Effect of insulin on the utilization of propionate in gluconeogenesis in sheep

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The effects of insulin on the utilization of propionate in glucose synthesis were studied in fed and fasted sheep. Insulin was infused at 0·40 μU/h into the mesenteric vein. Glucose was infused to prevent hypoglycaemia. The rate of incorporation of [2-^{14}C]propionate into glucose was determined before and during insulin infusion. After 150 min of insulin infusion endogenous glucose synthesis was about 70% of control values, whereas the incorporation of [^{14}C]propionate into plasma glucose was 94% of control values. In contrast, the incorporation of other glucose precursors into glucose was decreased 30–50% by insulin. Therefore, insulin does not appear to decrease the utilization of propionate in gluconeogenesis. These results are consistent with the proposition that insulin differentially affects the rate of incorporation of glucose precursors into glucose in ruminant animals.

Insulin: Propionate metabolism: Gluconeogenesis: Sheep

Ruminant animals convert dietary carbohydrate to short-chain fatty acids as a result of the fermentative nature of their digestion. Thus, little or no dietary carbohydrate is absorbed as hexose sugar (Bergman *et al.* 1970) and glucose needs must be met by gluconeogenesis. Gluconeogenesis is greatest in the fed state (Katz & Bergman, 1969*b*) when the absorption of precursors and insulin concentrations are greatest (Baird *et al.* 1980).

Previous studies (Brockman, 1985; Brockman & Laarveld, 1986) have shown that insulin inhibits gluconeogenesis in sheep by reducing the hepatic extraction efficiency of some glucogenic compounds, such as lactate, alanine, glutamine and glycerol. Since those studies were conducted on fasted sheep, propionate was not examined. However, the rate of utilization of propionate, a major glucogenic precursor (Bergman *et al.* 1966; Judson & Leng, 1973) in fed animals, may not be influenced by insulin. First, the hepatic extraction of propionate is high in fed animals (Bergman & Wolff, 1971). Second, the infusion of propionate into cows did not affect the hepatic extraction efficiency of propionate despite its elevation of insulin concentrations (Baird *et al.* 1980). The purpose of the present study was to determine if insulin alters the rate of utilization of propionate in glucose synthesis.

MATERIALS AND METHODS

Mature, non-lactating, non-pregnant, mixed-breed (predominantly Columbia × Suffolk) ewes weighing 51 (SE 1) kg (*n* 6) were used in these experiments. The sheep were maintained in a temperature-controlled (20°) room with constant lighting. They received a maintenance diet of 900 g lucerne (*Medicago sativa*) pellets daily. In the fed experiments feed was divided into twenty-four equal hourly portions. For the starved experiments the sheep were given 450 g twice daily and were last fed 36 h before experimentation. Water and salt blocks were provided *ad lib*. Treatment of the animals conformed with the principles outlined in the *Guide to the Care and Use of Experimental Animals* (Canadian Council on Animal Care, 1984).

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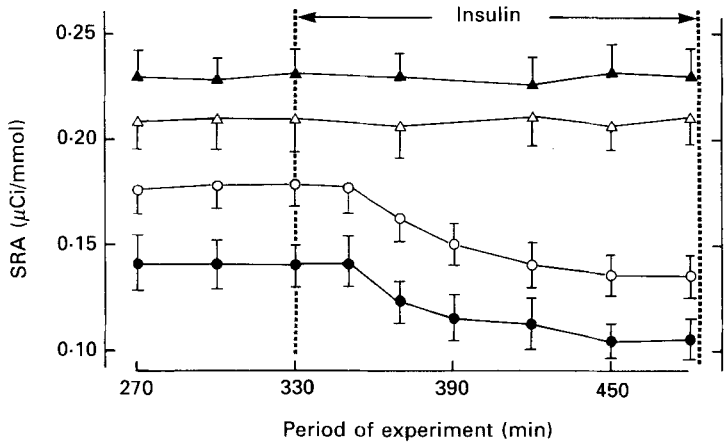

Fig. 1. ^{14}C specific radioactivities (SRA) of glucose (●, ○) and propionate (▲, △) in the plasma of fed (▲, ●) and fasted (△, ○) sheep before and during insulin infusion. Values are the means with their standard errors, represented by vertical bars, for five sheep.

At least 1 week before experimentation, polyvinyl catheters (2·50 × 1·50 mm) were surgically placed in the femoral artery and portal and femoral veins and a mesenteric vein tributary (Katz & Bergman, 1969*a*). A polyvinyl catheter (1·50 × 1·00 mm) was placed into a jugular vein the evening before experimentation. The patency of the catheters was maintained by filling them with heparinized saline (9 g sodium chloride/l; 500 U/ml). During the experiments the catheters were flushed every 15 min with saline.

