increased plasma insulin and glucagon concentrations in sheep (Sano et al., 1993). However, little propionate would normally escape from the liver into the general circulation, because most of the propionate absorbed from the alimentary tract is removed by the liver before reaching the pancreas.

Therefore, the present experiment was conducted to clarify the physiological significance of propionate absorbed from the alimentary tract in stimulating insulin and glucagon release by infusing propionate at a variety of doses into a femoral vein and a mesenteric vein in sheep.

Materials and Methods
Animals and Diets. Four yearling Suffolk rams, weighing 45 ± 2 kg, were used. They were surgically prepared under anesthesia with a skin loop enclosing the left carotid artery at least 3 mo before the experiment. Rams were kept in metabolism cages in a laboratory room at temperatures of 21 ± 2°C. They were fed 2% BW of alfalfa hay cube (12.4% moisture, 15.0% CP, 1.6% ether extract, 36.3% NDF, 12.5% crude ash, and 34.8% nitrogen-free extract) and .5%

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BW of commercial concentrate (12.3% moisture, 16.9% CP, 2.4% ether extract, 21.7% NDF, 6.7% crude ash, and 56.3% nitrogen-free extract) once daily at 1700. This diet was estimated to contain 2.4 Mcal of ME/kg (NRC, 1985). All sheep normally consumed their diet within 1 h after feeding. Water was available free choice. Polyvinyl catheters for infusions (Atom Co., Ltd., Tokyo, Japan, o.d. 2.0 mm, 60 cm length) were inserted into a femoral vein and a mesenteric vein under anesthesia at least 1 wk before the experiment. For blood sampling, an indwelling sterile needle (Top Co. Ltd., Tokyo, Japan, i.d. .9 mm, 70 mm length) was placed in the exteriorized carotid artery and was connected to a catheter at least 2 h before the initiation of each experiment. The catheters were filled with a sterile solution of 3.8% trisodium citrate.

Experimental Procedures. The concentration of infused propionate was 1.2 M and pH was adjusted to 7.4 with sodium hydroxide. Propionate was continuously infused with a multichannel peristaltic pump (Model AC-2120, Atto Co., Tokyo, Japan) for 30 min at rates of 1, 2, 4, 8, 16, 32, and 64 μmol·kg BW·min⁻¹ through the femoral or mesenteric vein catheter. Blood samples (8 mL) were taken into centrifuge tubes at −10, 0, 1, 2.5, 5, 10, 15, 30, 45, 60, 90, and 120 min after the initiation of propionate infusion. The tubes contained 30 units of heparin sodium and 60 mg of sodium benzamidine (Sigma Chemical, St. Louis, MO) and were stored in ice water until further treatment. Each of four sheep received all doses of propionate via both infusion routes in a random order with 4-d intervals between infusions.

Analyses. All analyses were carried out as reported previously (Sano et al., 1993). Chemical composition of diets was determined by methods described in AOAC (1970) and Van Soest and Wine (1967). An aliquot (5 mL) of each blood sample was used for VFA determination, and the remainder was centrifuged at 10,000 × g at 4°C for 10 min. After the plasma glucose concentration was determined with an automated glucose analyzer (GLU-1, Erma Optical Works, Tokyo, Japan), plasma was stored at −20°C until it was assayed for insulin and glucagon. Blood VFA were measured with gas chromatography (Model 208, Hitachi Ltd., Tokyo, Japan) after steam distillation. Insulin was assayed with a RIA kit (IRI 'Eiken,' Eiken Chemical Co. Ltd., Tokyo, Japan) based on a double-antibody method. Intra- and interassay CV for insulin were 5.7 and 9.0%, respectively. Pancreatic glucagon was assayed with a RIA based on a charcoal method (Ohneda et al., 1979) as described by Sasaki et al. (1982). Glucagon from a mixture of bovine and porcine pancreas (Sigma Chemical) as standard and antiglucagon serum G42-E, which reacts only with pancreatic glucagon, were used. Intra- and interassay CV for glucagon were 3.5 and 18.1%, respectively.

