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Molecular Basis of Antidiabetogenic Hormone Action

The research program under my direction is focused on the identification and characterization of novel therapeutic strategies that might be of use for the treatment of type 2 adult-onset diabetes mellitus. To this end, recent funding by the NIH and the American Diabetes Association has allowed my laboratory to initiate studies that should establish the efficacy of a new class of blood glucose-lowering agents collectively known as incretin hormones. One such incretin is GLP-1, a hormone secreted by endocrine L-cells of the distal intestine, and which when administered to type 2 diabetic subjects, lowers levels of blood glucose (*Fig.1*).

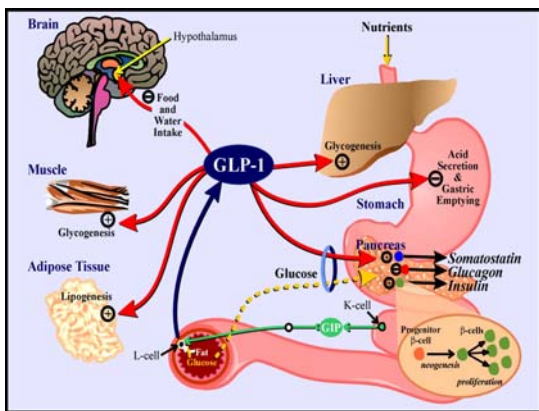


Fig. 1: GLP-1 is implicated in the regulation of systemic glucose homeostasis via its effects on multiple organ systems. In the pancreas, GLP-1 potentiates glucose-dependent insulin secretion from beta-cells located within the islets of Langerhans.

Why is GLP-1 of special interest? Earlier studies of pancreatic beta-cells located within the islets of Langerhans demonstrated that GLP-1 acts via its cognate G protein-coupled receptor to stimulate insulin gene transcription, insulin biosynthesis, and insulin secretion. More recently, it has become appreciated that GLP-1 also increases the number of beta-cells within the pancreas. This action of GLP-1 is explained by its ability to act as a growth factor. GLP-1 stimulates conversion of progenitor cells into new beta-cells, thereby increasing beta-cell “mass” substantially (*Fig.1*). Simultaneously, GLP-1 upregulates coordinate gene expression that maintains beta-cells in a fully differentiated state. In summary, this unique constellation of insulinotropic and growth factor-like actions of GLP-1 most likely explains its efficacy as a blood glucose-lowering agent .

With these points in mind, it is has been our interest to define at a molecular level the signal transduction properties of GLP-1 that confer such beneficial therapeutic effects. To this end, we have focused on ascertaining how GLP-1 exerts an acute stimulatory effect on pancreatic insulin secretion. Because the insulin secretagogue action of GLP-1 is dependent on metabolism of glucose by the beta-cells, it

appears likely that GLP-1 interacts with beta-cell glycolytic and/or mitochondrial metabolism, perhaps by facilitating the production of a metabolic coupling factor (ATP) important to glucose-dependent insulin secretion. Alternatively, GLP-1 might facilitate the action of such coupling factors, and in fact evidence exists that GLP-1 reinforces inhibitory effects of ATP at beta-cell ATP-sensitive K^+ channels. These channels play a critical role as determinants of the beta-cell membrane potential (Fig.2).

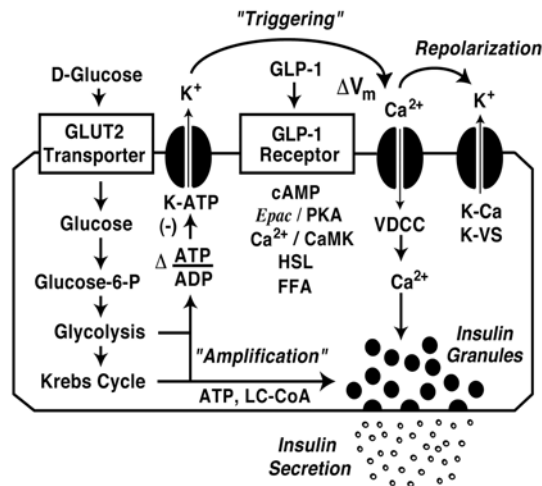


Fig. 2. Illustrated is the consensus model for glucose-dependent insulin secretion in the beta-cell. Metabolism of glucose produces an increase of ATP/ADP concentration ratio. This causes closure of ATP-sensitive K^+ channels (K-ATP), depolarization (ΔV_m), and influx of Ca^{2+} through voltage-dependent Ca^{2+} channels (VDCCs). We Hypothesize that GLP-1 accelerates glycolytic and mitochondrial metabolism of glucose while also rendering K-ATP channels more sensitive to the increase of ATP/ADP concentration ratio generated as a consequence of glucose metabolism.