The 'fed' experiments were initiated (at zero time) by giving a priming dose of 4·0 μCi [2-^{14}C]propionate (New England Nuclear, Boston, MA, USA) followed by continuous infusion at 4·5 μCi/h via the mesenteric catheter. In the 'fasted' experiments the priming dose consisted of 4 μCi radioactive isotope and 17 mmol unlabelled propionate followed by continuous infusion at 5·2 μCi/h and 22 mmol/h respectively. In the latter, unlabelled propionate was infused intramesenterically to approximate the entry rate of propionate to fed animals. After 150 min of infusion, [6-^{3}H]glucose (New England Nuclear) was infused at 20 μCi/h via the femoral venous catheter following a priming dose of 30 μCi. At 330 min (after 330 and 150 min of infusion of labelled propionate and glucose respectively) the insulin (crystalline bovine insulin, Lilly Research Laboratories, Indianapolis, IN, USA) infusion via the mesenteric venous catheter was initiated and continued for 150 min at 0·4 mU/kg per min. Insulin-free carrier was infused during the 0–330 min interval. Glucose (Dextrose, Rogar/STB, London, Ontario) was infused via the jugular venous catheter at rates to maintain euglycaemia (Brockman, 1985).

Blood samples were taken from the portal venous and arterial catheters at −20 min (for background radioactivity), 210, 240, 270, 300 and 330 min, and at 350, 370, 390, 420, 450 and 480 min after initiation of [2-^{14}C]propionate infusion. The blood samples were taken in heparinized syringes and transferred into tubes chilled on ice. The blood was centrifuged and the plasma was stored at −20° in vials containing 3·0 mg EDTA/ml plasma. Portions of plasma for glucagon analysis also contained 250 kallikrein inactivator units of aprotinin (Trasylol, Boehringer-Ingleheim, Dorval, Quebec) per ml.

All infusion solutions were made up in pyrogen-free sterile saline. The insulin solution also contained ovine plasma (50 ml/l) and ovine albumin (2 g/l). All solutions were infused by a peristaltic pump at a delivery rate of 0·30 ml/min.

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Propionate concentrations and radioactivities were determined in portal plasma with a Pye 104 gas–liquid chromatograph (Brockman & Greer, 1980). Glucose concentrations were determined in arterial plasma with a commercial kit (Statzyme, Worthington, Diagnostics, Freehold, NJ, USA). Arterial plasma glucose radioactivity was determined by isolation as potassium gluconate (Blair & Segal, 1960). Insulin and glucagon concentrations in portal plasma were determined by radioimmunoassay using a buffer system previously described (Brockman, 1979). Glucagon antiserum was GP26 (Brockman & Manns, 1985). Radio-iodinated insulin and glucagon were purchased from New England Nuclear.

The rate of appearance of glucose (RA_G) was calculated using the equation for non-steady state conditions (DeBodo *et al.* 1964) as validated for sheep (Brockman, 1984). Endogenous RA_G equals RA_G minus the rate of infusion of glucose. Propionate entry rate (RA_p) was determined from the infusion rate of [2-^{14}C]propionate (μCi/min):specific radioactivity of propionate (SRA_p, μCi/mmol) ratio in portal plasma. Portal plasma was used rather than arterial plasma because the larger amount of propionate in the former allows for more accurate determination of SRA_p. Since the source of plasma propionate is absorption from the gut, labelled and unlabelled propionate enter the plasma through the portal vein. Therefore, unless the liver removes labelled and unlabelled propionate at different rates, the SRA_p should be the same in portal venous and arterial plasma.

The proportion of propionate used in glucose synthesis was calculated as

$$(((SRA_G/2)/SRA_p) \times RA_G \times 2) \times (SRA_p/([^{14}C]\text{propionate infusion rate})),$$

which reduces to

$$(SRA_G) \times (RA_G)/([^{14}C]\text{propionate infusion rate}),$$

where SRA_G represents the specific radioactivity of glucose (μCi/mmol).

The glucose synthesized from propionate was calculated as:

$$(SRA_G/(SRA_p \times 2)) \times RA.$$

The factor 2 in the equation corrects for differences in the number of carbon atoms per molecule. These equations assume steady-state conditions existed. The calculations were based on SRA_G values measured during the last 0·5 h before insulin infusion was begun and the last 0·5 h of insulin infusion. Glucose concentrations, endogenous RA_G and ^{14}C SRA_G were constant during these intervals (Fig. 1), indicating that steady-state conditions existed.

