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BIOCHEM 551 SEMINAR
OCTOBER 23, 2023

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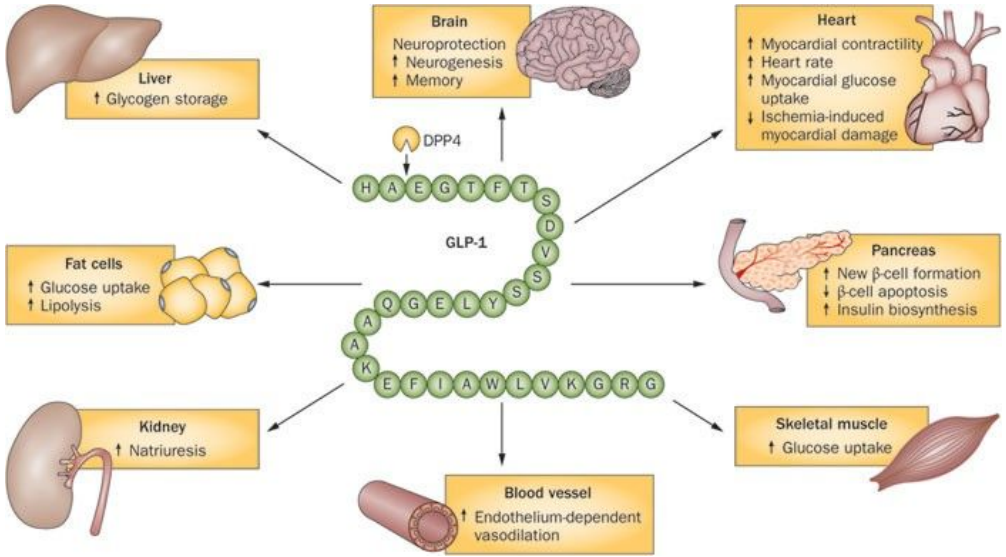
cAMP-dependent Mobilization of Intracellular Ca2+ Stores by Activation of Ryanodine Receptors in Pancreatic β-Cells

Background: Role of GLP-1

- GLP-1 is a peptide hormone synthesized from proglucagon in the intestine [7]
  - Released upon oral glucose load
- Broad variety of physiological effects
  - Includes “Incretin effect”
- Recently used as treatment for DMII



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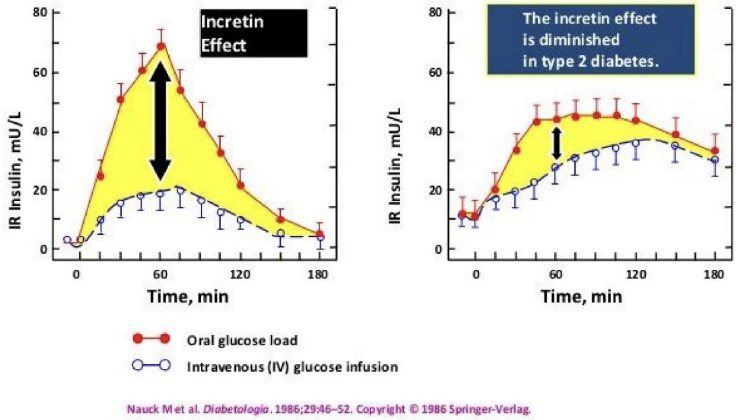
Relevance

- Increase understanding of pancreatic beta cell physiology
- DMII has been associated with diminished ‘incretin effect’ [3]
- The further understanding of glucose metabolism will increase the ability for researchers to treat related pathologies



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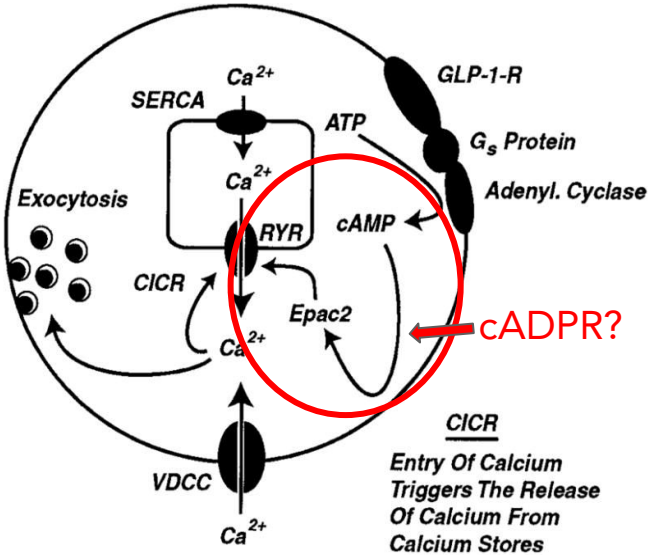
Background

- GLP-1 receptors are G-coupled receptors on beta cell membrane [5]
- Uses cAMP 2nd messenger pathway to activate Protein Kinase A [6]
- Recent contradictions on GLP-1s influence on specific mobilization of RyR Ca2+ stores [8,9]
  - Okamoto et al. hypothesized that cADPR influence mobilization of RyR Ca2+ stores [8]
  - Webb et al. found that mobilization is independent of cADPR [15]



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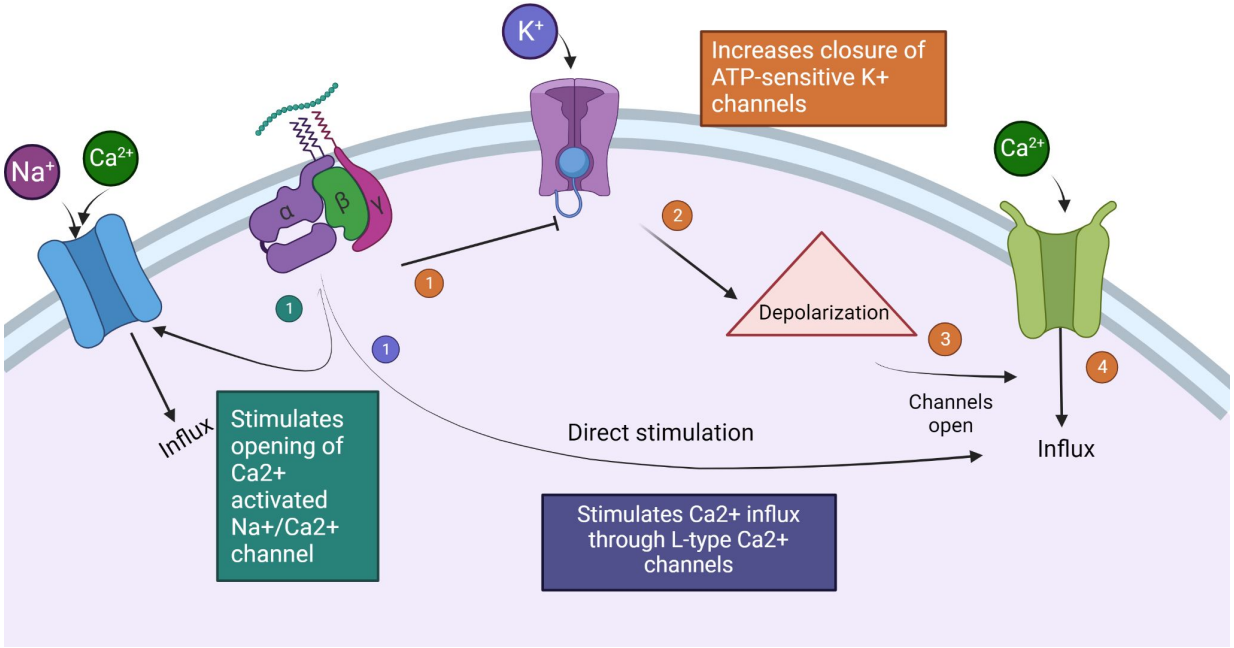
RELATED PREVIOUS STUDIES

