
# The control of insulin secretion (exocytosis) in pancreatic β cells.

Peter Thorn

## Lecture 1 Outline

- Regulation of insulin secretion in healthy and diseased β cells.

## Types of diabetes

Type 1 – No Insulin

Type 2- Less insulin+ Insulin resistance



Gestational Diabetes

SANOFI DIABETES

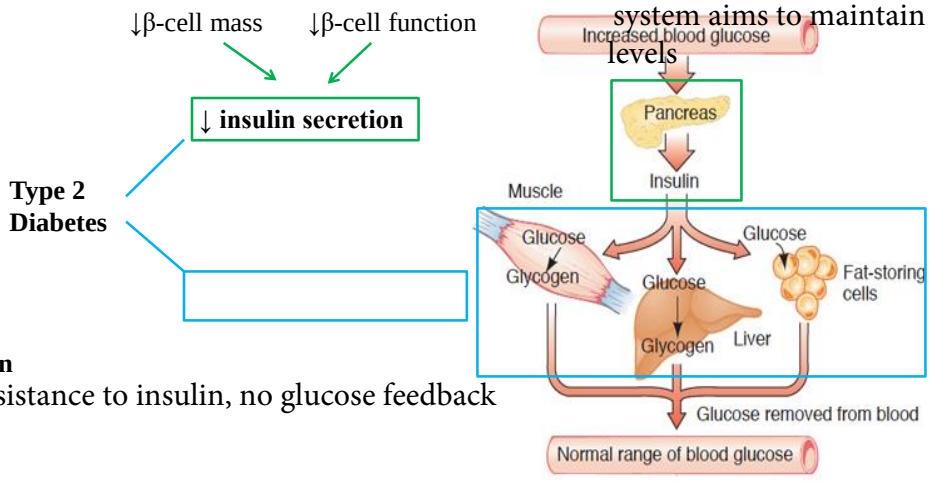
associated with old age, obesity, made from loss of insulin sensitivity in peripheral tissue and loss of insulin secretion from beta cells

- pancreas responds to increased glucose, releases insulin (circulating hormone) - which promotes glucose uptake and glucose storage, which brings glucose back down.

# T2D and beta-cell dysfunction

both loss of beta cell mass and function results in reduced insulin secretion

critically important in development of diabetes in mouse




system aims to maintain blood glucose levels

**Defect in insulin action**

type 2 diabetes - resistance to insulin, no glucose feedback

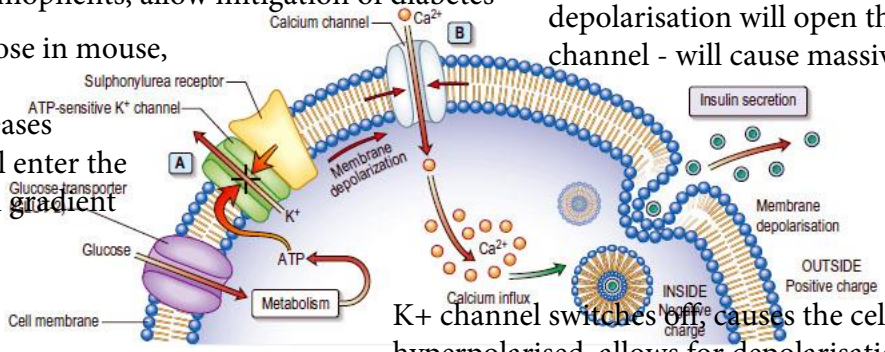
# Stimulus-secretion coupling in the β cell

specific for the beta cell - allow beta cell to 1. sense glucose and 2. transduce glucose sensation into a response (The secretion of insulin)

drugs attack these comopnents, allow mitigation of diabetes

GLUT2 detects glucose in mouse, GLUT1 in humans

- when glucose increases extracellularly, it will enter the beta cell by chemical gradient




depolarisation will open the voltage gated Ca2+ channel - will cause massive depolarisation