In calculating the appearance of ^{14}C from propionate into glucose, the mean value for ^{14}C SRA of propionate during the last 210 min of [^{14}C]propionate infusion was used since there was no change in SRA throughout both sets of experiments (Fig. 1).

Statistical significance was determined using paired t tests (Li, 1964).

RESULTS

Concentrations of insulin, glucagon, propionate and glucose in plasma are shown in Table 1. The concentrations of propionate in the control period were similar in fed and fasted sheep indicating that the infusion rate of exogenous propionate approximated physiological entry rates. Insulin concentrations, however, were higher in the propionate-infused group. Insulin concentrations increased about 30 μU/ml during insulin infusion in both fed and fasted animals. The glucose infusion prevented a fall in glycaemia during the insulin infusion. Propionate and glucagon concentrations did not change significantly in both groups.

The effects of insulin on glucose and propionate metabolism are presented in Table 2. The rate of appearance of glucose and the proportion of propionate converted to glucose

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Table 1. *Concentrations of insulin, glucagon, glucose and propionate, and specific radioactivities of glucose and propionate in plasma before and during insulin infusion in sheep*

(Values are means with their standard errors for five sheep)

Hormone or metabolite	Fed				Fasted + propionate			
	Control		Insulin		Control		Insulin	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Insulin (μU/ml)	12	2	39*	2	29	4	66*	8
Glucagon (pg/ml)	76	10	71	9	97	13	104	14
Glucose (mM)	3·2	0·1	3·3	0·1	3·4	0·2	3·6	0·3
Propionate (mM)	0·30	0·01	0·31	0·01	0·35	0·01	0·36	0·01

* Mean values were significantly different from the corresponding control values (paired t test): $P < 0{\cdot}05$.

Table 2. *Glucose rate of appearance (RA_G), propionate conversion to glucose and propionate rate of appearance (RA_p) before and during insulin infusion in sheep*

(Values are means with the standard errors of the differences between the respective control and insulin means for five sheep)

Metabolite	Fed			Fasted + propionate		
	Control	Insulin		Control	Insulin	
	Mean	Mean	SED	Mean	Mean	SED
RA_G (μmol/min)	418	510*	19	410	490*	17
Endogenous RA_G† (μmol/min)	418	311*	22	410	273*	29
Glucose from propionate						
μmol glucose/min	132	117	5	171	162	4
% endogenous RA_G	31	37*	2	42	59*	3
% RA_G	31	23*	2	42	34*	2
RA_p (μmol/min)	326	330	11	413	420	13

* Mean values were significantly different from corresponding control values (paired t test): $P < 0{\cdot}05$.
† Calculated as RA_G minus glucose infused.

were similar between fed and fasted + propionate groups during the control periods. Insulin + glucose infusion decreased endogenous RA_G in both fed and fasted groups. This was associated with only a marginal (5–10%) and non-significant reduction in the proportion of propionate converted into plasma glucose. In both groups a greater percentage of endogenous glucose was derived from propionate during insulin infusion. In contrast the utilization of other precursors in glucogenesis decreased 30 and 50% in fed and fasted sheep respectively. In fed sheep the other precursors accounted for 286 and 194 μmol glucose/min and in fasted sheep 239 and 111 μmol/min during the control and insulin infusion periods respectively (Table 2).

DISCUSSION

Ruminant animals absorb very little propionate after 36 h of fasting (Lomax & Baird, 1983). Therefore, exogenous propionate was administered to fasted sheep to provide propionate. However, administration of propionate probably changed the endocrine status away from a fasting condition. Insulin concentrations were elevated by propionate infusion

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(Brockman, 1982) such that they were higher than in fed sheep (Table 1), but glucose concentrations and hepatic glucose output were not different from those of fed animals (Table 2). The proportion of propionate converted to glucose was higher in the fasted group, probably reflecting that propionate is readily available from the infusion and elevated insulin suppresses the conversion of other glucose precursors to glucose (Brockman, 1985; Brockman & Laarveld, 1986).

The present study showed that the rate of incorporation of propionate into glucose is not decreased by changes in insulin concentrations in plasma. This conclusion is supported by observations in cattle where the propionate extraction efficiency did not change during propionate infusion despite increases in insulin concentrations (Baird *et al.* 1980). The linear relationship between propionate concentrations in plasma and its utilization in glucose synthesis (Bergman *et al.* 1966; Judson & Leng, 1973) is consistent with this conclusion. Certainly gluconeogenesis from propionate is less responsive than gluconeogenesis from lactate to changes in insulin concentrations (Table 1; Brockman, 1985; Brockman & Laarveld, 1986). This differential response to insulin among glucose precursors allows for the sparing of lactate and other glucogenic metabolites when propionate availability is high. Propionate is thereby preferentially used for gluconeogenesis while lactate and glucogenic amino acids would be more readily available for other synthetic processes. Thus, the proportion of glucose derived from propionate increases.