- GLP-1 mediates **sustained** Ca2+ influx for CICR through three mechanisms
  - Increases closure of ATP-sensitive K+ channels [10]
    - This causes a depolarization that reaches the activation threshold of voltage-dependent Ca2+ channels
  - Stimulates Ca2+ influx through L-type Ca2+ channels [11]
  - Stimulates opening of Ca2+ activated Na+/Ca2+ channel [12]
- The mechanism of **transient** Ca2+ influx was poorly characterized prior to this paper
- Clarify the localization of RYR and Insulin granules

GLP-1-Induced Sustained Increase In Calcium



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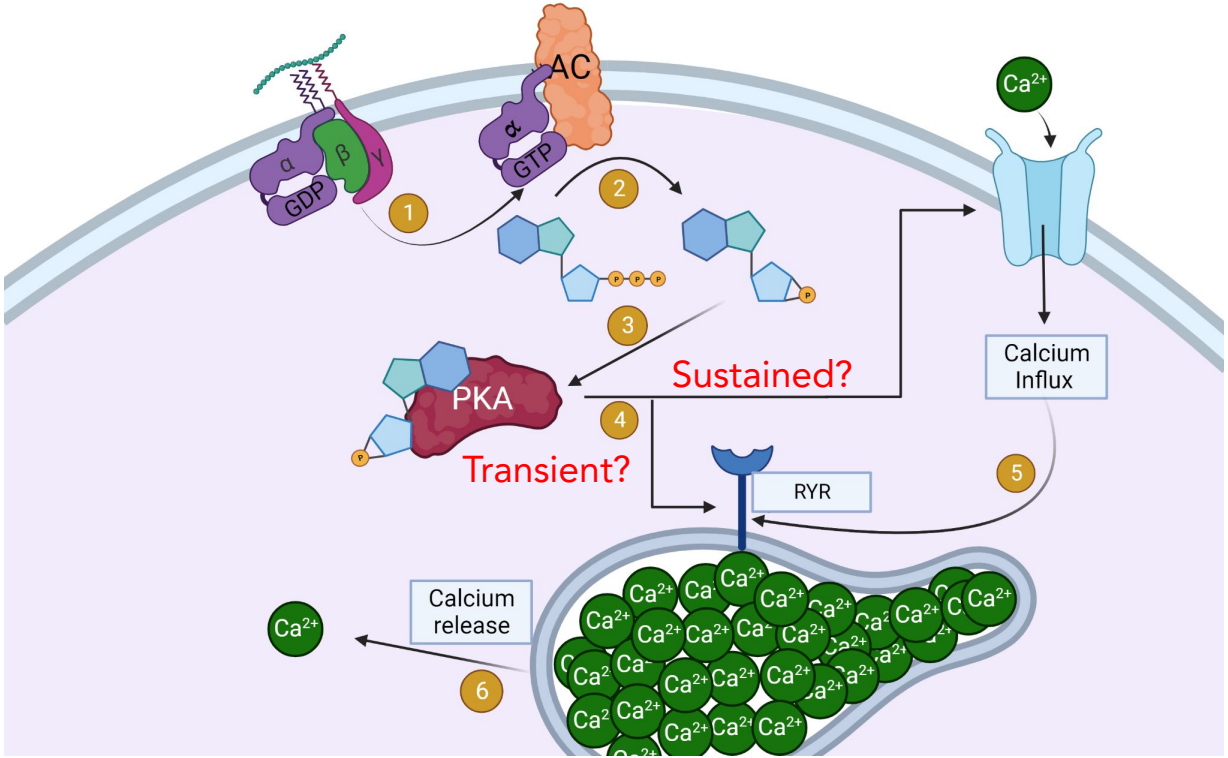
Rationale



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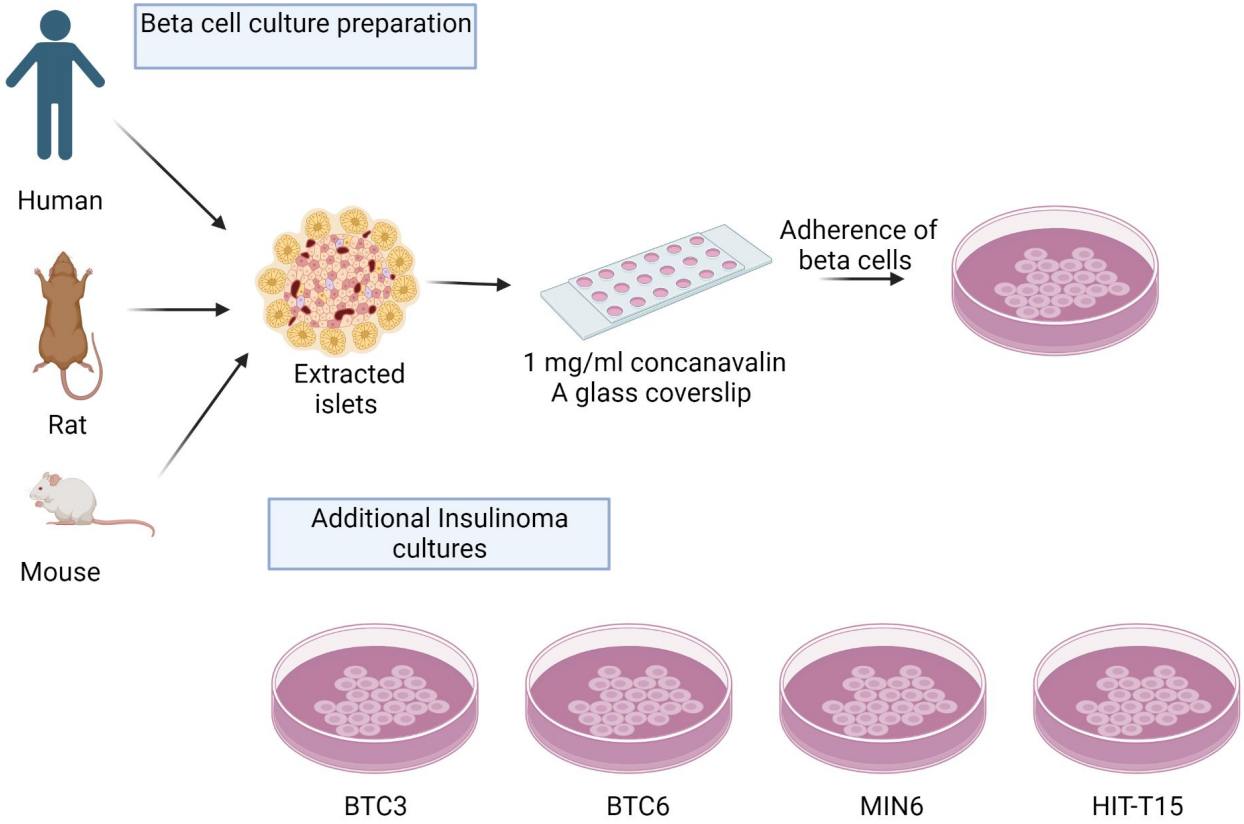
Hypothesis

The authors hypothesize that GLP-1 signaling relies on a transient increase in intracellular calcium concentration mediated by cAMP signaling and **activation of a ryanodine receptor** to allow efflux from intracellular calcium stores.

