FUNDAMENTAL CRYOBIOLOGY OF PANCREATIC ISLETS OF LANGERHANS

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ABSTRACT

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Coupled with the rapid development of clinical pancreatic islet transplantation, there is an increasing requirement for cryopreservation of islets. On an individual cell basis, fundamental cryobiology requires determination of several cryobiophysical parameters to predict optimal cryopreservation procedures. These include the hydraulic conductivity (water permeability; L_p) and its activation energy (E_a), the permeability of the cell plasma membrane to a cryoprotectant(s) (P_s) and its E_a, the osmotically inactive fraction of cell volume (V_b), and demonstration of linear osmometric behavior. The goal of this thesis was to determine the above parameters for individual hamster pancreatic islet cells and incorporate this knowledge into a mathematical model of an aggregation of islet cells, i.e. the whole islet.

Before initiating an investigation of islet cells, a much larger and more easily characterized cell type, the hamster and murine oocyte, was used for validation of the experimental methods. Hamster whole islets as well as the individual islet cells were then measured in a similar manner and found to behave as ideal osmometers. To determine L_p and P_s it was necessary to develop new methods of exposing islets and islet cells to cryoprotectant agents (CPAs) while dynamically measuring the volumetric response. Toward this goal we developed and validated (again using oocytes) both a micropipet

holding technique and a microperfusion chamber. Concurrently, an electronic particle measurement device (Coulter Counter) was used for most of the individual cell membrane permeability measurements. Hamster islet cell L_p at 22°C was found to be .25 μm/min/atm with an E_a of 16.2 Kcal/mole. For CPA permeability determination islet cells were measured at 4 different temperatures after exposure to 2 different CPAs, dimethyl sulfoxide (DMSO) and ethylene glycol (EG). Arrhenius plots ($\ln(L_p)$ or $\ln(P_s)$ vs. 1/Temperature (°K)) were created and found to be linear with correlation coefficients (r) of .99 for all four plots (L_p and P_s for both CPAs).

A mathematical model of water and CPA transport for whole hamster pancreatic islets purfused with DMSO, EG, and hypertonic PBS was then created. This model combined the effects of intracellular and intercellular transport of water and CPA throughout the islet and used the above experimentally derived cryobiologic characteristics from the individual islet cells. The model was developed using two differential equations for membrane flux and one second order differential equation to describe diffusion. The model was validated using a 3x3 factorial experimental method with whole islet experiments compared with model predictions at 3 temperatures using three perfusing solutions and three islet sizes. Three to six islets were compared at each of the 27 experimental conditions and found to fit with a coefficient of determination (R^2) of 0.87±.06 (mean±S.D.). We therefore conclude that a model has been constructed which accurately predicts volume deviation in response to osmotic and CPA challenges for whole islets.

TABLE OF CONTENTS