ISOLATION OF PANCREATIC ISLETS FROM SHEEP PANCREAS

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Introduction

There is general agreement that intestinal hormone, adrenargic nervous system and absorbed metabolites (short-chain fatty acids, glucose and amino acids) influence insulin secretion from the pancreatic B cell in ruminant.

In vitro insulin release from the intact pancreatic islets of rats have been studied extensively. In the ruminant, Jordan and Phillips (1978) described insulin release from the isolated islets from sheep pancreas. In sheep pancreas, as well as in cattle pancreas, wide bands of collagen are present between the pancreatic lobules.

Lacy et al. (1982) suggested that the liberation of intact islets from beef pancreas is interfered by gelatinous-form collagen during digestion with collagenase. And they reported that a large number of intact islets from beef were obtained using strips of Velcro with collagenase digestion.

The present study aimed at isolating the islets from the sheep pancreas using the procedure as described by Lacy et al. (1982).

Materials and Methods

Two castrated male sheep at 9 months of age (42 and 53 kg body weight) and two female sheep at 10 months (35.5 kg) and 7 years of age (66 kg) were used in this study. After slaughtering, the pancreas was removed and immediately placed in ice-cold Hanks' solution containing 10% fetal calf serum (FCS).

The outline of the procedure to isolate pancreatic islets from sheep in this study is as follows.

Hanks' solution containing 10% FCS and aprotinin (450 KIU/ml) were infused to distend the pancreas with a needle and syringe at various sites in the tail and the body of the pancreas. The serous membranes, blood vessels and fat tissue on the surface of the distended pancreas were removed, then the pancreatic tissue was chopped 4-5 mm in size to cover the hook surface of the siliconized Velcro (5x6 cm).

Pancreatic tissue on the Velcro was inserted into the plastic centrifuge tube (50 ml) and was digested with high (960 U/ml) or low level collagenase (740 U/ml) at 37°C for 20 min by sampling the contents at 1 min intervals. The digested tissues obtained from each interval were washed with Hanks' solution and placed on Ficoll or sucrose gradients. The density gradient centrifugation was performed at 1000 rpm for 10 min. The layer containing islets was removed and washed with tissue culture medium RPMI1640 containing D-glucose (1.0 mg/ml). The islets and tissue fragments were transfered to a petri dish and then examined with a dissecting microscope at x10 magnification.

The islets were picked up with a siliconized capillary pipette and transfered to a 10 ml test tube. About 50 islets were incubated in 3 ml of RPMI1640 containing D-glucose (1 mg/ml) for 30 min, then incubated in the medium (3 ml) containing 5 mg/ml of glucose. Insulin concentration in the medium were determined by means of an EIA.

Results and Discussion

The islets isolated from the sheep pancreas were found at the interface between the top and second layers of the density gradients after the centrifugation. It is described that the islets isolated from beef (Lacy et al., 1982) and from rat (Lindall et al., 1969) were found at the same interface. Under a dissecting microscope, the pancreatic islets isolated from sheep appeared a gray spherical structure. 173 islets on the average were obtained from 1 gram of the pnacreas. The sheep pancreas used in this study weighed approximately 60 grams, therefore, it is assumed that about 10,000 islets could be obtained from a single pancreas of sheep.

The effect of the collagenase level on isolation of the islets from sheep pancreas is shown in figure 1. 75 percent of islets isolated in 20 min were

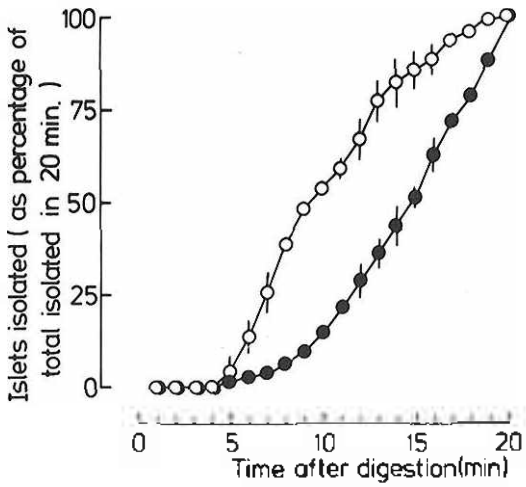

Figure 1. Effect of collagenase level on isolation of the islets from sheep pancreas. The mean value with their standard errors represented by vertical bars. The sheep pancreas were digested with collagenase 740 U/ml (●) or 960 U/ml (○).

obtained by digestion for 13 min with high level collagenase (960 U/ml). In contrast to low level collagenase (740 U/ml), 75 percent of islets were obtained for 18 min. This difference in the isolation rate must be due to the level of collagenase. It is suspected that prolonged digestion with collagenase reduced the viability of isolated islets, therefore, digestion with a high level of collagenase for 15 min would be sufficient to isolate islets from sheep pancreas.

Insulin concentration on the average (± SE) in the medium of incubation with glucose (5 mg/ml) was 256.5 ± 150.0 μU/ml. The insulin release stimulated in this study by glucose confirmed the in vitro result of Jordan and Phillips (1978).

We concluded that the procedure to isolate pancreatic islets using Velcro as described by Lacy et al.(1982) was available to isolate islets from sheep pancreas.

(Key Words: Pancreatic Islet, Isolation, Sheep)

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