Methods: Preparation of Cell Cultures



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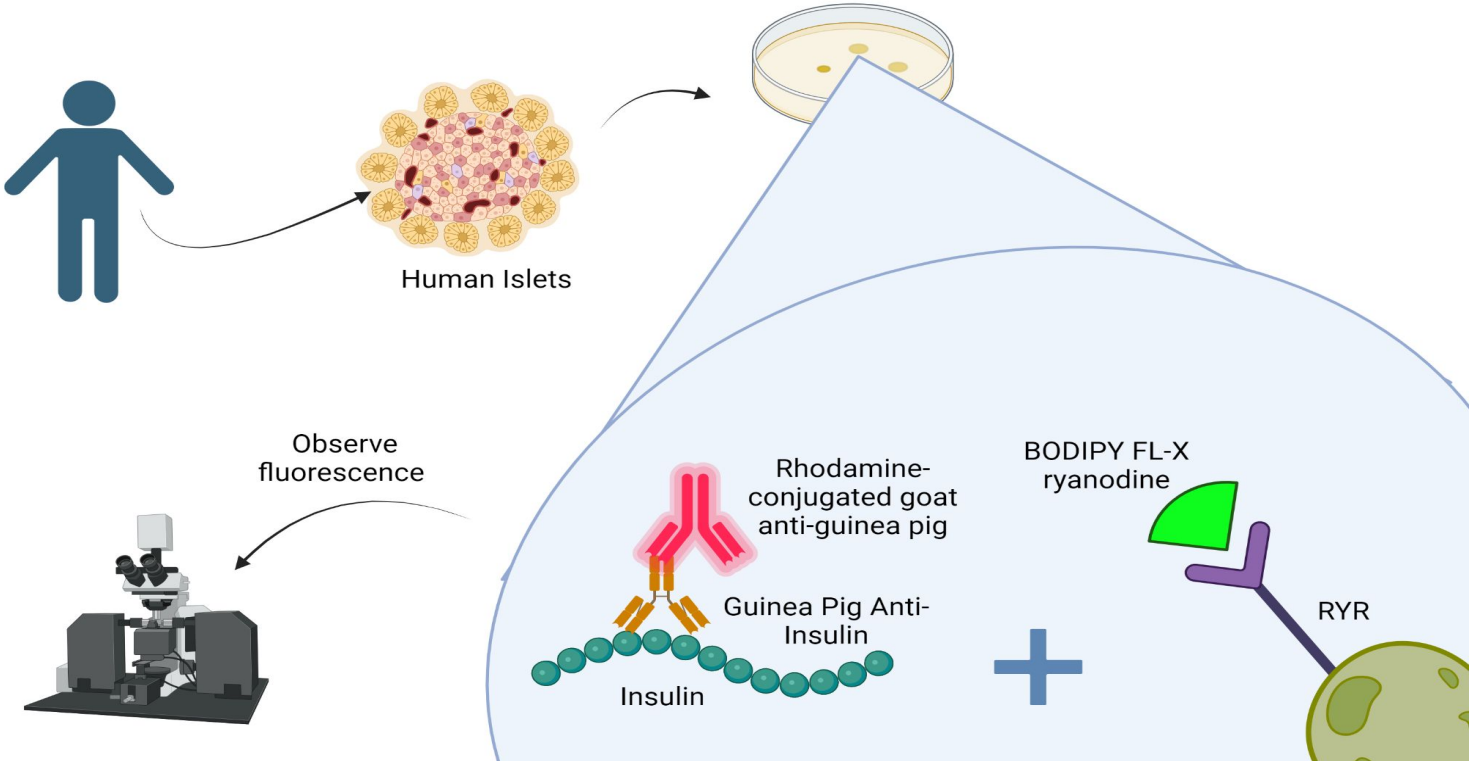
Methods: Fluorescent Detection of RYR

Goal: Probe ryanodine receptors in islet cells and visualize colocalization with insulin activity



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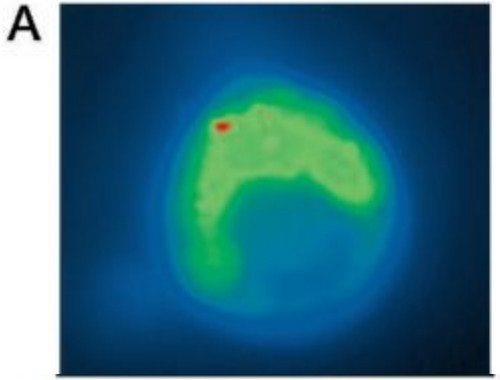
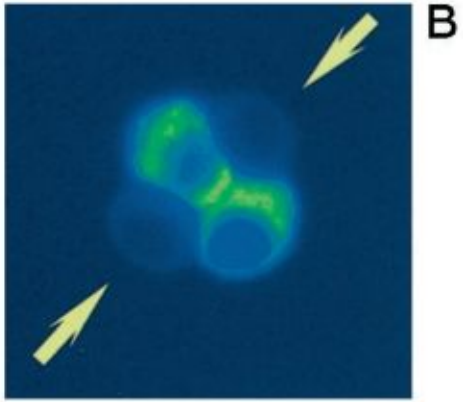
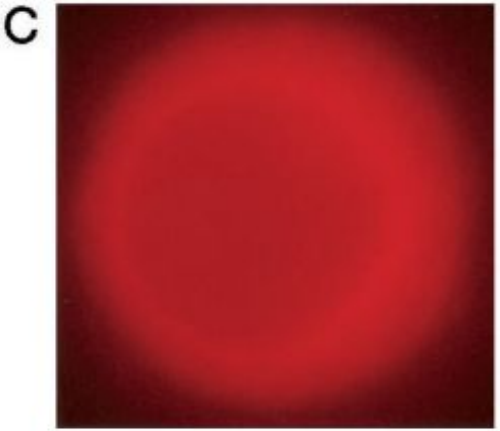
Results: Fluorescent Detection of RYR



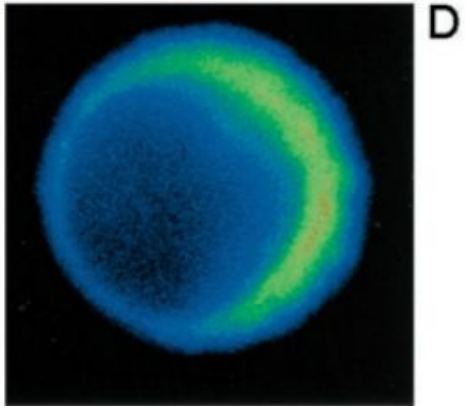
Localization of RYR in a single islet. Perinuclear fluorescence. Likely on the ER



Cluster of islet cells. Not all demonstrate fluorescence!