Statistics. Mean values with SE are given. The incremental areas of propionate, insulin, and glucagon concentrations above basal were calculated as the areas beneath the curve for concentrations measured during the 30-min propionate infusion as described by Sasaki et al. (1984). All data were statistically analyzed with the GLM procedures of SAS (1985). The repeated statement was used to compare the preinfusion means with values obtained after the initiation of propionate infusion. Analyses for incremental areas of propionate, insulin, and glucagon were performed with an incomplete Latin square design. The main effects were sheep, period, propionate infusion rate, propionate infusion site, and propionate infusion rate × site interaction. Results were considered significant at the P < .10 level.

Results

Blood propionate concentrations for the femoral vein infusion increased to greater than the preinfusion values (P < .01) during propionate infusions at rates of ≥ 1 μmol·kg BW⁻¹·min⁻¹ (Figure 1). Arterial concentrations for the mesenteric vein infusion remained unchanged during infusion at rates of ≤ 4 μmol·kg BW⁻¹·min⁻¹ and increased (P < .05) from the preinfusion values at rates of ≥ 8 μmol·kg BW⁻¹·min⁻¹. Blood propionate concentrations were virtually constant during the 30-min infusion period for both infusion sites, and then they decreased to preinfusion values after the end of the infusion. The incremental areas of blood propionate concentrations were greater (P < .001) for the greater infusion rates and smaller (P < .001) for the mesenteric vein infusion than for the femoral vein infusion (Table 1). A propionate infusion rate × site interaction was detected (P < .001).

Plasma glucose concentrations during propionate infusion remained unchanged at rates of ≤ 8 and 32 μmol·kg BW⁻¹·min⁻¹ for the femoral and mesenteric vein infusions, respectively (Figure 2). Concentrations increased (P < .05) from the preinfusion concentrations at rates of ≥ 16 and 64 μmol·kg BW⁻¹·min⁻¹, respectively, and reached peaks at 10 min after the initiation of infusion. With the greater infusion rates, concentrations then decreased (P < .05) to less than the preinfusion values by 45 min after the initiation of propionate infusion.

For both infusion sites, plasma insulin concentrations during propionate infusion remained unchanged at rates of ≤ 4 μmol·kg BW⁻¹·min⁻¹ (Figure 3). Concentrations increased (P < .10) from the preinfusion values at rates of ≥ 8 μmol·kg BW⁻¹·min⁻¹ for both infusion sites, reached peaks at 5 to 15 min after the initiation of propionate infusion, and then decreased gradually to the preinfusion values. The incremental response areas of plasma insulin concentrations increased (P < .001) with increasing propionate infusion rates, and they tended to be smaller (P < .112) for the mesenteric vein infusion than for the
femoral vein infusion (Table 1). There was no significant interaction between infusion rate and site of infusion ($P < .297$).

For the femoral vein infusion, plasma glucagon concentrations during propionate infusion remained unchanged at rates of $\leq 4 \mu$mol·kg BW·min$^{-1}$. They increased ($P < .05$) from the preinfusion values at rates of $\geq 8 \mu$mol·kg BW·min$^{-1}$ and decreased to the preinfusion values after the end of propionate infusion (Figure 4). Concentrations for the mesenteric vein infusion increased ($P < .05$) at rates of 16 and 64 $\mu$mol·kg BW·min$^{-1}$. The concentrations of plasma glucagon during propionate infusion were relatively stable compared with those of plasma insulin. The plasma glucagon response areas above basal increased ($P < .008$) in a dose-dependent manner, and they were smaller ($P < .011$) for the mesenteric vein infusion than for the femoral vein infusion (Table 1). A rate x site interaction was detected ($P < .04$).

