Hyperlipidemic Modulation of Pancreatic β-cells Increases Calcium Sensitivity of Insulin Exocytotic Machinery

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Introduction

- Type II diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and progressive β-cell dysfunction, leading to hyperglycemia. It affects over 11% of Americans, around 38 million people, and is closely associated with obesity.
- Hyperlipidemia, a common comorbidity of T2DM, exacerbates the pathophysiological changes in insulin secretion and glucose metabolism. Exposure to excess nutrients in the form of glucose and fatty acids leads to glucolipotoxicity and subsequent lipid accumulation in pancreatic β-cells (INS-1).

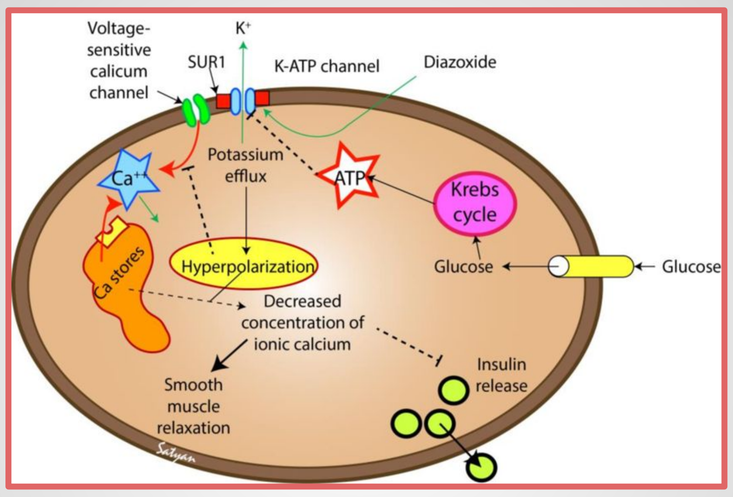

Fig. 1 Mechanism of Insulin Secretion with Diazoxide Present. Diazoxide inhibits the closing of ATP-sensitive K^+ channels, preventing K^+-induced membrane depolarization, thereby stopping normal insulin secretion. KCl becomes the driving force for triggering insulin secretion by artificially allowing Ca^{2+} into the cell through voltage-sensitive channels. [3]

- Previous studies on permeabilized β-cells have demonstrated an increase in calcium sensitivity under elevated glucose conditions. The present research investigates the effects of excess exposure to nutrients and the accumulation of lipids on the calcium sensitivity of intact INS-1 cells in glucolipotoxic conditions.
- By isolating the exocytotic mechanism to be dependent on concentrations of K^+ ions instead of normal glucose stimulated insulin secretion (GSIS), we investigate whether manipulation of lipid presence, which subsequently alters enzyme activity through certain signaling molecules, can change calcium thresholds in the signaling pathway of insulin secretion that may influence the development of T2DM.

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Methods

- INS-1 cells were grown at 37℃ in RPMI 1640 media (11 mM glucose) for 5 days and then switched to either 4 mM or 11mM glucose media 24 hours prior to the experiment.
- INS-1 cells were pre-incubated at 37℃ in 0.05% bovine serum albumin (BSA) Krebs-Henseleit (KREBS) solution buffer for a 45 minute period.
- The media was replaced with a 400 µM diazoxide (DZ) KREBS solution for another pre-incubation period of 15 minutes.
- Then, INS-1 cells were incubated with the same 400 µM DZ KREBS solution containing varying concentrations of potassium chloride (KCl) from 5 mM to 30 mM for 60 minutes.
- Additionally, half of our samples were exposed to an acute addition of fatty acid along with our test solution for the 60 minute incubation period
- Subsequently, insulin antibodies were added to a 1536 well plate along with our samples to measure insulin content using an HTRF (fluorescence-based) insulin assay kit. A TECAN M1000 Pro Plate Reader was used to measure the fluorescence-resonance energy transfer (FRET) signal insulin-binding antibodies.

