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COVER SHEET FOR FINAL REPORT

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Name of Project: Insulin and Glucagon Secretion In Vitro

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TMC/NASA Cooperative Agreement Program NCC 9-36, Round II FINAL REPORT

PRINCIPAL INVESTIGATOR: Arun S. Rajan, M.D. Baylor College of Medicine

TITLE: Insulin and Glucagon Secretion *In Vitro*

Objectives

Long-duration space flight is associated with many physiological abnormalities in astronauts. In particular, altered regulation of the hormones insulin and glucagon may contribute to metabolic disturbances such as increased blood sugar levels, which if persistently elevated result in toxic effects. These changes are also observed in the highly prevalent disease diabetes, which affects 16 million Americans and consumes over $100 billion in annual healthcare costs. By mimicking the microgravity environment of space in the research laboratory using a NASA-developed bioreactor, one can study the physiology of insulin and glucagon secretion and determine if there are alterations in these cellular processes. The original specific objectives of the project included: 1) growing ('cell culture') of pancreatic islet beta and alpha cells that secrete insulin and glucagon respectively, in the NASA bioreactor, 2) examination of the effects of microgravity on insulin and glucagon secretion, and 3) study of molecular mechanisms of insulin and glucagon secretion if altered by microgravity.

Description

The first goal of the research was to optimize conditions for cell culture of the islet cells in the bioreactor. Two experimental cell models, HIT (hamster insulin tumor) and αTC-6 (mouse glucagonoma) cells were used to study insulin and glucagon secretion respectively. Studies of morphology, growth and cell viability were performed. In addition, 3-dimensional cell culture of co-cultured beta and alpha cells was undertaken. The second experimental aim was to examine islet cell function by measurement of hormone secretion from the bioreactor-cultured islet cells. A dynamic perifusion system was used with the cultured cells placed in Swinnex (Millipore) filtration chaambers and continuously perifused with defined physiological media. Time-based, effluents were collected and analyzed for hormone content by radioimmunoassay. Our final objective was to characterize the molecular mechanisms of secretion by analysis of common second messenger signaling pathways including cytosolic Ca^{2+} and cAMP transduction. These latter studies could not be completed during the present project period.

Significance

The ability to grow differentiated tissue in the shear-free environment of the bioreactor enables examination of the influence of microgravity on the structure and function of cultured cells. Thus, these studies may contribute to a better understanding of the cell-cell interactions in the pancreatic islet and physiology of islet secretions. Ultimately, new insights into the pathophysiology of diabetes may be gained that could facilitate development of novel therapeutic measures for diabetics on earth and for space travelers.

Progress

Our initial studies began with microcarrier-based cell culture of the islet beta (HIT) or alpha (αTC-6) cells in the STLV bioreactor using protocols previously established with other cell types. We examined cell adherence to collagen substrates (Cytodex-3 (Pharmacia) and Solo Hill Engineering), Cultospheres (Sigma) and Biosilon plastic-coated beads (Nunc). In general, islet cell attachment to the microcarriers was poor with all surfaces tested and collaged-coated substrates being the most preferred. The beta cells attached better to the Cytodex-3 spheres than alpha cells and also propagated over the surface of the bead to a limited extent. We determined that the cells attached to the microcarriers remained viable. These results were unexpected because the islet cells adhere well to, and grow as monolayers on, conventional tissue culture ware. Apparently, similar results are observed in smooth muscle cells grown in the bioreactor. An interesting observation during these studies was that cells tended to attach together and form small clumps. These small masses then organized themselves with surrounding cells attached to the beads into bits of tissue. In essence, islet cells (both beta and alpha) preferred to attach to themselves rather than to artificial substrates.

Based on these results, we initiated islet cell cultures in the STLV bioreactor without the use of microcarriers. Remarkably, islet cell aggregates were formed and there was continued growth of the individual cells. Viability testing using dual fluorescent labeling (live-dead assay) demonstrated that the cells showed good viability over a long-term period (cultures maintained for up to a month). This result was identical for both the islet beta and alpha clonal cells. Having constructed tangible cell masses that could be grown in simulated microgravity, we examined the functional response of the cells to this environment. Using a static incubation protocol, we assayed the insulin secretory response of beta cell aggregates to physiological stimulants. Our early studies suggested that compared to conventionally grown beta cells, beta cells grown in the bioreactor show a blunted insulin secretory response to glucose challenge while retaining normal secretory capacity to a high potassium stimulus. Further characterization revealed that microgravity-grown beta cells show diminished insulin secretion (compared to controls) to a variety of physiological insulin secretagogues including amino-acids, glyburide (a clinical agent that restores insulin secretion in diabetics) and forskolin (a cAMP elevator). Thus, under the conditions tested (i.e. static incubations), microgravity appears to induce a global suppressive effect on hormone secretion from pancreatic islet cells.

