Simultaneous Measurements of Intracellular \([\text{Ca}^{2+}]\) i and \([\text{cAMP}]\) i in Intact Islets to Study the Mechanism Underlying Dopaminergic Inhibition of Insulin Secretion

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Pancreatic islets secrete multiple hormones, including insulin, that are required to maintain glycemic levels and respond to insulin secretagogues in everyday activities. We are interested in understanding the interplay between molecular mechanisms that precisely regulate these secretions. One specific paracrine modulator, dopamine, functions as a negative regulator of insulin secretion in the context of the pancreatic islet. It is secreted by the insulin-producing \(\beta\)-cells, activates an autocrine negative feedback that decreases the frequency of glucose-stimulated \([\text{Ca}^{2+}]\) i oscillations, and in turn, inhibits insulin secretion. The \(\beta\)-G protein coupled dopamine receptors are present in islet cells, but it is not clear how activation of these receptors results in the observed changes in \([\text{Ca}^{2+}]\) i in intact pancreatic islet cells. We are using an mTurquoise-Based cAMP biosensor with an improved dynamic range (Klarenbeck, J.B., et al., PLoS One, 2011), along with organic and genetically encoded \([\text{Ca}^{2+}]\) i indicators. Labeled cells are studied by live imaging using perfused pancreatic islet. Spectral unmixing is used to extract the fluorescence emissions. This experimental setup allows us to monitor the effect of specific dopamine receptor agonists and antagonists on the two main cellular second messengers. Also, islets from mice with a genetic target mutation of the DRD3 (D3-KO) are used to measure how the deletion of the dopaminergic feedback changes the second messenger dynamics. With the same approach we are measuring the effects of the overexpression of dopamine receptor D3 in wild-type and D3-KO islets. The information from these experiments will help elucidate the mechanism of dopamine signaling in the pancreatic islet.

Determining the Dopaminergic Feedback Pathway in Pancreatic \(\beta\)-Cells with Fluorescence Fluctuation Spectroscopy

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Tight regulation of insulin and glucagon, two hormones secreted by the pancreatic islet, allows the body to maintain glucose homeostasis. Insulin resistance coupled with insufficient insulin secretion leads to type 2 diabetes. Insulin secretion from pancreatic \(\beta\)-cells is regulated by multiple signaling inputs including the neurotransmitter dopamine. Upon treatment with dopamine, the amplitude and frequency of intracellular free calcium oscillations in \(\beta\)-cells decrease, leading to a concomitant decrease in insulin secretion. We hypothesize that activation of the dopamine receptor (DRD3) by dopamine releases the Gbg subunit which is expected to increase the diffusion of calcium, as well as each of the Gbg subunit. To test this hypothesis, we are utilizing fluorescence fluctuation spectroscopy to determine protein interactions in stable \(\beta\)-cell lines. Fluorescence fluctuation spectroscopy provides a method to determine interaction between two proteins in a live cell that surpasses many of the limitations of FRET. Single and two color studies allow us to determine diffusion constants of each protein before and after dopamine stimulation and the cross-correlation, or amount of interaction, between two proteins. Additionally, brightness analysis can be used to determine homo- and heterodimerization of the tagged proteins. Diffusion constants, cross-correlation rates, and protein clustering among the DRD3, Gbg subunit and Gbg subunit will be presented. Through this analysis, the dopamine signaling pathway in pancreatic \(\beta\)-cells can be determined, which will reveal novel potential therapeutic targets that can increase insulin secretion during diabetes mellitus.

Making an Aquaporin Water-Tight: Structural Basis of Selectivity in Plant Nodulin 26 Intrinsic Proteins

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The evolution of land plants led to the amplification and diversification of the aquaporin superfamily of membrane channels. Among the subfamilies of plant specific aquaporin-like changes are the nodulin-26 intrinsic proteins (NIPs) which are multifunctional transporters of water, ammonia, glycerol and metalloid nutrients that participate in a number of osmoregulatory and metabolic functions. NIPs share the conserved hourglass fold of the aquaporin family, but possess substitutions within the aromatic arginine (ar/R) selectivity filter. The nine proteins of the NIP subfamily in the model plant Arabidopsis thaliana can be subdivided into two ar/R pore subgroups: the NIP subgroup I, which form aquaglyceroporins that are permeated by glycerol, water and ammonia, and the NIP subgroup II, which form metalloporins which are lack aquaporin activity and are essentially “water tight”. These two NIP pore families differ principally by the substitution of a conserved alanine (NIP subgroup II) for a conserved tryptophan (NIP subgroup I) in the helix 2 position (H2) of