Fluorescent labelling of insulin activity in a single islet cell



Colocalization of RYR and insulin reactivity in a single beta cell

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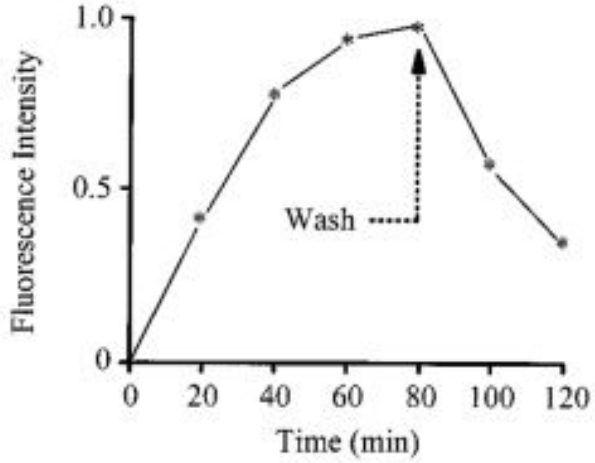
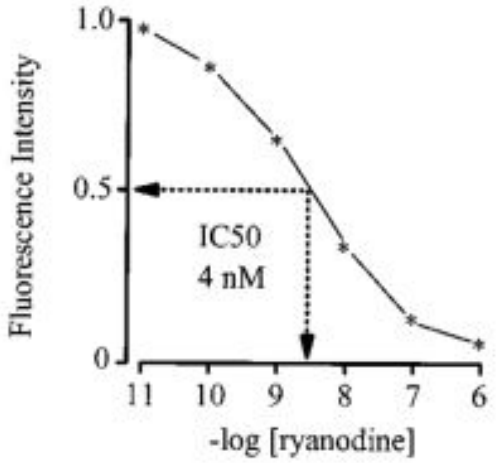
Results: Fluorescent Detection of RYR

A



Quantification of fluorescence following incubation with fluorescently-labelled Ryanodine

B



Prior treatment of cells with non-fluorescent Ryanodine decreases fluorescent intensity

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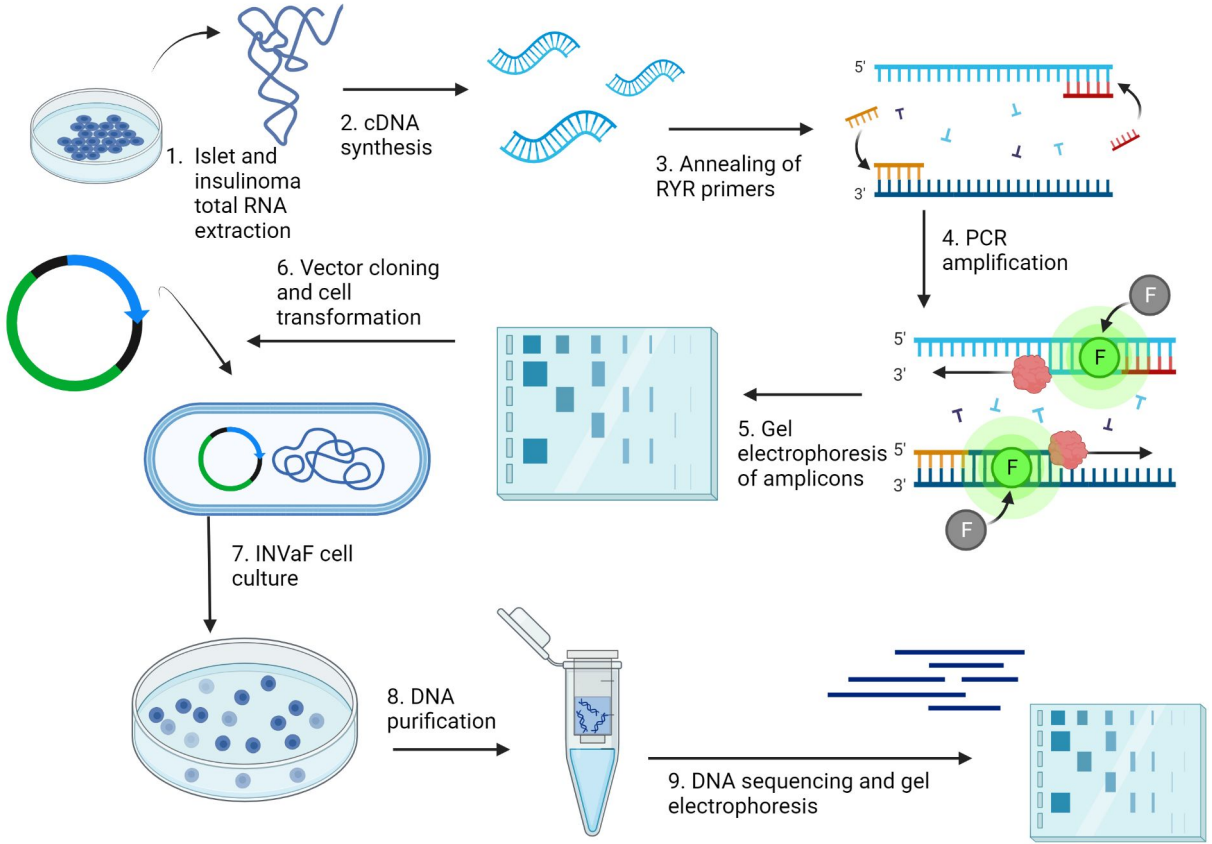
Methods: RT-PCR Analysis of Ryanodine Isoforms

Goal: Determine which RYR isoforms are present in rat islet cells and Beta TC3 cells



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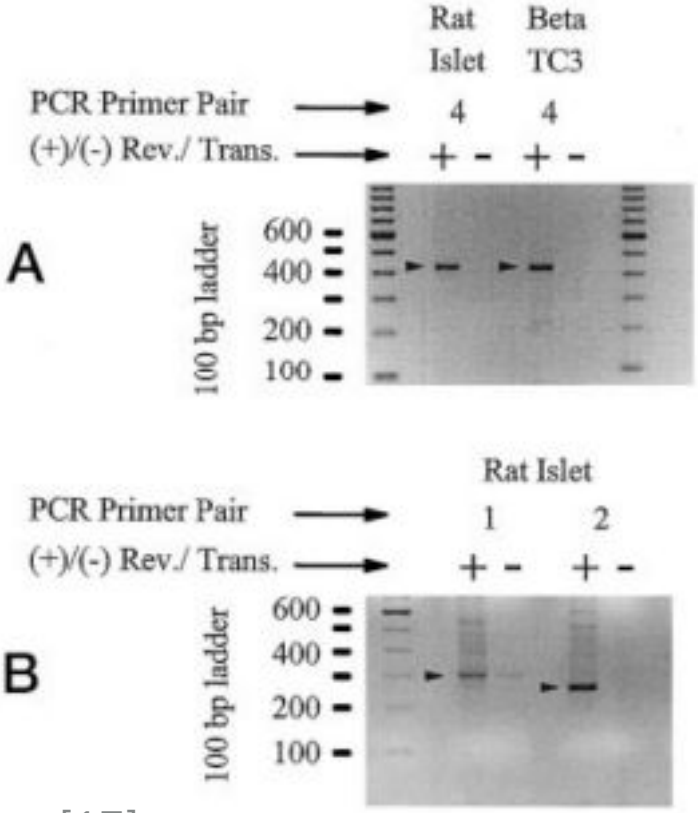
Results: RT-PCR Analysis of Ryanodine Isoforms