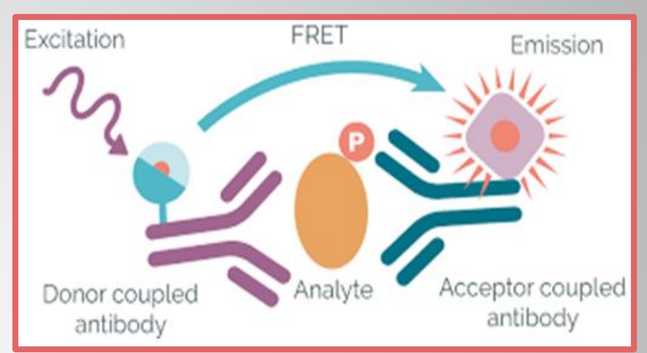


Figure 2. FRET Excitation and Emission through Insulin-Binding Antibodies. Excitation of the donor fluorophore triggers fluorescence emission, detected by the acceptor fluorophore, to reveal insulin content in HTRF assays. [4]

Results

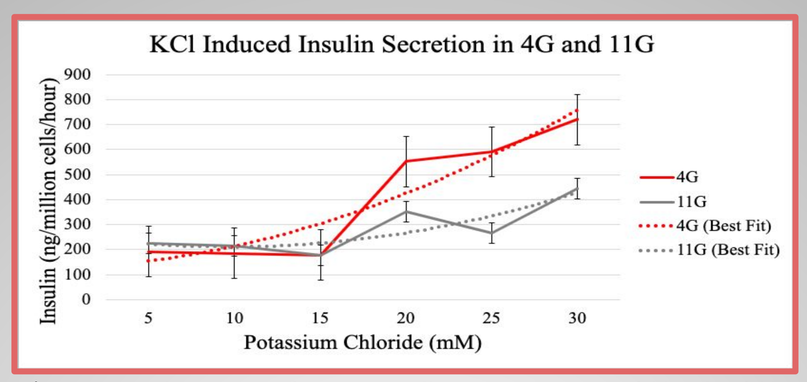
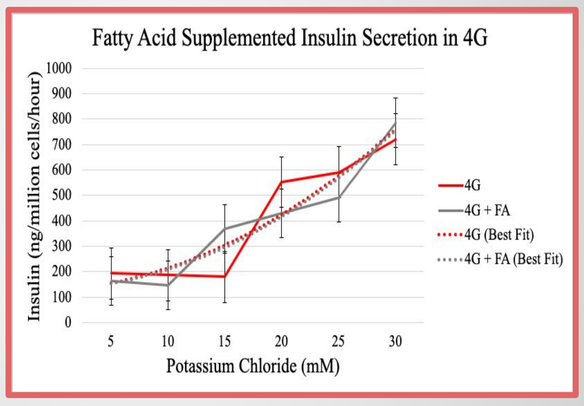


Figure 3. Insulin secretion from INS-1 cells increased over 3.8-fold and 2-fold in cells cultured in 4G and 11G respectively when KCl concentration was increased from 5 mM to 30 mM.

Figure 4. Insulin secretion from INS-1 cells was left-shifted in 4G cells supplemented with fatty acids. Basal and maximal secretion levels were similar in 4G and 4G + FA samples. Significant stimulation in secretion occurs between 10-15 mM KCl in the 4G sample and occurs between 15-20 mM KCl in the 4G +FA sample.



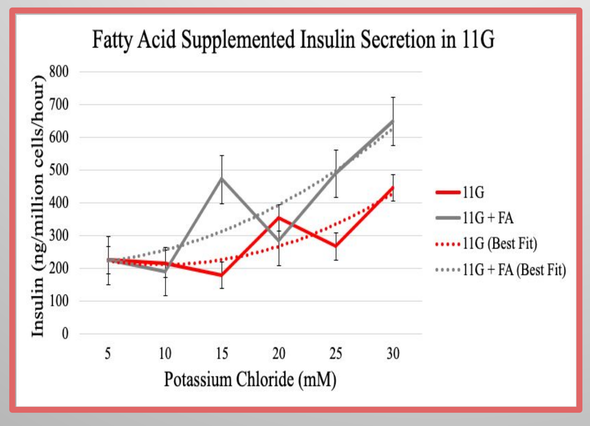


Figure 5. Insulin secretion from INS-1 cells was also left-shifted in 11G cells supplemented with fatty acids. Basal level remained similar, but maximal levels had a 2-fold and 3-fold increase in 11G and 11G + FA samples respectively. Significant stimulation in secretion occurs between 10-15 mM KCl in the 11G sample and occurs between 15-20 mM KCl in the 11G +FA sample.

