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THE DEPENDENCE OF EXOCRINE PANCREATIC SECRETION ON INSULIN IN SHEEP

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SUMMARY

Exocrine pancreatic secretion, blood insulin, glucose and free fatty acid concentrations were measured on days 1, 4 and 5 of a 5 d experimental period. Sheep were treated with alloxan (day 2) and insulin (day 5). The volume of pancreatic juice decreased markedly in the diabetic state and returned to the initial value after insulin treatment. The daily secretion of lipase and amylase decreased during development of diabetes. Injection of insulin restored the secretion of lipase but not amylase. It is suggested that insulin potentiates the stimulatory action of the vagus nerve on the pancreatic secretion of sheep.

INTRODUCTION

Many species differences can be observed in the regulation of pancreatic secretion (Chey, 1980). Certain peculiarities exist in the digestive processes of adult ruminants, among them the activity of the pancreas. The classical experiments of Harrison & Hill (1962) on sheep showed a relatively constant secretion rate of bile and pancreatic juice during the day, with a slight increase in response to an increase of digesta flow in the duodenum. The daily output of pancreatic juice and its enzymatic activity is lower in sheep than in dogs, pigs or other non-ruminants (Hill, 1961). Also the pancreatic response to secretin is less in sheep than in dogs (Caple & Hill, 1975). Experiments by Reynold & Heath (1981) indicate that the vagus nerve plays an important role in the stimulation of enzyme output and juice flow in the pancreas of sheep; treatment with cholecystokinin produced only a slight effect on enzyme secretion and no effect on the flow of pancreatic juice.

Recently the significance of insulin in the exocrine activity of the pancreas has been demonstrated by Saito & Kanno (1980) and Saito, Williams & Kanno (1980*a*, *b*). The insulin secretion in sheep depends significantly on the rate of fermentation processes in the rumen (Ostaszewski & Barej, 1979) and observations (W. Barej, unpublished) show a parallel increase of the blood insulin level and pancreatic juice flow after feeding. The present experiments therefore were undertaken to study the effect of insulin deficiency on the exocrine pancreatic activity in sheep.

METHODS

Experiments were carried out on five sheep about 2 years old. The animals were accustomed to a ration consisting of sugar beet silage, with urea–mineral concentrate and hay. The rumen cannula, as well as cannulas to the duodenum and pancreatic duct, were inserted according to techniques described by Pierzynowski (1978). The cannulation of the pancreatic duct allowed the temporary collection of pure bile-free pancreatic juice, which after quantitative measurement and sampling was reintroduced into the duodenum. The cannula to the rumen was used for feeding throughout experimentation. Such procedure standardized the amount of feed obtained by each animal. Every

Table 1. *The mean values of insulin, glucose and FFA concentrations in the blood of sheep during the experimental period*

Compound	Day of experiment		
	1 (before treatment)	4 (diabetic state)	5 (insulin treatment)
Plasma insulin ($mu.l^{-1}$)	18·3±7·37*	3·87±4·38†	103·9±16·1‡
Blood glucose ($mmol.l^{-1}$)	2·48±0·47*	13·21±3·4†	4·95±2·47*
Plasma FFA ($\mu mol.l^{-1}$)	174±47*	416±151†	197±45*

*†‡ given with the results describe the significant differences of values when $P \leqslant 0{\cdot}01$. Values are expressed ±S.D. ($n = 35$; five sheep; seven samplings during the day).

Table 2. *The mean values of the flow rate, pH and enzyme activities of pancreatic juice in sheep during the experimental period*

Estimations	Day of experiment		
	1 (before treatment)	4 (diabetic state)	5 (insulin treatment)
Flow rate ($ml.h^{-1}$)	14·0±2·9*	5·01±2·05†	16±7·5*
pH	7·58±0·36	7·76±0·19	7·65±0·09
Amylase activity			
($u.ml^{-1}$)	480±141*	742±373†	193±84‡
($u.h^{-1}$)	6748±2434*	3566±1793†	3220±988†
Lipase activity			
($u.ml^{-1}$)	150±26*	225±43†	149±25*
($u.h^{-1}$)	2092±341*	1111±135†	2273±666*

*†‡ given with the results describe the significant differences of values, when $P \leqslant 0{\cdot}01$. Values are expressed ±S.D. ($n = 35$; five sheep; seven samplings during the day).

day sheep were administered sugar beet silage intra-ruminally (0·4 kg dry matter) at 7.00 a.m. and hay orally (0·5 kg) at 5.00 p.m.

On days 1, 4 and 5 the pancreatic secretions and blood samples were collected from the jugular vein in 1–2 h intervals between 6.00 a.m. and 5.00 p.m. On the morning of the second day the animals were treated intravenously with alloxan (40 $mg.kg^{-1}$). According to our previous observations diabetes developed within 48 h after alloxan treatment. Immediately after collection of pancreatic juice (5.00 p.m.) on the fourth day of the experiment, 3 d after alloxan treatment, the animals were given insulin intramuscularly (1·5 $u.kg^{-1}$). A similar injection of insulin was administered 14 h later. In addition to measuring the flow rate of pancreatic secretion, the pH, amylolytic and lipolytic activities were estimated. Amylolytic activity was determined colorimetrically using maltose as a standard (Caraway, 1959). Lipolytic activity was determined by the method of Cherry & Crandall (1932). Blood glucose was estimated by the glucose oxidase and peroxidase method using the 'Blut–Zucker Test-Fermognost'. Plasma concentrations of free fatty acid (FFA) were determined according to Duncombe's method (1963) and plasma insulin by a radioimmunoassay method (IBJ Test, Swierk). The results were statistically evaluated by analysis of variance.