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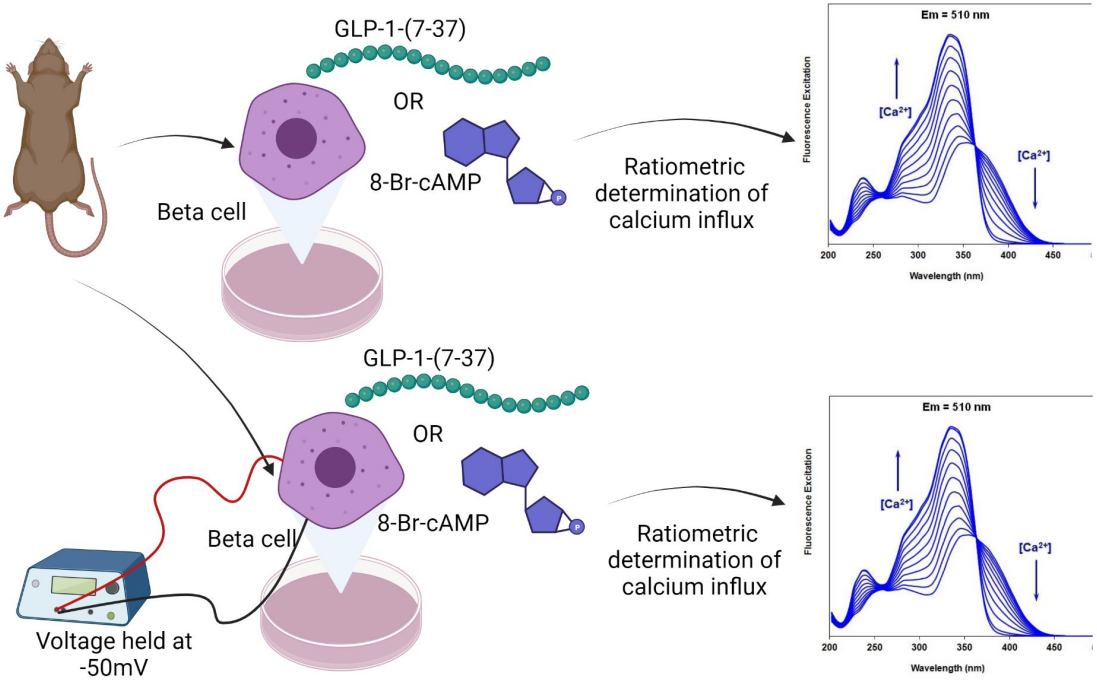
(-) Reverse Transcriptase acts as a negative control

Black triangles point to the RYR isoform(s) present in each of the cell types

Methods: GLP-1 Induced Calcium Influx

Goal: Characterize the influx of calcium following incubation with GLP-1 and its dependence on cell depolarization



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* 8-Br-cAMP: known activator cAMP-dependent protein kinase. Acts as a positive control

Results: GLP-1 Induced Ca influx

Response type	Fraction of cells responding	Increase of $[Ca^{2+}]_i$ (mean ± S.D.)
		nM
Transient	12/50	490 ± 65
Sustained	14/50	621 ± 82
Biphasic	8/50	443 ± 30 (transient)
No effect	16/50	587 ± 78 (sustained)

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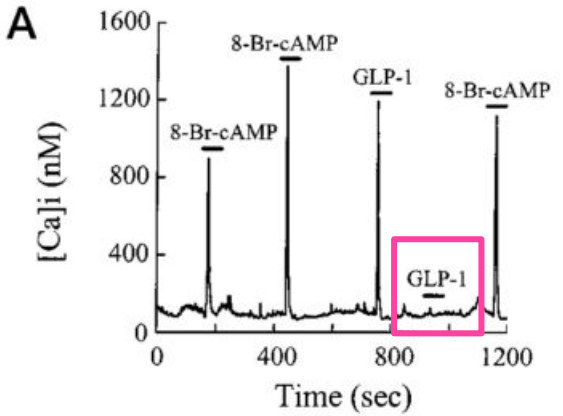
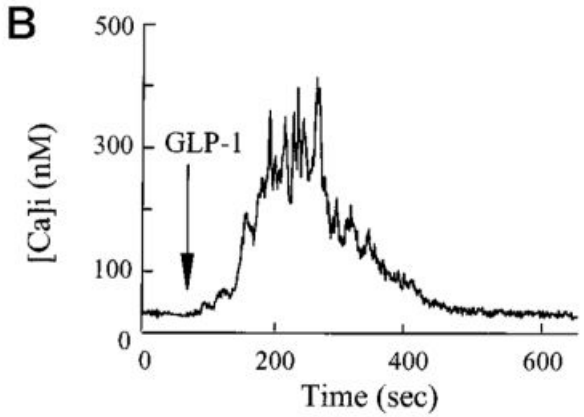
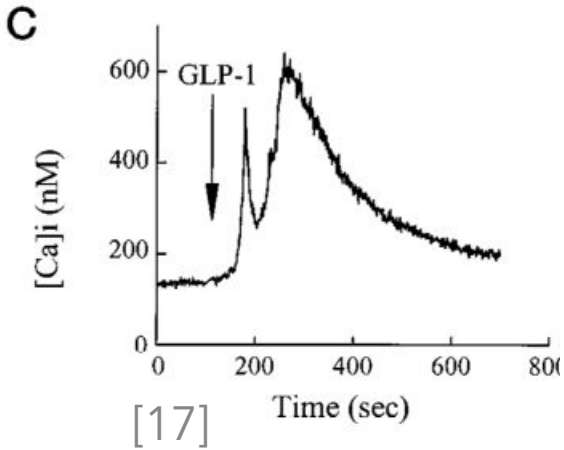
Results: GLP-1 Induced Calcium Influx



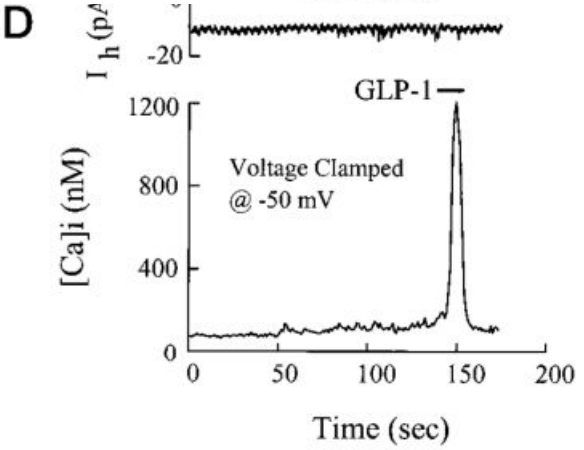
Fast influx of calcium following 30sec stimulation with GLP-1. Can be exhausted 8-Br-cAMP acts as a positive control



Sustained rise in calcium following 30sec stimulation with GLP-1



Biphasic influx of calcium following 30sec stimulation with GLP-1.



Transient influx of calcium when membrane potential is held at -50mV. Transient increase does not rely on VGCCS!

