INSULIN SECRETION FROM A CONTINUOUSLY SUBCULTIVABLE ISOLATE DERIVED FROM FOETAL BOVINE PANCREATA BETA CELLS: I. MONOLAYER CULTURES

Anthony M. Sun, George M. Healy, Ivan Vacek and Hilda G. Macmorine

Connaught Laboratories Limited
Toronto, Ontario, Canada
M2N 5T8

Received September 1,1977

SUMMARY: Monolayer cultures have been derived from bovine embryonic pancreatic cells grown in medium CMRL-1969 supplemented with foetal calf serum. The isolate has been subcultivated up to 10 population doublings. Insulin secretion from the cells into the culture medium declined with increasing passages. Of several insulin secretagogues, glucagon was found to be effective in potentiating insulin release from the cultivated cells into the medium. Insulin secretion rose to approximately 600 μU/culture/day in the presence of glucagon as compared to an average of 10 μU/culture/day in the control. This may be the first demonstration of a beta cell line developed from bovine embryonic pancreas.

INTRODUCTION: Monolayer cultures of pancreatic cells from rodents and humans have been the subject of many reports (1, 2, 3, 4). Most of the investigations involved the growth of primary cultures from fresh explants. It is now known that glucose induces only a small insulin release in embryonic beta cells, but marked stimulation follows the addition of caffeine (2). It has also been suggested that insulin secretion could depend upon, and be regulated by, hormones such as glucagon, corticotrophin, and thyrotropin (5).

The development of bovine, pancreatic cell cultures which have been subcultivated through ten passages and remained viable and functional is reported. The effects of glucagon upon the insulin release into the culture fluids from the subcultivated cells were also examined.

MATERIALS AND METHODS: Foetal, bovine pancreata were collected under aseptic conditions from local slaughter-houses. To obtain Islets of Langerhans the collagenase-digestion techniques of Lacy (6) were used with some modifications. Briefly, pancreatic tissue was dressed and perfused by multiple puncture with Hanks' balanced salt solution. The minced tissue was digested for 20 to 30 minutes at 37°C with collagenase (Worthington Type IV, 15 mg/4 ml of tissue), and hyaluronidase (Worthington,

ISSN 0006-291X

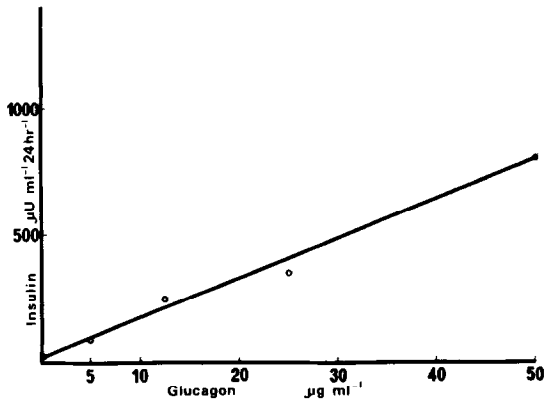

Figure 1: Dose response of insulin secretion from calf, embryonic β cells (9th passage) in the presence of glucagon. Cells were cultivated for 24 hours in medium CMRL-1969 supplemented with 7.5% foetal calf serum and 300 mg% of glucose.

10 mg/4 ml of tissue). Isolated islets were partially purified from acinar cells by washing and decanting (7). They were then digested for 5-10 minutes at 37°C in 2 ml of warm trypsin solution (Sigma, 0.25%), prepared in Ca- and Mg-free phosphate-buffered saline (8). To prepare the cell cultures, the cells released by the trypsin digestion alongwith the intact islets were washed 3 times with medium CMRL-1969 (9) supplemented with 7.5% foetal calf serum, Hepes buffer (0.36%) and Gentamicin (20 μg/ml). The cells were then seeded into Falcon flasks (25 cm^2) in the same medium as described above, and cultivated at 37°C.

To study insulin secretion in response to secretagogue challenges, the culture medium was replaced with serum-free medium after 3 washes. Twenty-four hours later the cells were challenged with medium (serum-free) containing insulin secretagogues for 24 hours. Samples were taken from the culture media for insulin determinations.

Glucagon was obtained from both Connaught Laboratories and Calbiochem. Bovine growth hormone (NIH-GH-B-17) was made available through the National Institutes of Health, Washington. Insulin in the culture fluid was determined by the immunoassay of Hales and Randle (IRI) (10). Each sample was assayed in triplicate. The cells were grown in Leighton tubes, fixed in Bouin's solution and stained with Gomoris' aldehyde-fuchsin stain (11). Specimens of the culture were also stained by the fluorescent antibody method using guinea pig anti-insulin serum (12).

