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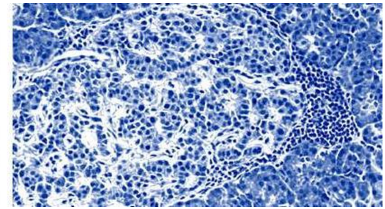
Network for Pancreatic Organ Donors with Diabetes



The Role of Glycolytic Flux in the Control of Glucagon Secretion

AUTHORS

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PURPOSE

The pathophysiology of type 1 diabetes (T1D) is bihormonal, with both insufficient insulin production due to loss of beta cell mass and dysregulated glucagon release by alpha-cells. To date, despite the increased acknowledgment that abnormal glucagon secretion is central to the pathophysiology of both T1D and T2D, the precise mechanisms controlling glucagon release from alpha-cells and, even more so, its dysregulation in diabetes are unclear. It was recently shown that *G6PC2,* encoding the islet-specific glucose-6-phosphatase 2, is dramatically downregulated in alpha cells of T1D patients. *G6PC2* is active exclusively in pancreatic islets and multiple GWAS have linked polymorphisms in *G6PC2* with variations in fasting blood glucose levels. *G6PC2* limits glycolytic flux by creating a futile cycle with Glucokinase (*GCK*), thus limiting glucose-mediated stimulation of insulin secretion. However, the same mechanism would be expected to lead to stimulation of glucagon secretion in alpha-cells. We hypothesize that G6PC2 functions in alpha-cells as part of the glucose-sensing mechanism, therefore altering its levels will affect glucagon secretion. Downregulation of G6PC2 in alpha cell from T1D could therefor halt glucagon release in the fasting state which could worsen the hypoglycemic episodes seen in these patients.

METHODS

In order to elucidate the role of glucose metabolism and the glycolytic flux in controlling glucagon secretion, we manipulated the levels of G6PC2 in mouse and human islets and measured their response to glucose suppression of glucagon release. We transduced dispersed islet cells with lentiviral particles carrying the G6PC2 coding sequence and GFP under control of a CMV promoter or with an shRNA against G6PC2. Islets were allowed to reaggregate for 5 days to form pseudo-islets before being assayed for glucagon and insulin secretion.

SUMMARY OF RESULTS

G6PC2 overexpression affected both glucagon and insulin secretion, albeit in opposite directions; while glucagon release was increased at basal glucose levels (3mM, $p<0.05$), insulin secretion was significantly decreased at high glucose levels as compared to controls (9mM, $p<0.05$). Next, we employed shRNA to suppress G6PC2 levels in pseudo-islets. We found that low concentrations of glucose were more effective in reducing glucagon secretion in this paradigm, as predicted by our model.

CONCLUSIONS
These preliminary data support our hypothesis that glycolytic flux and glucose cycling are critical to the control of alpha-cell function, which will be further evaluated by utilizing two new genetically modified mouse strains to ablate and overexpress *G6pc2* specifically in alpha cells.