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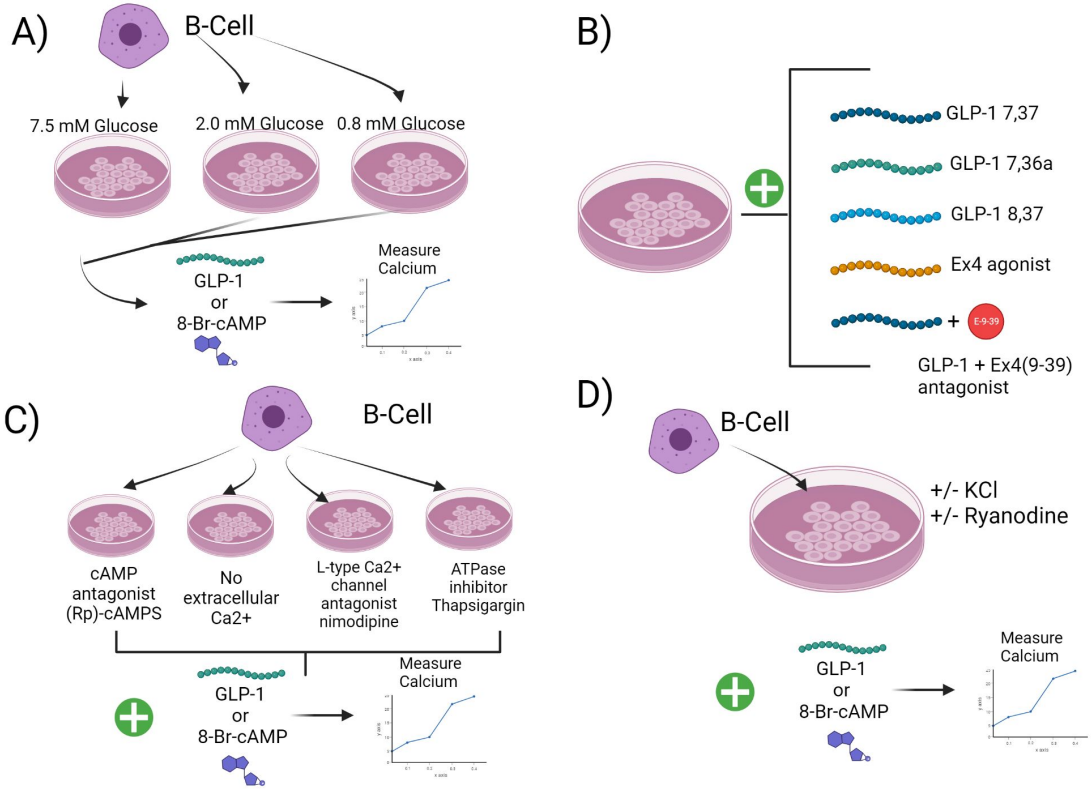
Methods: Pharmacology of GLP-1 Signal Transduction

Goal: Identify necessary conditions for the influx of calcium following incubation with GLP-1



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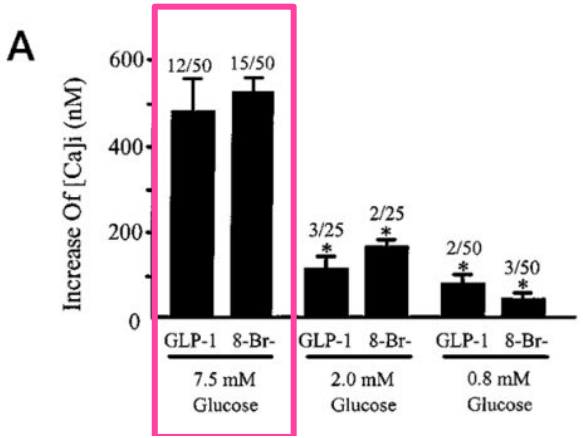
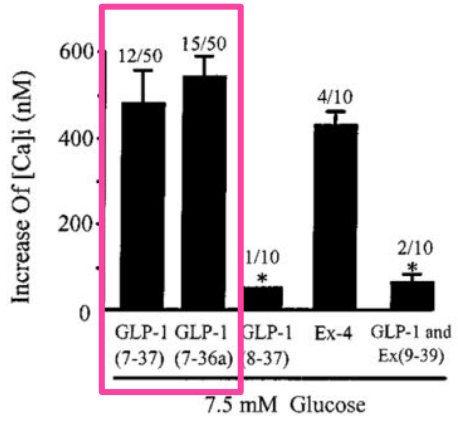
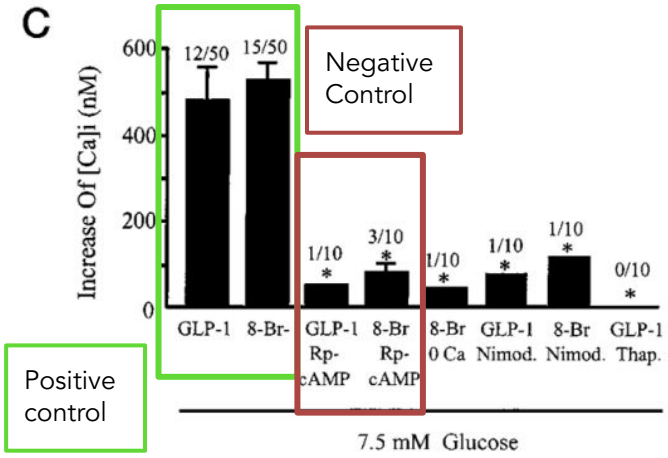
Results: Pharmacology of GLP-1 Signal Transduction



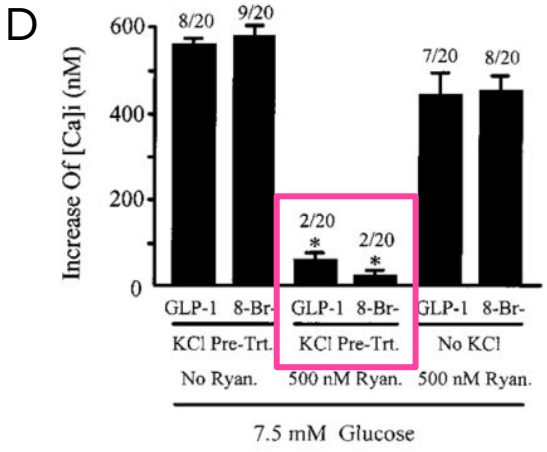
7.5mM extracellular glucose generates highest calcium influx
8-Br is a positive control



GLP-1(7-37) and GLP-1(7-36a) generate highest calcium influx
Ex-4 is a positive control
Ex-9-39 is a negative control



Calcium influx relies on extracellular calcium, L-type calcium channels, and ATPase activity