A basis for this differential hormonal response between propionate and lactate undoubtedly is related to the fact that pyruvate carboxylase (PC; *EC* 6.4.1.1), but not phosphoenolpyruvate carboxykinase (GTP) (PEPCK; *EC* 4.1.1.32), is responsive to changes in physiological status or hormonal concentrations in ruminants (Brockman & Manns, 1974; Filsell *et al.* 1969). The PC-catalysed reaction is not involved in the conversion of propionate to triose phosphate, whereas the conversion of lactate to triose phosphate utilizes both the PEPCK- and PC-catalysed reactions. Therefore, hormonal-induced changes in PC activity would alter the rate of utilization of lactate and other glucose precursors in carbohydrate synthesis without altering the rate at which propionate is used for this process.

The present study has a number of limitations. In the gluconeogenic pathway, propionate is converted to oxaloacetate (OAA). In the direct conversion of propionate to glucose the label from [2-^{14}C]propionate is not lost (White *et al.* 1978). However, the labelled OAA may enter the tricarboxylic acid (TCA) cycle where the label may be lost in the cycling (White *et al.* 1978). If the unlabelled OAA re-enters the gluconeogenic pathway the conversion of propionate to glucose is undetected, thereby causing an underestimation of the rate of gluconeogenesis. This underestimation may be minimal (Leng & Annison, 1963). It would be particularly small in the fasted sheep, since 80% of the label appeared in plasma glucose (Table 2).

However, underestimation of the rate of gluconeogenesis would not alter the conclusions if the loss of label by entry of OAA into TCA was not changed by the insulin–glucose infusion. If such infusion changes the entry of OAA into the TCA cycle, it probably would increase it. This contention is supported by the ability of insulin to increase the production of $^{14}CO_2$ from [2,3-^{14}C]succinate in isolated rat hepatocytes (Bessman *et al.* 1986). Moreover, insulin–glucose infusions seem to be thermogenic (Ravussin & Bogardus, 1982); presumably this increases TCA cycle activity. This would cause a reduction in the incorporation of label into glucose and would tend to exacerbate any inhibitory effect of insulin on gluconeogenesis.

Gluconeogenesis was calculated as the glucose released into the plasma. Any newly synthesized hexose phosphate that was converted to hepatic glycogen and not released in the blood as glucose would not be included in the calculations of gluconeogenesis. It is

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possible that insulin increased the rate of hepatic glycogen synthesis (Bishop *et al.* 1965). Thus, the rate of appearance of ^{14}C into blood glucose probably underestimated the utilization of propionate for carbohydrate synthesis during insulin infusion and overestimated any inhibitory effect of insulin on incorporation of propionate into hexose sugars. However, the effects of increased glycogen synthesis, rather than masking any inhibitory effect of insulin on gluconeogenesis, would exacerbate it. This would be of major concern only if insulin decreased hepatic output of [^{14}C]glucose significantly. In the present study the appearance of ^{14}C into plasma glucose decreased only 5–10%.

Finally, significant error could result from the rate of change in SRA_G (Brockman & Laarveld, 1986). The glucose pool has a relatively slow turnover time of 1 h (Leng, 1970). A decrease in the rate of incorporation of propionate into glucose would take some time to be reflected accurately in the SRA_G. Measurements taken before equilibration cause overestimates of the rate of gluconeogenesis. However, this error was minimized by using SRA_G values from the last two samples during insulin infusion. The SRA of [^{14}C]glucose was relatively constant at that time. In conclusion, the methodological errors do not alter the validity of the findings of the present study.

It is possible that insulin increases the absolute amount of propionate converted to hexose sugar. Studies with rat liver cells suggest that insulin enhances glucose production from propionate (Soling & Seuffert, 1975). It is not clear from the study of Soling & Seuffert (1975) whether the estimates of glucose production included newly synthesized glycogen. If it did, release of glucose from hepatic cells may have been decreased while the total production of carbohydrate (glucose + glycogen) increased. If hexose phosphate is converted to glycogen at an increased rate and the rate at which propionate is converted to triose phosphate is not impaired, the flux of propionate to glycogen + glucose may increase. In the present study hepatic glycogen synthesis was not determined. Thus, the possibility of increased utilization of propionate in gluconeogenesis cannot be ruled out.

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