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Single Islet Metabolomics using Capillary LC-MS

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Summary

Dysfunction in insulin secretion profiles from islets of Langerhans is a hallmark of type II diabetes. Insulin secretion is metabolically driven from various sources including glucose, glutamine, fatty acids, and lactate. Interestingly, when individual islets, comprising about 500 cells are stimulated using glucose, their secretion profiles fall into one of three categories. To date, the metabolic changes that are associated with these different profiles are not known. This is due to metabolite analysis from islets being available only from batches of islets. Using chemical tagging and capillary based LC-MS, we are able to analyze over 80 metabolites from a single islet.

Derivatization and analysis: First, 3-(diethylamino) propionyl chloride tag was synthesized by reacting 3-(diethylamino) propanoic acid and 1-chloro-N,N,2-trimethyl-1-propenylamine (Ghosez's reagent) in dimethylformamide. Islet lysate was reacted with 3-(diethylamino)propionyl chloride with pyridine for 30 minutes. The first step reaction was quenched with N,N-Diethylenediamine followed by addition of HATU/HOBt and allowed to react for 2 hours.

Sample injection and Separation: Individual islets were injected using a bomb injection method to load the entirety of the sample. Analytes are separated with 5mM ammonium carbonate buffer (pH= 9)/acetonitrile on RPLC capillary 50µm x 23 cm column packed with Kinetex Evo C18 2.6µm particles reverse phase column (RP). nESI analysis on a Q-Exactive mass spectrometer operating at resolution = 70k.

The primary/secondary amines and alcohols was derivatized to amides and esters from the acyl chloride tag (1st step) and is stable under neutral condition Ghosez's reagent offers. Organic acids and other carboxylic acids were derivatized to amide using the diamine tag (2nd). The reaction after second step has an average reaction efficiency of 98% and average % RSD of 7. 80 specific metabolites were targeted for analysis and all 80 were detected in single islets as well as from populations down to 25 cells. Islet secretions were also analyzed for metabolite changes, as insulin is co-secreted with a number of neurotransmitters and biogenic amines. Islets were subjected to low glucose, stimulatory glucose for 20 minutes and stimulatory glucose for 40 minutes. Our results show substantial metabolic differences in energy metabolism and amino acid profiles between these groups.

Our dual stage derivatization increases sensitivity and S/N of analytes to allow the analysis of 8o metabolites from individual islets down to single cell levels.

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