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Glucose-dependent phosphorylation signaling pathways and crosstalk to mitochondrial respiration in insulin secreting cells

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Abstract

Background: Glucose is the main secretagogue of pancreatic beta-cells. Uptake and metabolism of the nutrient stimulates the beta-cell to release the blood glucose lowering hormone insulin. This metabolic activation is associated with a pronounced increase in mitochondrial respiration. Glucose stimulation also initiates a number of signal transduction pathways for the coordinated regulation of multiple biological processes required for insulin secretion.

Methods: Shotgun proteomics including TiO_2 enrichment of phosphorylated peptides followed by liquid chromatography tandem mass spectrometry on lysates from glucose-stimulated INS-1E cells was used to identify glucose regulated phosphorylated proteins and signal transduction pathways. Kinase substrate enrichment analysis (KSEA) was applied to identify key regulated kinases and phosphatases. Glucose-induced oxygen consumption was measured using a XF96 Seahorse instrument to reveal cross talk between glucose-regulated kinases and mitochondrial activation.

Results: Our kinetic analysis of substrate phosphorylation reveal the molecular mechanism leading to rapid activation of insulin biogenesis, vesicle trafficking, insulin granule exocytosis and cytoskeleton remodeling. Kinase-substrate enrichment identified upstream kinases and phosphatases and time-dependent activity changes during glucose stimulation. Activity trajectories of well-known glucose-regulated kinases and phosphatases are described. In addition, we predict activity changes in a number of kinases including NUAK1, not or only poorly studied in the context of the pancreatic beta-cell. Furthermore, we pharmacologically tested whether signaling pathways predicted by kinase-substrate enrichment analysis affected glucose-dependent acceleration of mitochondrial respiration. We find that phosphoinositide 3-kinase, Ca2+/calmodulin dependent protein kinase and protein kinase C contribute to short-term regulation of energy metabolism.

Conclusions: Our results provide a global view into the regulation of kinases and phosphatases in insulin secreting cells and suggest cross talk between glucose-induced signal transduction and mitochondrial activation.