2012 Pilot & Feasibility Award Recipients

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"Two pore-domain potassium channels: Pancreatic islet expression and function"

Pancreatic islet hormone secretion is imperative to maintaining glucose homeostasis and becomes perturbed in 7.8% of the United States population that develops diabetes. Thus, identifying the mechanisms that regulate islet hormone secretion may reveal new therapeutic targets for treating diabetes. Glucose stimulated insulin secretion results from calcium entry that occurs due to beta-cell membrane potential depolarization, which activates voltage dependent calcium channels (VDCCs). The activity of two-pore domain potassium (K2P) channels regulates the membrane potential in neurons, which influences calcium entry and electrical activity. However, a role of K2P channels in regulating the pancreatic β-cell membrane potential is unknown. We have determined that K2P channels are expressed in pancreatic islet cells where their currents modulate the membrane potential and influence glucose stimulated calcium influx. Therefore, this project plans to test the hypothesis that K2P channels modulate the membrane potential of mouse and human islet cells regulating calcium influx and hormone secretion. The specific objectives are to: A. Define the K2P channels of mouse and human islet cells, B. Identify the biophysical regulation of islet K2P channels and their influence on membrane potential and hormone secretion, and C. Characterize the pharmacology of islet K2P channels and utilize this pharmacology to address the influence of K2P channels on human and mouse islet cell electrical activity and hormone secretion. These goals will significantly contribute to our understanding of the ion channels that influence islet hormone secretion and provide important insights of potential therapeutic targets relevant to diabetes.

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"Small molecule modulators of the Y4 receptor for treatment of obesity"

Pancreatic beta cells faced a variety of ongoing stresses that lead to the accumulation of damaged proteins and organelles. Recent evidence indicates that a housekeeping process known as autophagy plays a critical role in clearing these damaged components, as genetic disruption of autophagy in pancreatic beta cells leads to beta cell dysfunction and loss of beta cell mass in mice. In addition, ubiquitinated protein aggregates accumulate in stressed beta cells, suggesting that a form of autophagy known as selective autophagy becomes compromised in response to conditions that lead to beta cell stress. However, very little is known about the molecular mechanisms that lead to the formation of these potentially toxic protein aggregates, what steps in selective autophagy are rate limiting for their clearance, or how their presence impacts beta cell function under conditions where selective autophagy fails. As a first step toward addressing these questions, in this pilot project we will test the hypothesis that selective autophagy is responsible for the clearance of both cytoplasmic and nuclear protein aggregates that form in response to beta cell stress, and that failure of this process is associated with beta cell dysfunction. To do so, we will utilize a combination of live cell imaging and immunocytochemical approaches to 1) test the hypothesis that two key components of the selective

autophagy pathway, LC3 and p62, cooperate to remove ubiquitinated protein aggregates from the nucleus as well as the cytoplasm, and that this pathway functions to clear aggregates formed in response to oxidative stress in pancreatic beta cells in vitro and 2) determine if the accumulation of cytoplasmic and nuclear ubiquitinated protein aggregates is a general characteristic of mouse models of beta cell dysfunction and human islets from type 2 diabetics. In addition to testing a novel role for selective autophagy in nuclear quality control, these pilot studies will enable us to develop model systems to study how selective autophagy contributes to beta cell homeostasis, determine how this process becomes compromised in type 2 diabetes, and identify steps in this pathway that could serve as potential targets for therapeutic intervention in order to prevent or reverse beta cell damage in humans.

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The 375 amino acid G-protein coupled receptor (GPCR) neuropeptide Y4 receptor (Y4) is expressed both in the periphery, including the gastrointestinal tract, and in the central nervous system. The Y4 subtype is the only receptor of the Y-receptor family with a very high affinity for the 36-residue pancreatic polypeptide (PP). Selective small molecule modulators of this receptor would not only be valuable probe molecules to study its pharmacology, they also present attractive strategies for treatment of obesity. In fact, variants of PP are currently in phase II clinical trials for treatment of obesity (TM30339, 7TM Pharma). However, as PP is a peptide, issues of stability and bioavailability remain. The development of subtype-selective orthosteric ligands has failed in the past hampering development of effective probe molecules and therapeutics6. It is the objective of the present study to identify small molecule allosteric modulators of Y4 as tools for pharmacological research and to test their potential in a therapeutic strategy. We argue that allosteric modulators of GPCRs have a higher chance to be selective as allosteric binding sites tend to be evolutionary less conserved between subtypes. The therapeutic potential of allosteric modulators is further increased by their ability to fine-tune the receptor instead of turning it entirely on or off. Side effects are reduced not only through subtype selectivity but also as the therapeutic acts only at times when the receptor is engaged by its native ligand. It is the central hypothesis of the present proposal that identification of small molecule allosteric modulators selective to Y4 will allow the development of probe molecules to study subtype-selective pharmacology and can seed drug discovery programs in obesity. Preliminary data demonstrate the adaptation of an Y4 functional assay for high-throughput screening (HTS). A pilot screen of 2,000 compounds of the Spectrum Collection (MicroSource) yielded several hit compounds. Among those, Niclosamide displayed a robust, selective allosteric potentiation with an EC50-value of 410 nM. Cell lines for orthogonal GIRK-based assay for hit validation and to establish the initial selectivity profile through testing allosteric modulation of Y1/2/5 have been established.