**Discussion**

One of the nutritional characteristics of ruminant species is that most of the VFA are produced from dietary carbohydrates by the ruminal microorganisms, supplying 50 to 80% of the DE available to the host animal (Ballard et al., 1969). Propionate is the second most abundant VFA after acetate in the rumen and blood, and the net absorption rate of propionate from the rumen is influenced by type of diet and intake level (Gross et al., 1988; Reynolds et al., 1988). Smaller incremental areas of blood propionate concentrations for the mesenteric vein infusion compared with the femoral vein infusion would reflect hepatic removal of propionate, which is well established (Bergman, 1975; Reynolds et al., 1988). Our infusion of 1 to 64 $\mu$mol·kg BW·min$^{-1}$ is a physiological dose rather than pharmacological dose. In lactating dairy cows consuming a 40% concentrate diet ad libitum (2.4% BW), net portal absorption of propionate was 20.4 $\mu$mol·kg BW$^{-1}$·min$^{-1}$ (Reynolds et al., 1988). In sheep fed alfalfa hay at maintenance or intragastrically infused with varying ratios of VFA, net portal absorption of propionate varied from 4.5 to 18 $\mu$mol·kg BW$^{-1}$·min$^{-1}$ (Gross et al., 1990a,b).

Increases in plasma glucose concentrations at the greater propionate infusion rates may be related to changes in endocrine status, but they do not simply reflect enhanced gluconeogenesis from propionate (Peters and Elliot, 1984) because of the smaller changes in plasma glucose concentrations with the mesenteric vein infusion than with the femoral vein infusion. For the mesenteric vein infusion, plasma insulin concentrations were increased by propionate infusion at rates of $\geq 8 \mu$mol·kg BW·min$^{-1}$, but plasma glucose concentrations remained unchanged as reported previously (Sano et al., 1993). Therefore,
Table 1. The response areas of arterial blood propionate and of plasma insulin and glucagon concentrations during propionate infusion into the femoral and mesenteric veins in sheep (n = 4)

<table>
<thead>
<tr>
<th>Propionate infusion rate</th>
<th>Femoral vein</th>
<th>Mesenteric vein</th>
<th>SEM</th>
<th>Propionate area, mmol·L⁻¹·min⁻¹</th>
<th>Significance</th>
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|                          |              |                 |     | Insulin area, mU·mL⁻¹·min⁻¹ |              |
|                          |              |                 |     |                                |              |
|                          |              |                 |     | 1                               | .0           |
|                          |              |                 |     | 2                               | .1           |
|                          |              |                 |     | 4                               | .0           |
|                          |              |                 |     | 8                               | .7           |
|                          |              |                 |     | 16                              | .3           |
|                          |              |                 |     | 32                              | .9           |
|                          |              |                 |     | 64                              | 6.5          |

|                          |              |                 |     | Glucagon area, ng·mL⁻¹·min⁻¹ |              |
|                          |              |                 |     |                                |              |
|                          |              |                 |     | 1                               | .5           |
|                          |              |                 |     | 2                               | .5           |
|                          |              |                 |     | 4                               | .5           |
|                          |              |                 |     | 8                               | .7           |
|                          |              |                 |     | 16                              | .3           |
|                          |              |                 |     | 32                              | 2.3          |
|                          |              |                 |     | 64                              | 6.1          |

aPropionate infusion rate, µmol·kg⁻¹·min⁻¹.  
bThe response areas of blood propionate and plasma insulin and glucagon concentrations were the incremental areas beneath the curve for the concentrations above basal over time, and they were calculated over 30 min during propionate infusion.

