Modulating PLC-DAG-Munc13-1 Signaling in INS-1 β-cells to Mitigate Basal Insulin Hypersecretion in Glucolipotoxicity

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Introduction

- **Hyperinsulinemia**, a hallmark of insulin resistance and type 2 diabetes, arises from glucolipotoxicity (GLT) induced by chronic glucose and free fatty acid (FFA) excess.
  - GLT conditions exhibit basal insulin hypersecretion and a left-shifting of the glucose dose-response curve (Erion et al., 2015).
  - Ca^{2+} influx drives phospholipase C (PLC) activity (Sabatini et al., 2019), increasing diacylglycerol (DAG) production via $PtIns(4,5)P_2$ cleaving, which activates Munc13-1 to promote insulin exocytosis (Das et al., 2023).
- **Diazoxide** curbs insulin secretion by binding SUR1, opening K_{ATP} channels to limit Ca^{2+} influx from membrane depolarization (Raphemot et al., 2014).
- **Orlistat** acts as an inhibitor of pancreatic lipases and PLC while U73122 blocks calcium-sensitive PLC but with greater specificity (Farley et al., 2022).
- **What specifically regulates basal insulin secretion is not definitively known.**
  - Extracellular FFA can amplify K_{ATP}-independent insulin secretion by binding to GPR-40 (Fig. 1).
  - **What is the role of PLC-DAG-Munc13-1 lipid signaling and lipase activity in basal insulin hypersecretion?**
  - **What are the differential effects of inhibiting $PtdIns(4,5)P_2$ lipid hydrolysis and broadly inhibiting lipolysis?**

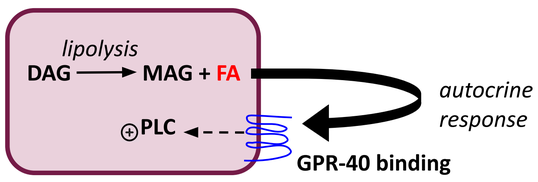


Fig. 1: FFA generated from lipolysis activates PLC via G-protein coupled receptors.

Methodology

- INS-1 rat insulinoma-derived β-cells (passage 64, RPMI 1640, BME 1:1000) were cultured in 4 mM (normal) or 11 mM (diabetic) glucose and measured glucose-stimulated insulin secretion (GSIS) at 1 mM or 12 mM glucose. Cells were treated with diazoxide, orlistat, and U73122. Insulin (ng/million cells) was quantified using **Homogeneous Time-Resolved Fluorescence (HTRF) (CisBio)**.
  - Insulin in ng/ml was obtained from the ratios plugged into the equation of the standard curve ($R^2 = 0.8954$) then converted to ng/million cells knowing the cell count for 4 mM and 11mM glucose (Glc) (88000 and 105000 respectively).

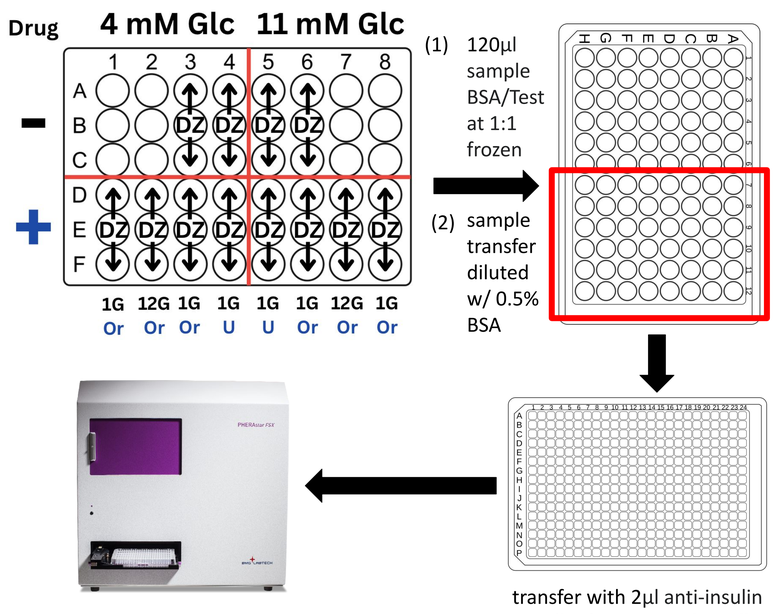


Fig. 2: GSIS Assay Protocol

- Lipid droplet accumulation in 4mM and 11mM glucose cultures was assessed using **fluorescence microscopy (LAICA)** with nile red staining.
- DAG in 3 conditions (diazoxide (DZ), DZ + orlistat, and DZ + U73122), all treated with 20mM KCl was measured by using an **expressed fluorescent DAG protein reporter (Molecular Montana)** ran through a fluorescence plate reader (NanoQuant Infinite M200 Pro, 20 scs. kinetic interval for 45 mins. at 494 nm excitation and 530 nm emission).