RESULTS: The culture, established in medium CMRL-1969 supplemented with 7.5% foetal calf serum, consisted primarily of epithelioid cells which proliferated rapidly after the first passage. In general, the epithelioid

Table 1: Insulin$^{(IRI)}$ secretion from subcultivated bovine embryo, pancreatic beta cells (6-10th passage) in the presence of various insulin secretagogues

Insulin Secretagogues	IRI (μU/ml/24 hrs)[a]	
	Pre-challenge	Post-challenge
Glucose 1 mg/ml	5.7 ± 0.71	8.1 ± 0.63
Glucose 3 mg/ml	6.6 ± 0.69	8.6 ± 2.16
Glucagon 50 μg/ml + Glucose 1 mg/ml	10.7 ± 2.35	652.1 ± 39.10
Glucagon 50 μg/ml + Glucose 3 mg/ml	6.5 ± 1.44	780.2 ± 56.37
Bovine Growth Hormone 25 μg/ml + Glucose 1 mg/ml	15.0 ± 9.81	14.8 ± 6.48

[a] Mean ± S.E. Number of experiments = 6.

cells possessed the morphological characteristics of pancreatic beta cells. Histological examination showed that these cells were stained by aldehyde-fuchsin and also by immunofluorescence stain using bovine insulin anti-serum.

Insulin secreted into the medium declined exponentially with increasing passages. Large quantities of insulin were always present in the medium from primary cultures. This high insulin concentration progressively declined until only a trace of insulin could be detected in the culture medium, usually after the fourth or fifth passage. Cells cultivated beyond this stage no longer responded to high glucose challenges (Table 1). However, when these cells were cultivated in a medium containing glucagon, insulin secretion into the culture medium was markedly increased (Table 1). In twenty-four hours the insulin output increased from 10.7 ± 2.35 μU/ml/

culture before glucagon challenge to 652.1 ± 39.10 μU/ml/culture, after glucagon was added to the medium.

The stimulation was found to be effective both in the presence of low (100 mg%) and high (300 mg%) glucose concentrations. The dose response study showed that glucagon, at a concentration as low as 5 μg/ml/culture fluid, can stimulate insulin secretion significantly. By increasing the glucagon concentration from 5 to 50 μg/ml, the increase in insulin release was almost linear (Figure 1).

When bovine growth hormone was used as an insulin stimulant, no significant increase in insulin secretion into the surrounding medium was obtained (Table 1).

DISCUSSION: Using the technique described for the development of mixed, bovine, pancreatic cell cultures, viable cultures were derived with high frequency. In most cases these primary, mixed cultures were functional and secreted appreciable amounts of insulin into the surrounding medium, suggesting that beta cells were present in the cultures. However, with increasing passages the quantity of insulin secreted (in the absence of secretagogues) decreased sharply. The ability of the cells to secrete insulin in considerable quantities in response to stimulation by glucagon suggests that the physiological competence of the constituent beta cells has been retained. The observed rise in insulin secretion probably resulted from a direct effect of glucagon on the beta cells and may be similar to that in the liver cell where glucagon activates 3', 5' adenosine-monophosphate which in turn activated phosphorylase and thereby increases glycogenolysis (13). Studies on the effect of other secretagogues such as theophylline, c-AMP and c-GMP on insulin secretion from the cell cultures are in progress.

The results of this study show clearly that pancreatic islet beta cells retain their functional, physiological competence in tissue culture after as many as ten passages and indicate the feasibility of using such in vitro systems for further studies of beta cell functions.

ACKNOWLEDGEMENTS: This work was supported by a grant under the Industrial Research Assistance Programme of Canada. We are grateful for their support and encouragement. We wish to acknowledge the assistance of our technical staff: Mrs. B. Hexham, Mrs. A. Wood, Mr. A. Haviko, and Mr. E. Nossek.

REFERENCES:

1. Lambert, A.E., Blondel, B., Kanazawa, Y., Orci, L., and Renold, A.E. (1972) Endocrinology 90, 239-248.
2. Lambert, A.E., Junod, A., Stauffacher, W., Jeanrenaud, B., and Renold, A.E. (1969) Biochim. Biophys. Acta. 184, 529-539.
3. Lambert, A.E., Jeanrenaud, B., and Renold, A.E. (1967) Lancet I, 819-820.
4. Hilwig, I., and Schuster, S. (1970) Z. Zellforsch, Mikrosk. Anat. 103, 282-290.
5. Malaisse, W.J., Malaisse-Lagae, F., and Mayhew, D. (1967) Clin. Invest. 46, 1724-1734.
6. Lacy, P.E., and Kostianovsky, M.M. (1967) Diabetes 16, 35-39.
7. Sun, A.M., Lin, B.J., and Haist, R.E. (1973) Can. J. Physiol. Pharmacol. 51, 175-182.
8. Kostianovsky, M., McDaniel, M.L., Still, M.F., Codilla, R.C., and Lacy, P.E. (1974) Diabetologia 10, 337-344.
9. Healy, G.M., Teleki, S., Seefried, A.v., Walton, M.J., and Macmorine, H.G. (1971) Appl. Microbiol. 21, 1-5.
10. Hales, C.N., and Randle, P.J. (1963) Biochem. J. 88, 137-146.
11. Gomori, G. (1950) Amer. J. Clin. Path. 20, 665-666.
12. Nairn, R.C. (1969) in Fluorescent Protein Tracing, 3rd ed. (Nairn, R.C., ed.) pp. 232-295, E. & S. Livingston Ltd., Edinburgh and London.
13. Samols, E., Marri, G., and Marks, V. (1966) Diabetes 15, 855-866.