Pharmacological doses of VFA stimulate insulin release in ruminants (Manns and Boda, 1967; Horino et al., 1968; Ambo et al., 1973). However, their physiological significance has not been clarified (Stern et al., 1970). Bines and Hart (1984) investigated plasma hormone and metabolite responses to intraruminal infusion of VFA mixtures in cattle and reported that insulin concentrations were less when propionate was omitted from the infusate. They concluded that propionate was a major stimulant of insulin secretion in bovine. Istasse et al. (1987) infused propionate (approximately 70 µmol·kg⁻¹·min⁻¹ for 3 h) into the rumen of nonlactating cows and found that plasma insulin concentrations increased without any change in plasma glucose concentrations. De Jong (1982) infused a mixture of acetate, propionate, and n-butyrate (30.1, 7.4, and 3.0 µmol·kg⁻¹·min⁻¹ for 4 h, respectively) intraportally into goats and found a transient increase in plasma insulin concentrations, whereas the increments of blood propionate and n-butyrate were within the physiological range (approximately 10 and 5 µmol/L, respectively). He suggested a significant role for propionate and n-butyrate in stimulating insulin release. Our previous report (Sano et al., 1993) indicates that in sheep plasma insulin concentrations increased during propionate infusion into a femoral vein at rates (> 4 µmol·kg⁻¹·min⁻¹) less than the net rate of portal propionate absorption (6 µmol·kg⁻¹·min⁻¹; Bergman and Wolff, 1971). In the present experiment, the propionate infusion rates at which plasma insulin concentrations were increased were similar to those reported previously (Sano et al., 1993) for both infusion sites. Blood propionate concentrations at the minimum dose of propionate required to stimulate insulin release were comparable to those measured after the feeding of cows and goats (Evans et al., 1975; Emmanuel and Kennelly, 1984; Sutton et al., 1988) and differed between the infusion sites (approximately 150 and 20 µmol/L for the femoral vein infusion and the mesenteric vein infusion, respectively). The tendency for the plasma insulin responses to be less with the mesenteric vein infusion may be related to the reduced incremental blood propionate concentration areas.

Plasma glucagon responses to VFA are generally less clearcut than are insulin responses regardless of infusion doses (De Jong, 1982; Mineo et al., 1990).
Figure 2. Effect of propionate infusion (1, 2, 4, 8, 16, 32, and 64 μmol·kg BW⁻¹·min⁻¹ for 30 min) into the femoral vein (square) and the mesenteric vein (circle) on arterial plasma glucose concentrations in sheep (n = 4). Vertical bars indicate standard errors. The solid horizontal bars represent the period of infusion. Open symbols indicate differences (P < .10) from the preinfusion values. The dose of propionate is shown above the response curve.

For both propionate infusion sites, the propionate infusion rates at which plasma glucagon concentrations were increased were comparable to those obtained previously (Sano et al., 1993). The reduced plasma glucagon response areas for the mesenteric vein infusion indicate that the magnitude of plasma glucagon responses is also influenced by hepatic propionate removal. The significant propionate infusion rate x site interaction for plasma glucagon responses was due to the different responses between infusion sites at the greater propionate infusion rates. It seems that plasma glucagon responses to propionate

Figure 3. Effect of propionate infusion (1, 2, 4, 8, 16, 32, and 64 μmol·kg BW⁻¹·min⁻¹ for 30 min) into the femoral vein (square) and the mesenteric vein (circle) on arterial plasma insulin concentrations in sheep (n = 4). Vertical bars indicate standard errors. The solid horizontal bars represent the period of infusion. Open symbols indicate differences (P < .10) from the preinfusion values. The dose of propionate is shown above the response curve.
infusion could not totally be explained with the variables measured in the present experiment. Because most of propionate is absorbed through the portal vein and is removed by the liver (Bergman, 1975), it is possible that propionate infused into the femoral vein has a different effect on plasma glucagon responses from propionate absorbed from the alimentary tract, even though blood propionate concentrations were within the physiological range. Alternatively, other factors are involved in causing the differential plasma glucagon responses induced by the two routes of propionate infusion.

**Implications**

The results of the present experiment indicate that plasma insulin and glucagon concentrations in sheep are increased by a physiological dose of propionate infused into the mesenteric or the femoral veins, with no change in plasma glucose concentrations. This may indicate that propionate produced in the rumen and absorbed into the circulatory system stimulates insulin and glucagon secretion. Moreover, the magnitude of plasma insulin and glucagon responses to propionate is reduced by hepatic propionate removal.

**Literature Cited**


Figure 4. Effect of propionate infusion (1, 2, 4, 8, 16, 32, and 64 µmol·kg BW⁻¹·min⁻¹ for 30 min) into the femoral vein [square] and the mesenteric vein [circle] on arterial plasma glucagon concentrations in sheep (n = 4). Vertical bars indicate standard errors. The solid horizontal bars represent the period of infusion. Open symbols indicate differences (P < .10) from the preinfusion values. The dose of propionate is shown above the response curve.