Discussion/Conclusions

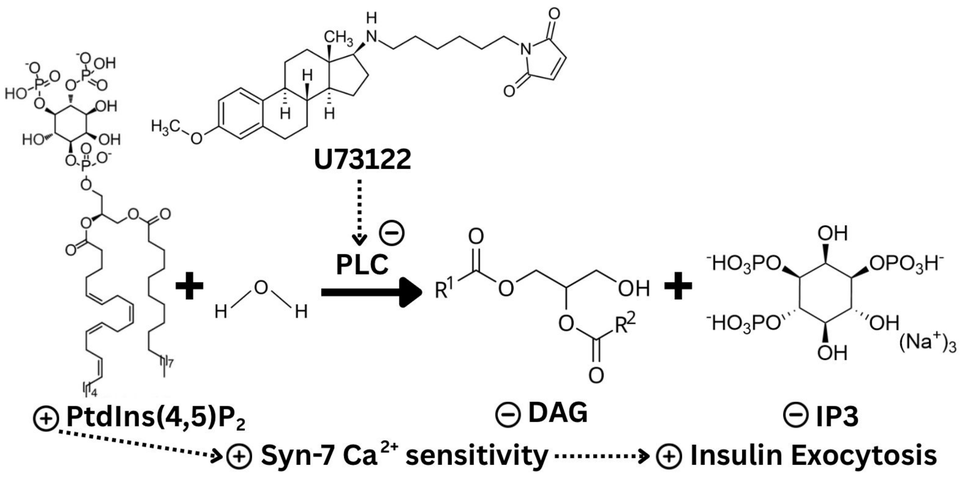
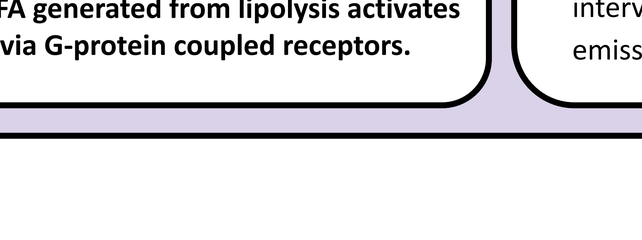
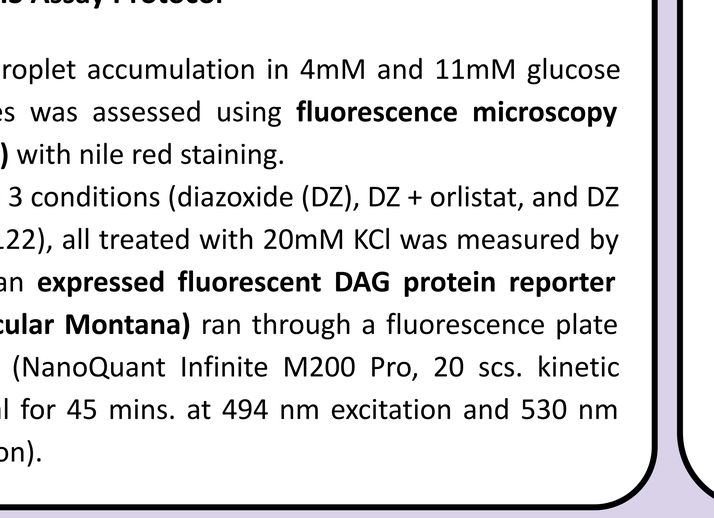


Fig. 5: Our proposed mechanism for U73122's activation of insulin secretion via $PtdIns(4,5)P_2$ buildup.

- The inhibition of PLC, an enzyme that catalyzes hydrolysis to produce DAG, using **U73122 unexpectedly resulted in the 57.16% increase in insulin secretion (Fig. 4C), possibly due to the buildup of $PtdIns(4,5)P_2$** (the substrate of the reaction).
  - Downstream effects of minimizing DAG production were expected to result in a decrease in insulin secretion because DAG enhances Munc13-1's activity in priming insulin-containing large dense core vesicles (Kang et al., 2006).
  - Surplus of $PtdIns(4,5)P_2$ would lower the $Ca2^+$ threshold required for synaptotagmin to initiate SNARE-complex-mediated exocytotic fusion (Dolai et al., 2016).
  - Diazoxide drops insulin secretion to basal levels because the open K_{ATP} channels would prevent the depolarization of the cell.
  - While modulating PLC activity might offer short-term benefits (e.g., in acute glucose dysregulation), it would need careful control to avoid adverse long-term effects.
- GLT's effect on insulin confirmed with the decrease in secreted insulin because of the β-cells' dependence for glucose instead of CPT-1-dependent lipid oxidation for ATP; CPT-1 regulates the rate limiting step for lipid transport to the mitochondria (Choi et al., 2024).
- DAG fluorescence under orlistat treatment being only slightly lower than the U73122 inhibitor (Fig. 4A) **could suggest the persistence of pre-existing DAG pools stored in lipid droplets** (Fig. 4D).
  - Lipid droplet dynamics may complicate efforts to regulate DAG levels, necessitating a deeper understanding of lipid storage and mobilization in glucotoxic conditions.

Results





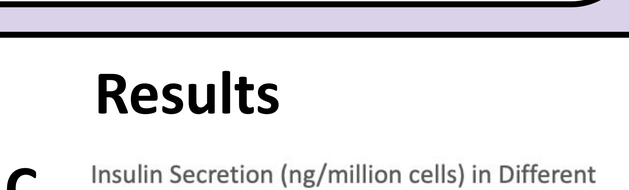


Fig. 4A: DAG is lowest with Orlistat and slightly higher with U73122 in β-cells cultured n 4 mM glucose treated with 12 mM glucose. DAG expression without the inhibitors (control) is the highest at around 100 AFU. **Fig. 4B:** DAG fluorescent expression inside the β-cells-cells used for Fig. 4A is confirmed.

Fig. 4C: There is a 57.16% **increase** in insulin secretion in β-cells (in both glucose cultures) treated with 1 mM glucose, diazoxide, and U73122 compared to those treated with 1 mM glucose, diazoxide, and Orlistat. Cells treated with 12 mM glucose only in 11 mM glucose culture conditions saw a decrease (-25.30%). Orlistat addition decreases insulin secretion for all experimented cases when compared to controls.

Fig. 4D: Lipid droplets show heavier accumulation in 11 mM glucose-cultured cells (diabetic) than in 4 mM glucose-cultured cells.

References

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