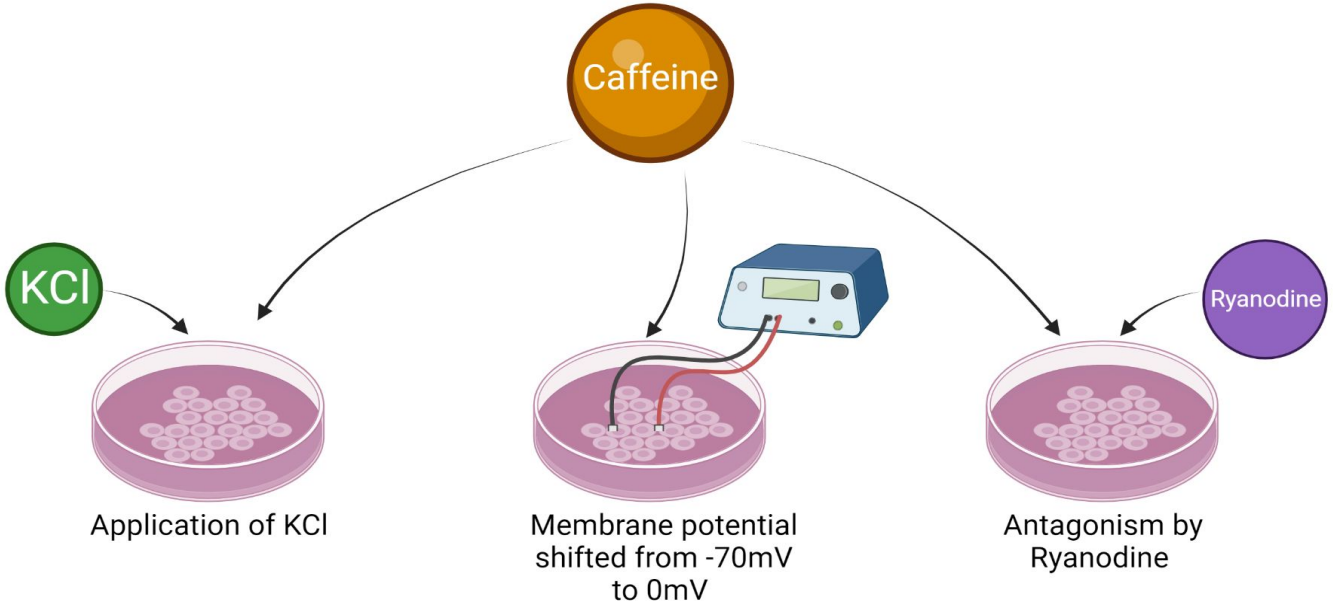
Calcium influx relies on cellular depolarization and lack of RYR saturation

[17]

Methods: Sensitization of Ca2+-induced Ca2+ release by caffeine

Goal: Determine if caffeine has an agonist effect on RYRs in HIT-T15 insulinoma cells

- Caffeine is a known agonist of calcium-induced calcium release (CICR) that acts on RYRs in cardiomyocytes



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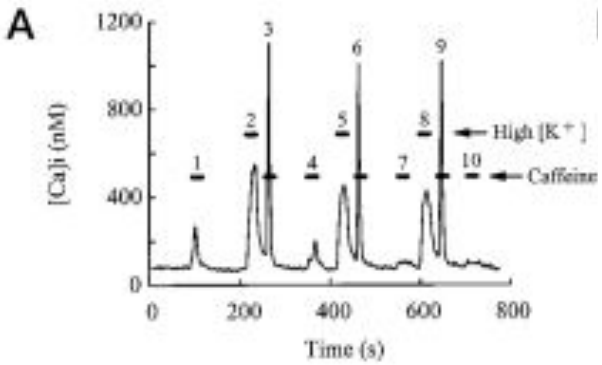
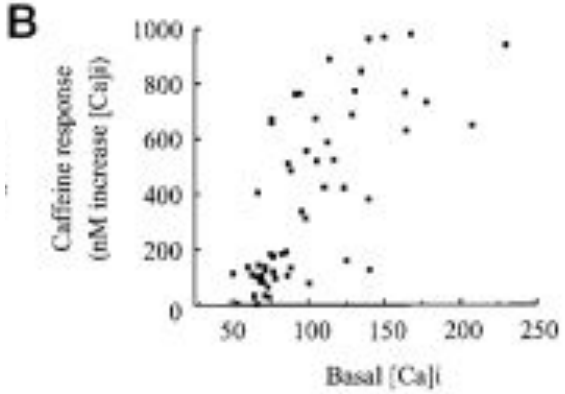
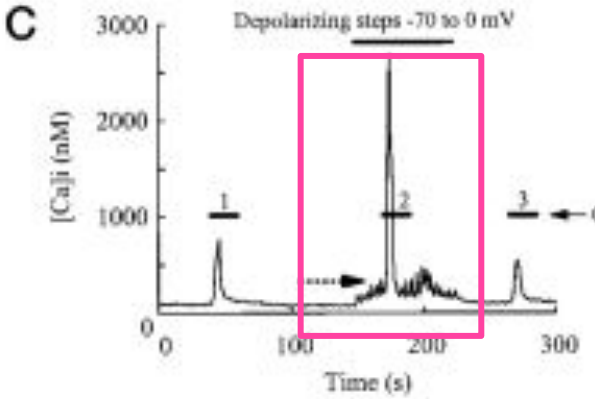
Results: Sensitization of Ca2+-induced Ca2+ release by caffeine



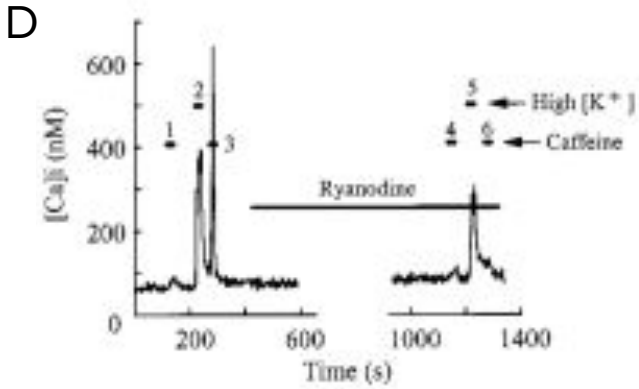
Increase in calcium can be exhausted with repeated caffeine exposure. Prior depolarization negates this



CICR demonstrated by the effects of caffeine. Higher initial calcium allows for a greater increase with caffeine



Depolarization allows for better mobilization of caffeine-sensitive calcium stores

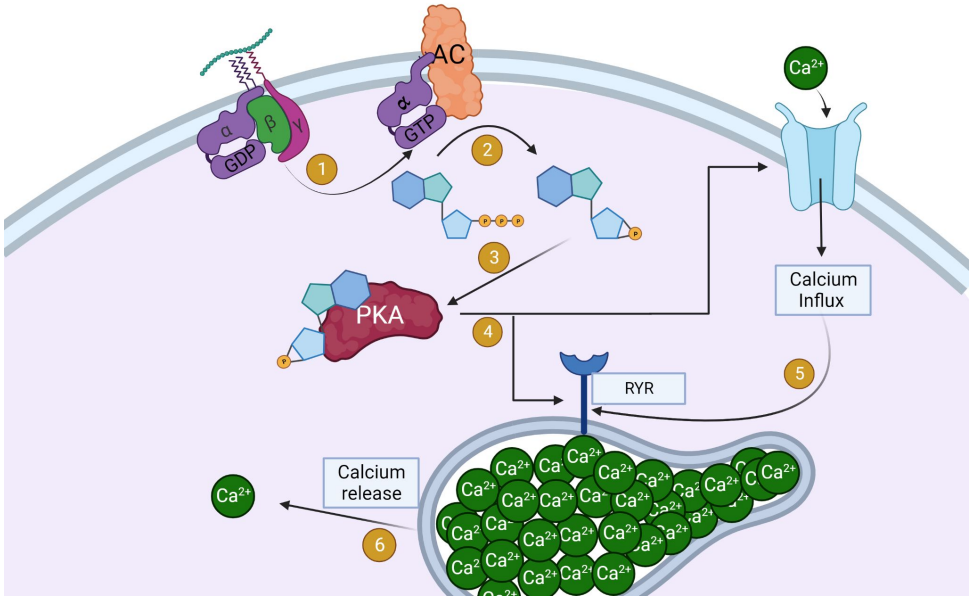


Treatment with Ryanodine blocks mobilization of caffeine-sensitive calcium stores