Our most exciting results were obtained in the co-culture experiments. When the beta and alpha cells were seeded together for bioreactor culture, islet cell aggregates were formed within 24 to 48 hours. These aggregates were analyzed extensively as follows. First, phase contrast microscopy revealed spherical constructs that appeared to have a central 'core' of cells surrounded by a mantle layer of cells, a pattern which was confirmed by hematoxylin-eosin staining of fixed sections. Further characterization involved fluorescent labeling ('Cell tracker') of either the beta or alpha cells prior to initiating the culture to determine the distribution of the labeled cells in the generated aggregates. These studies showed that beta cells constitute the core of the aggregate and are encircled by the alpha cells. This result was confirmed by the use of dual label immuno-histochemistry using insulin and glucagon antibodies. This remarkable non-random arrangement of cells is significant because it mimics the native cytoarchitecture of normal pancreatic islets. In addition, this result is achieved although we are using clonal (tumor) islet cells and despite the fact the cells originate from different species – beta (HIT) cells from hamster, and alpha (αTC-6) cells from mouse. Thus, the microgravity-like environment enhances the inherent recognition and confluence of islet cells to recreate near-normal islet architecture. Having assembled a perifusion system in this period, we also examined the secretory response of the bioreactor-generated cell aggregates. Preliminary experiments suggest that both first and second phase glucose-stimulated insulin secretion is enhanced in beta-alpha constructs relative to the beta-only cell aggregates (Note that the perifusion system now allows evaluation of hormone secretion in a dynamic manner permitting dissection of the phases of secretion versus a cumulative single time point analysis of static experiments).

The results from the co-culture experiments have initiated an unexpected and novel line of investigation. The significance of this new research is that microgravity biotechnology may enable the synthetic construction of islets with near-native structure and function. If successful, these 'islets' may potentially be used in islet transplantation, a therapeutic remedy that is presently handicapped by a severe shortage of donor islets. Thus, in vitro-constructed islets may be a vital tool in combating diabetes mellitus.

Outcomes and future plans

An abstract of the research (see attached) has been accepted for presentation at the FASEB meeting in December 1998. As a result of the results achieved and its potentially important impact on health we are continuing the studies initiated here, to characterize the biophysics of islet cell aggregation, structure and function using the bioreactor and comparing the efficiency of different culture vessels. In addition, we are also exploring strategies of tissue engineering (islet co-culture with carrier cells e.g. Sertoli cells) that may prevent immune rejection of potential islet grafts, and also of using islet precursors to induce differentiation into mature islets in the bioreactor. We have submitted a grant application in response to a NASA Microgravity Biotechnology NRA (97-HEDS-02) and continue to seek other funding to continue this exciting research, made possible by this NASA-TMC grant.

To be presented at the FASEB Meeting in December 1998

ASSEMBLY OF FUNCTIONAL ISLETS FROM ALPHA AND BETA CELL LINES IN SIMULATED MICROGRAVITY. A. S. Rajan, Dept. of Medicine, Baylor College of Medicine, Houston, Tx 77030 and S. Navran, Synthecon, Inc., Houston, Tx 77054

The technique of cell culture in simulated microgravity has recently been used to construct a variety of three-dimensional tissue-like aggregates from dispersed cells in a horizontally rotating bioreactor. These aggregates exhibit more differentiated functions than conventional two-dimensional culture. We have used this principle to construct artificial islets from alpha and beta cell lines in the NASA-designed rotating bioreactor.

Incubation of dispersed hamster islet beta cells (HIT) and mouse islet alpha cells (alpha TC-6) in the rotating bioreactor resulted in cell aggregates of 0.5-1.0 mm diameter within 48-72 hr. Immunohistochemical analysis of formalin-fixed aggregates showed a central core of insulin positive cells surrounded by a peripheral layer of glucagon positive cells, mimicking the native islet cytoarchitecture. This cellular organization occurs despite the different species sources of the cells. Islet cell aggregates composed of alpha plus beta cells or beta cells alone were compared for their ability to secrete insulin in response to a glucose challenge. Insulin secretion from the alpha/beta aggregates was enhanced compared to beta cell aggregates confirming that interactions between alpha and beta cells are important for islet secretory function.

These results show that cell culture in simulated microgravity can produce complex, three dimensional islet-like aggregates which respond to physiologic stimuli. This technique should prove useful for studying islet cell interactions and may eventually lead to a source of artificial islets for transplantation.