Understanding the metabolic reprogramming linked with (dys)regulation of islet amyloid polypeptide (IAPP) expression in Type 2 Diabetes mellitus

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Specific problem being addressed: Human islet amyloid polypeptide (hIAPP), or amylin, is a peptide hormone that is co-secreted with insulin from the pancreatic β-cells. In Type 2 Diabetes mellitus (T2DM), to meet the increased nutrient (high glucose and free fatty acid) supply, imbalance in the expression of IAPP and insulin occurs. Additionally, pro-IAPP and hIAPP have been known to aggregate and form amyloid fibrils that mediate toxicity in β-cells. Detailed studies are required to gain insights into the metabolic reprogramming that occurs as a result of hIAPP aggregation in pancreatic cells, its effect on the insulin secretion, and its regulation. Here, we aim to investigate the metabolic disturbances that occur in pancreatic β-cells upon IAPP exposure. We will also attempt to understand the influence of identified metabolites on IAPP expression and aggregation in pancreatic cells and gain further insights into the pathways involved in regulation of these processes.

Project summary: Our lab recently established a method of producing recombinant hIAPP (rhIAPP) in large amounts using a cost-effective method. Using rhIAPP, we will establish cytotoxic conditions in pancreatic insulinoma (INS-1E) and will investigate the dose-dependent changes in metabolite patterns associated with the same. Recombinant hIAPP fibrils will be produced using the method developed in our laboratory. The rhIAPP fibrils mediate cytotoxicity in the pancreatic insulinoma cells - INS-1E and affected insulin release from the pancreatic islets in a dose-dependent manner. Taking leads from these experiments, we will expose INS-1E cells to sub-optimal and IC50 concentrations (increasing concentrations) for different time points – 1 hour, 2-hour, 6-hour, 24 hour and 48 hours. Post-incubation, the cells will be pelleted, washed with PBS and subjected to metabolite extraction using ice-cold methanol. The extracts will be lyophilized, reconstituted in NMR buffer (10 mM phosphate buffer at pH 6.5) prepared in D2O and will be transferred to NMR tubes. Identification of the metabolites will be performed using ^{1}H NMR spectroscopy and ^{1}H ^{1}H 2D total correlation spectroscopy (TOCSY). DSS (4,4- dimethyl-4-silapentane-1-sulfonic acid) will be used as an internal calibration standard for chemical shift referencing as well as absolute concentration measurement of metabolites. The different metabolites that show time and dose-dependent changes with hIAPP exposure will be identified. Pathway analysis will also be performed to understand the major metabolic pathways affected thereby. In addition to this, supporting data will also be generated by measuring, mitochondrial membrane permeability, lipid peroxidation, reactive oxygen species (ROS) levels, protein and transcript levels of genes involved in apoptosis, ER stress and mitochondrial stress generation, insulin signaling and the β-cell.

Our study will provide a comprehensive overview of the metabolic pathways affected by hIAPP aggregation. In addition to this, the effect of the significantly affected metabolites on hIAPP aggregation and expression will also be investigated in INS-1E cells expressing hIAPP. Our studies would also provide information on a new therapeutic regimen of drugs that can be used for the treatment of T2DM. Also, since hIAPP aggregates form much earlier in time, much before the actual disease manifests in, these drugs might be able to delay the onset of T2DM.