Discussion

- Our results indicate a decrease in maximal KCl-induced insulin secretion of β-cells exposed to hyperlipidemic conditions. (Fig. 3)
- Fatty acid exposure left-shifted the KCl induced insulin secretion curve for 4G samples, indicating higher sensitivity for the exocytotic machinery at lower levels of KCl. (Fig. 4)
- Fatty acid exposure also left-shifted the KCl induced insulin secretion curve for 11G samples, again indicating higher sensitivity of the exocytotic machinery at lower KCl concentrations. (Fig. 5)

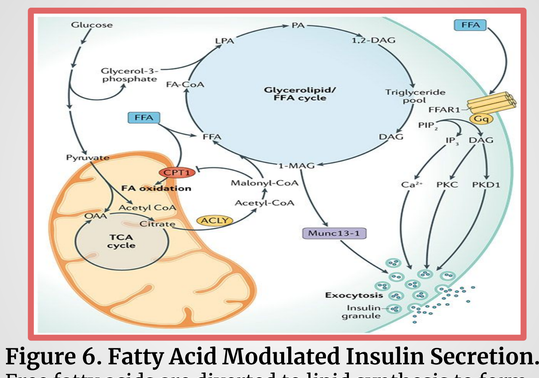


Figure 6. Fatty Acid Modulated Insulin Secretion. Free fatty acids are diverted to lipid synthesis to form DAG, facilitating interactions of the insulin granule trafficking protein Munc13-1. [6]

- The significant difference in maximal secretion levels between 11G and 11G + FA samples, in contrast to the similar maximal secretion levels between 4G and 4G + FA samples, suggests that excess nutrient exposure alters fatty acid oxidation when cells are exposed to excess nutrients.
- At 11G, the surplus of nutrients leads to reduced fatty acid oxidation, and an exacerbated accumulation of lipids.
- Future work could look to verify the consistency of calcium levels across test samples or image the content of specific lipids known to influence the insulin exocytotic machinery.

Conclusion

- Our research demonstrates that hyperlipidemic modulation of pancreatic β-cells induces a leftward shift in KCl induced insulin secretion curves.
- Acute fatty acid exposure elicits increased secretory responsiveness
- A more pronounced effect of fatty acids under conditions of lipid excess and resultant reduced fatty acid oxidation may account for differences observed in maximal secretion between normal and glucolipotoxic samples.
- Our hypothesis posits that the increase in calcium sensitivity could be mediated through a reduction in fatty acid oxidation, leading to elevation of phosphatidylinositol 4,5-bisphosphate (PIP2) and diacylglycerol (DAG), which could modify the electrostatic potential of SNARE proteins, altering their calcium-sensitive interactions with nearby proteins to lower the calcium threshold for exocytosis, thereby increasing responsiveness to lower KCl concentrations.
- Further investigation into the interplay between lipid metabolism and the calcium sensitivity of the SNARE exocytotic complex could offer new therapeutic targets for addressing glucose dysregulation in hyperlipidemic conditions and improving β-cell function in T2DM.

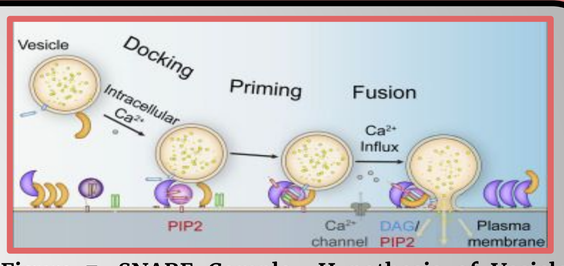


Figure 7. SNARE Complex Hypothesis of Vesicle Fusion and Release. DAG and PIP2 modulate interaction of SNARE proteins—such as syntaxin, SNAP-25, synaptobrevin, and Munc13 —thereby influencing the efficiency of membrane fusion and vesicle exocytosis. [5]