MONOLAYER CULTURE OF PANCREATIC ISLETS FROM THE SYRIAN HAMSTER

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SUMMARY

Pancreatic islets of Langerhans of the Syrian hamster were maintained in culture for as long as 43 wk. Islets were prepared by collagenase/hyaluronidase digestion of minced pancreas. The islets quickly attached to the plastic culture flasks and lost their spherical form as they flattened out to form circular monolayers. Few fibroblastoid cells were observed. As outward migration continued, the islets became vacuolated with the ultimate formation of monolayer rings. Throughout the culture period the beta cells continued to synthesize and secrete insulin. Furthermore, the cells maintained a responsiveness to glucose stimulation with increased rates of hormone secretion in the presence of elevated concentrations of the sugar. These studies demonstrate the suitability of Syrian hamster islets for studies involving long-term culture.

Key words: islets of Langerhans; Syrian hamster; monolayer culture.

INTRODUCTION

Culture of pancreatic islets has been of considerable interest for some time and the various methods employed have been reviewed (14). Many of these systems, however, suffer two major shortcomings: the islets invariably lose their functional responsiveness to physiologically significant stimuli and the cultures are not truly long term. To date there has been no report on the maintenance of islets from the Syrian golden hamster. We have recently developed such a system in which the islets continue for many months to secrete high levels of insulin in response to glucose. This report describes the behavior of these islets both immediately after isolation and during prolonged culture.

METHODS

Male Syrian golden hamsters, 8 wk old (Charles River Breeding Laboratories, Wilmington, MA), were used for all experiments. Pancreatic islets were isolated by a modification of the collagenase method (5). Under ether anesthesia the three-lobed pancreas was removed and placed in Hanks' solution (pH 7.4), containing 4.5 mM glucose and 1 mg/ml bovine serum albumin (BSA). Adjacent fat was trimmed away and three pancreases were pooled and finely minced with scissors. The pancreas pieces were washed three times in the same buffer and suspended in a volume of 4 ml in a 16 x 100 mm siliconized, screw-capped glass vial. After the addition of 8 mg crude collagenase (Type IV, 202 U/mg, Worthington Biochemical Corp., Freehold, NJ) and 3 mg hyaluronidase (from bovine testes, Type IV, 810 NF U/mg, Sigma Chemical Co., St. Louis, MO) the capped vial was shaken by hand in a 37° C water bath. After 10 min the digest was washed seven times in 200 ml of buffer to remove most of the acinar tissue. Islets were individually picked from the washed digest under a dissecting microscope with the aid of a Pedersen constriction pipette. Only clean islets, free of surrounding acinar tissue, were selected.

Islets were washed five times with 4 ml of alpha-modified Eagle's medium containing 10 mM glucose, 25 mM HEPES, penicillin (100 U/ml), and streptomycin (100 μg/ml), supplemented with 15% vol/vol heat-inactivated fetal bovine serum. Groups of 100 islets were seeded in 4 ml of culture medium in 25-cm² plastic flasks and maintained in a water-saturated atmosphere of 5% CO₂ in air. Except where stated, medium was changed twice weekly. The spent medium was centrifuged to remove any floating cells and debris, and aliquots were frozen for the subsequent determination of insulin content utilizing a radioimmunoassay kit (Amersham Corp., Oakville, Ontario, Canada). Aliquots of the medium were also assayed for glucose by the glucose oxidase method, using a Beckman glucose analyzer II. During some weeks, cultures were maintained for two sequential 2-d periods in the presence of 4 and then 20 mM glucose.

Some experiments were performed in which freshly isolated, noncultured islets were evaluated with respect to their ability to secrete insulin in response to glucose challenge. Batches of 20 islets were incubated in a Dubnoff metabolic shaking incubator under an atmosphere of 95% O₂:5% CO₂. The incubation medium consisted of 500 μl of Krebs-Ringer bicarbonate buffer supplemented with 5 mg/ml BSA and 4 mM glucose. After 60 min the buffer was removed completely and replaced with 200 μl of buffer containing various concentrations of glucose. After a further 60 min, the incubation medium was removed and centrifuged at 60 ×g for 10 min and aliquots were frozen for the subsequent determination of insulin. Islets were extracted with 500 μl acid alcohol for 24 h at 4° C. The extracts were also assayed for insulin.
RESULTS

Pancreatic islets were easily prepared by the collagenase method. Typically 200 to 250 well-preserved islets could be isolated from each pancreas. Figure 1 shows the secretion of insulin in batch-type incubations of freshly isolated islets. At the lowest concentrations of glucose, or in the complete absence of the sugar, insulin secretion amounts to less than 3 μU/islet per hour. The threshold for glucose stimulation lies between 4 and 5 mM. Half-maximum rates of secretion occur in the presence of about 8 mM. Maximum secretion, about 40 times basal, is observed at 15 mM, with no further increase at higher concentrations. The insulin content of the islets, as measured by the amount in acid alcohol extracts, was 600 ± 100 μU/islet.

Soon after plating, the islets became attached to the bottoms of the culture flasks. At the first medium change (4 d) more than 85% of the islets had attached. The remainder were removed with the spent medium. At this point the attached islets had started to lose their original shape, and began to flatten at their margins (Fig. 2 A). A few fibroblastoid cells were observed at the peripheries of some islets. Over the next 2 to 3 mo the islets continued to flatten, ultimately forming circular monolayers, about 4 to 8 mm in diameter, consisting of well-granulated cells (Fig. 2 B). During the process of flattening, two or more neighboring islets often fused into one monolayer. Some of the larger islets did not flatten completely and remained multicellular at their centers.

FIG. 1. Insulin secretion by freshly isolated hamster islets. Groups of 20 islets were incubated at various glucose concentrations as described in the text. Results are expressed as the mean ± SEM for three observations at each glucose concentration.

FIG. 2. Appearance of hamster islets in culture. A, At 1 wk the islet has started to flatten. Note the absence of fibroblasts. X106. B, Portion of an islet, 4 mo after plating. Note the monolayer arrangement of cells. Vacuolation has started with the appearance of a cell-free “hole” at the top center of the photograph. X74. C, At 5 mo, vacuolation is more pronounced as several “holes” fuse. X148. D, At 8 mo the central region of the islet monolayer is almost completely lost leaving a ring of cells. A patch of cells remains near the center of the ring. X32.