Recent studies provide evidence in support of the model presented in Fig.2. Using mitochondrially-targeted luciferase to measure the [ATP] within the inner mitochondrial matrix, it was demonstrated that GLP-1 stimulates mitochondrial oxidative phosphorylation. This action of GLP-1 was dependent on exposure of beta-cells to glucose. It was also associated with a transient increase of cytosolic Ca^{2+} concentration that resulted from the release of Ca^{2+} from intracellular Ca^{2+} stores. Interestingly, the Ca^{2+} mobilizing action of GLP-1 was shown to be secondary to this hormone's known ability to stimulate cAMP production in beta-cells. Thus, there appears to exist a novel process of cAMP-dependent Ca^{2+} mobilization in beta-cells that is intimately linked to the stimulation of mitochondrial ATP production. This concept is in agreement with the established ability of Ca^{2+} to stimulate TCA cycle and respiratory chain dehydrogenases important to oxidative phosphorylation.

Working with my associates Drs. Oleg G. Chepurny and Guoxin Kang, I have begun to test elements of the model discussed above. Our methods of analysis include patch clamp electrophysiology, confocal microscopy for measurement of $[Ca^{2+}]_i$, imaging of mitochondrial [ATP], and the primary cell culture of human beta-

cells. Using these methods, we have established the existence of a novel cAMP signaling mechanism that utilizes protein kinase A (PKA) and the *Epac* class of cAMP-regulated guanine nucleotide exchange factors (cAMPGEFs) to influence intracellular Ca^{2+} homeostasis. In our model, GLP-1 utilizes PKA and *Epac* to sensitize intracellular Ca^{2+} release channels (ryanodine and IP_3 receptors) to stimulatory effects of cytosolic Ca^{2+} , thereby promoting Ca^{2+} -induced Ca^{2+} release (CICR). The ensuing increase of cytosolic $[\text{Ca}^{2+}]$ acts as a signal for increased ATP production (Fig.3).

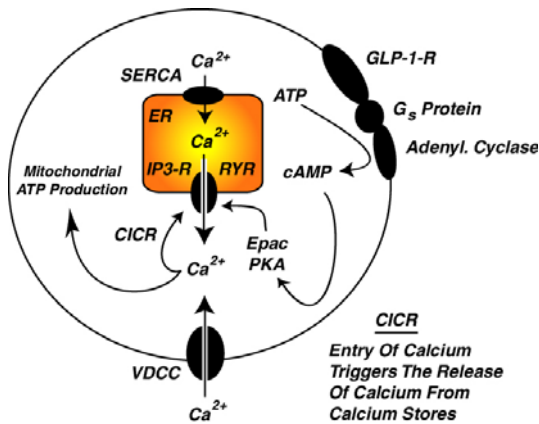


Fig.3 Glucose metabolism stimulates VDCC-mediated Ca^{2+} influx, thereby triggering CICR when Ca^{2+} release channels are sensitized by GLP-1. The source of Ca^{2+} released in this manner is the endoplasmic reticulum (ER) which we believe to be intimately associated with the mitochondria, thereby explaining how ER Ca^{2+} release drives mitochondrial ATP production. A feed-forward mechanism exists because increased ATP production favors an additional round of K-ATP closure, Ca^{2+} influx, and CICR.

It is noteworthy that we find *Epac* to be expressed not only at the plasma membrane but also the nuclear envelope of beta-cells (Fig.4). Expression of *Epac* at the plasma membrane is expected because this is where adenylyl cyclase is located. In contrast, expression of *Epac* at the nuclear envelope is unexpected. Perhaps nuclear envelope-associated *Epac* plays some role in the regulation of gene expression in beta-cells? If so, findings described here may provide the impetus for new avenues of research concerning how GLP-1 influences beta-cell growth and differentiation.

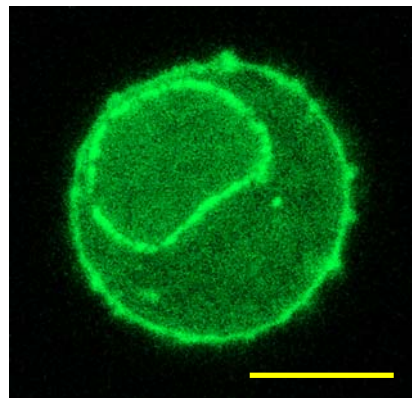


Fig.4 Illustrated is the pattern of *Epac1* expression imaged in a rat INS-1 insulin-secreting cell. These cells were transfected with an *Epac1*-EGFP fusion protein and visualized by confocal microscopy (calibration bar indicates 5 microns). The plasma membrane localization of *Epac* is of interest because it suggests a possible role of *Epac* in ion channel modulation and exocytosis. Given that CICR in beta-cells can originate at the nuclear envelope, *Epac* may play also some role in nuclear Ca^{2+} signaling.