RESULTS

The changes in the concentrations of plasma insulin, glucose and FFA (Table 1) show the diabetic state in sheep on the fourth day of the experiment. The level of insulin dropped

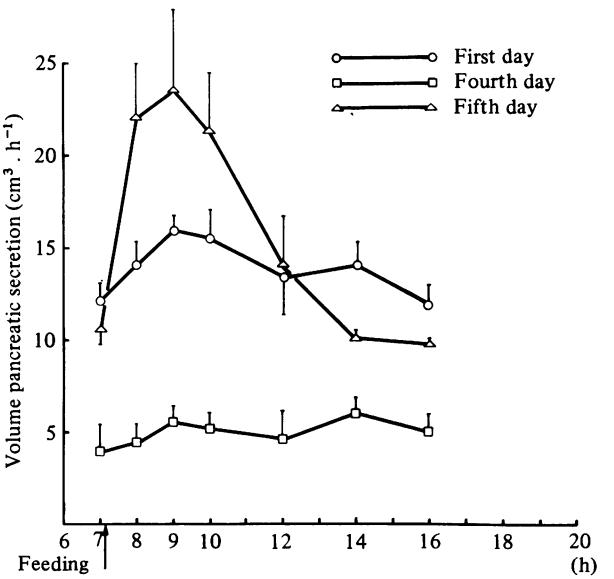

Fig. 1. Daily changes in the pancreatic flow rate during experimental period ($n = 5$).

significantly from 18 mu.l^{-1} on the first day to 3·9 mu.l^{-1} on the fourth day. The subsequent injections of insulin caused the significant increase of the insulin level in the blood.

The level of glucose increased significantly on the fourth day of the experiment, from 2·48 to 13·21 mmol.l^{-1}. On the fifth day the glucose concentration dropped significantly but it did not reach the initial value in spite of a high level of insulin. The FFA concentration in the blood increased significantly on the fourth day of the experiment and returned to the initial value after insulin treatment.

Data in Table 2 present the flow rate, pH and enzyme activities of pancreatic juice during the successive days of the experiment.

On the first day (control value) the average output of pancreatic juice was 14·0 ml.h^{-1} with a small increase immediately after feeding (Fig. 1).

The pancreatic juice output showed a significant decrease (5·0 ml.h^{-1}) on the fourth day of experiment when diabetes developed (Table 2). Intra-ruminal feeding in this state did not change pancreatic secretion (Fig. 1). Injection of insulin into diabetic sheep restored the control value of the pancreatic juice secretion (Fig. 1). There was no significant change in the pH of the pancreatic secretion throughout the experiment, except for a small increase on the fourth day.

On the fourth day of the experiment (diabetic state) the concentration of amylase and lipase in the pancreatic juice increased compared to the first day. However, the daily secretion of these enzymes was significantly lower because of the scanty outflow of juice (Table 2). In the diabetic sheep injection of insulin caused a return of lipase secretion to the original level while it did not change the daily secretion of amylase. The concentration of amylase at this time was much lower than on the first and fourth days of the experiment. Injection of insulin into the diabetic sheep caused a return of lipolytic activity to the original values and a significant decrease of amylolytic activity.

DISCUSSION

The flow of pancreatic juice in the diabetic sheep was greatly reduced (day 4) but was restored after insulin treatment. The intra-ruminal feeding of healthy sheep (day 1) caused a small temporary increase in the volume of pancreatic secretion. Similar changes in the insulin and glucagon levels were observed by Ostaszewski & Barej (1979) who suggested that the increase of rumen fermentation after feeding stimulated endocrine pancreatic activity. The intra-ruminal administration of food to the insulin-treated animals significantly stimulated pancreatic juice secretion but it was ineffective in diabetic sheep (Fig. 1). All these observations show the importance of insulin in the regulatory mechanism of pancreatic juice production in sheep.

The stimulatory role of insulin in the mouse and rat pancreatic acini was explained by Korc, Sankaran, Wong, Williams & Goldfine (1978) and Korc, Iwamoto, Sankaran, Williams & Goldfine (1981). According to these findings, insulin directly stimulates the synthesis of enzymatic protein. Saito *et al.* (1980*a*) suggested that insulin potentiated the cholecystokinin action in isolated rat pancreas in both juice flow and amylase secretion. The daily secretion of pancreatic lipase and amylase in diabetic sheep was greatly reduced (Table 2). The insulin treatment of diabetic sheep restored the secretion of lipase but not of amylase.

Some species differences regarding sheep pancreatic juice secretion have been reported by Caple & Heath (1975) and Reynolds & Heath (1981). They found a dominant role of the vagus nerve in the production of pancreatic juice. Probably the insulin dependence of vagal action in the pancreatic secretion is developed much more in ruminants than in monogastric animals. The continuous digestive processes in the fore-stomach and similar digesta flow into duodenum offset the regulatory mechanisms in the digestive tract. Under such conditions the changes of internal factors, like insulin concentration, may play an important role in the pancreatic juice production. A stimulatory effect of insulin in the acetylocholine-induced secretory response in the perfused rat pancreas was observed by Saito *et al.* (1980*b*). It seems however that exogenous insulin is less effective than the endogenous insulin, since the experimental hyperinsulinaemia in sheep did not restore the production of pancreatic amylase. In the latter, however, the damage of pancreatic acini as the suppressing effect of hyperglycaemia should also be taken into consideration.

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