[17]

Discussion: Readdressing the hypothesis

- Authors sought to characterize the mechanism of transient calcium influx in GLP-1 signalling
  - Hypothesized that this was mediated by cAMP signaling and activation of RYR to induce calcium-induced calcium release
  - L-type channel is required for sustained increase only



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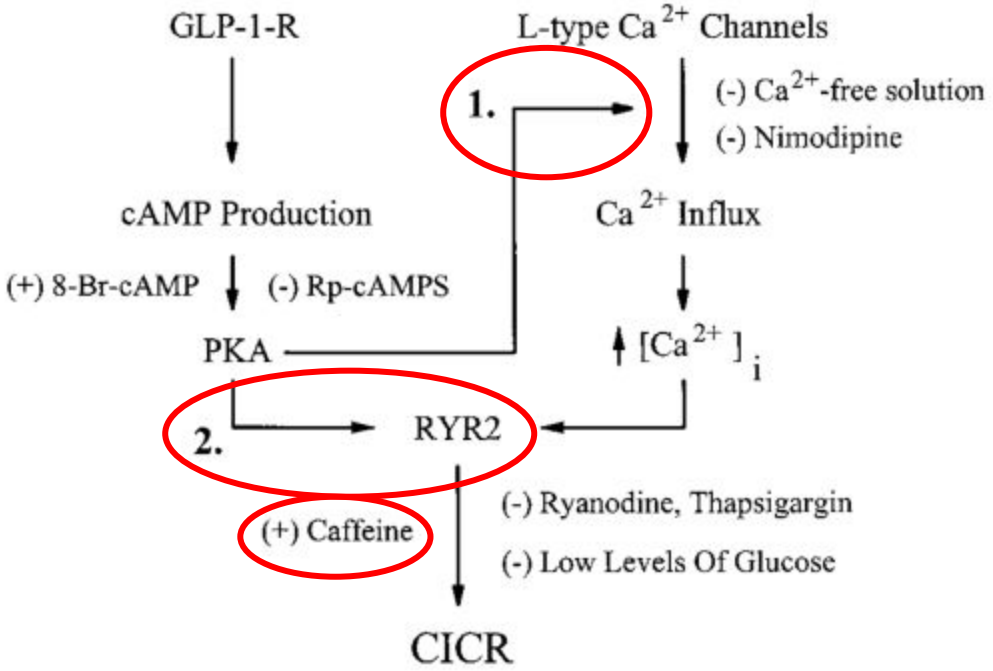
Discussion: Readdressing the hypothesis



[17]

Figure 7

Summary

- RYR colocalizes with insulin
  - Some but not all islet cells
- Ryanodine and RYR have high and reversible affinity
- RYR isoforms expressed in a cell-specific manner
- GLP-1 causes a transient Ca2+ influx independent of voltage-gated channel
  - Acts on RYR
  - This can be desensitized

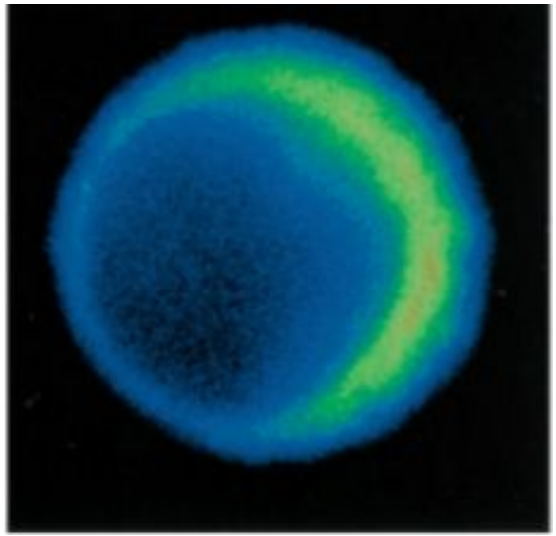


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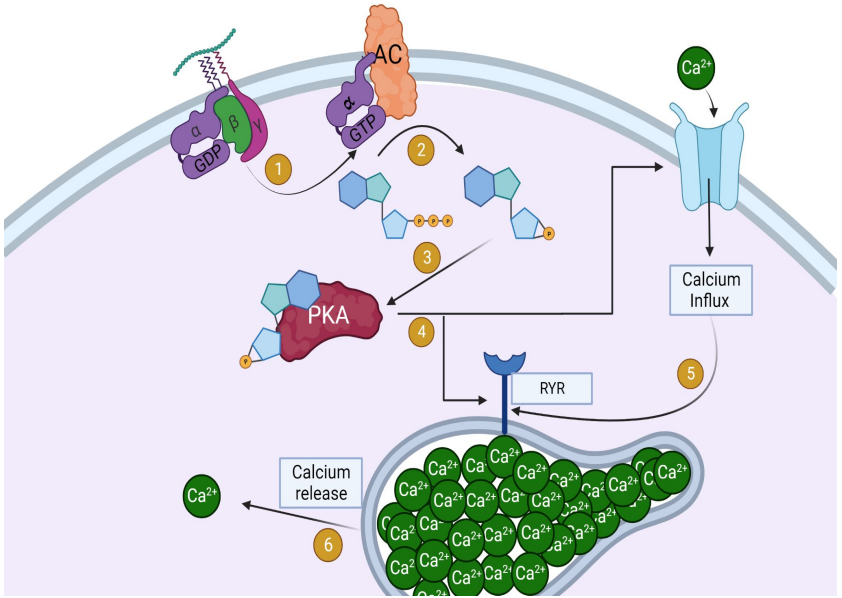
Fig. 1D

Summary

- Clarified parameters required for GLP-1 to induce Ca2+ influx
- Distinguished the potency of several GLP-1 Isoforms
- Caffeine had a synergistic effect with depolarization of the membrane to augment the Ca2+ release from intracellular stores
- GLP-1 found to be involved in phosphorylation of RYR to induce Ca2+ release



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Contribution

- Clarifies GLP-1 involvement in intracellular pathways involved in Ca2+ influx
- Compares GLP-1 to other agonists of cAMP pathway
- Provides an understanding of the effect of common pharmaceuticals such as caffeine and associated effects on Ca2+ influx
- Characterizes the signalling pathway of GLP-1 that can be exploited by GLP-1 pharmaceutical agonists

Relevance

- Understanding the influence of GI hormones on insulin secretion will increase knowledge of glucose metabolism
- DMII affects nearly 7% of the global population and 10% of Americans [13]
- Increased knowledge of insulin secretion would provide potential treatment options for insulin related pathologies
  - Metabolic syndrome
  - Polycystic ovary syndrome

Future Studies

- Look at the potential influence of GLP-1 on pancreatic alpha cells and glucagon release
- Research the interaction of GLP-1 on the orexigenic/anorexigenic axis (satiety)
- Look at possible synergistic effects between GLP-1 and other GI hormones

Questions We Have

- Can caffeine mimic the effects of modern-day GLP-1 agonist drugs and beta cell insulin release?
- What advantage do insulinoma cell cultures provide for this experiment?
- Why do certain islet cells not express RYR?

Questions?

Works Cited

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