K+ channel switches off, causes the cell to no longer remain hyperpolarised, allows for depolarisation

metabolism of glucose will generate ATP to fuse wit

beta cell - ATP then activates the ATP-sen

channel (ATP levels go up in beta cell, it s

on the channel)

http://www.youtube.com/watch?v=yavhcGGxB5E

calcium response will cause

insulin containing vesicles

within the

h the membrane

sitive K+

then secretion of insulin

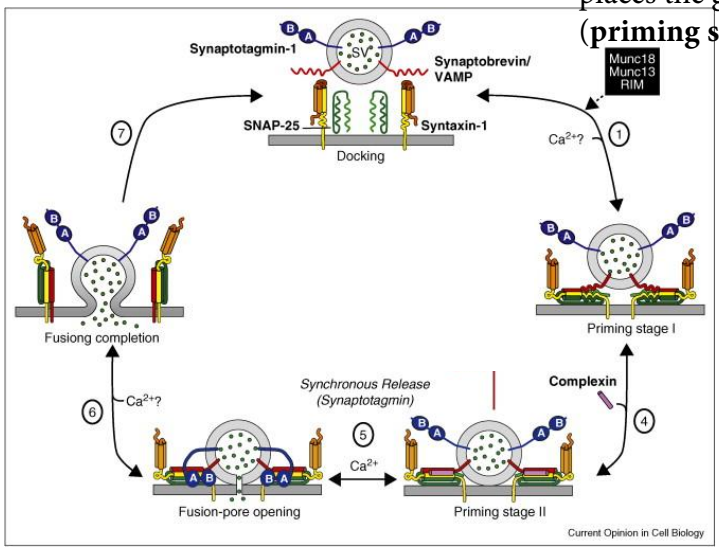
witches

# Fusion: docking and priming

SNARE hypothesis - fusion of membrane compartments require proteins on both membrane compartments - in beta cell, insulin containing granules fuse with the cell membrane
common proteins on target membrane and membrane of granule (VAMP = Vesicle associated membrane protein), SNAP25 on the membrane

fusion requires SNAP25 and VAMP

places the granule in right place (**priming stage)**




Adapted from

granule is brought close to cell membrane - again called priming

conformational change of synaptotagmin-1

## Two key unknowns:

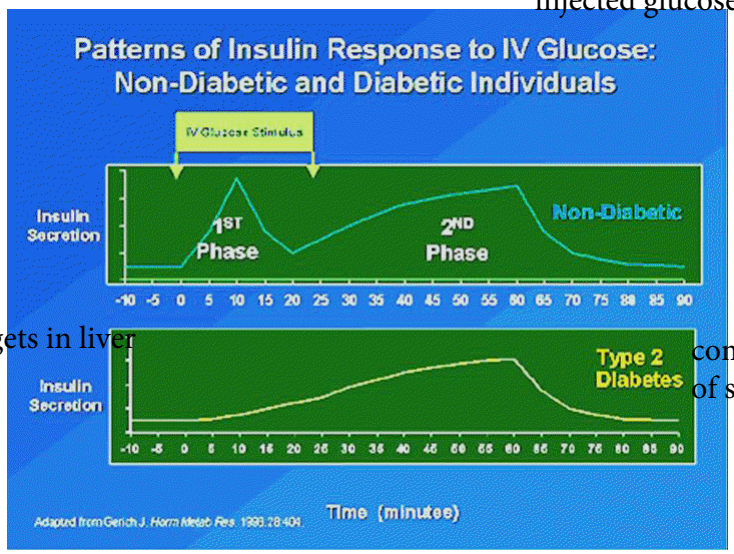
timing of everything

- Temporal complexity
- Spatial complexity

everything in their correct place

## Temporal: two phases in the glucose-induced response

injected glucose stimulant




1st diagram doesnt indicate why this happens

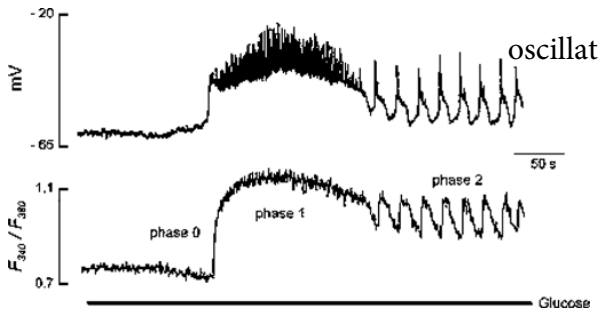
1st phase from glucose trgets in liver

complete loss of 1st phase of secretion

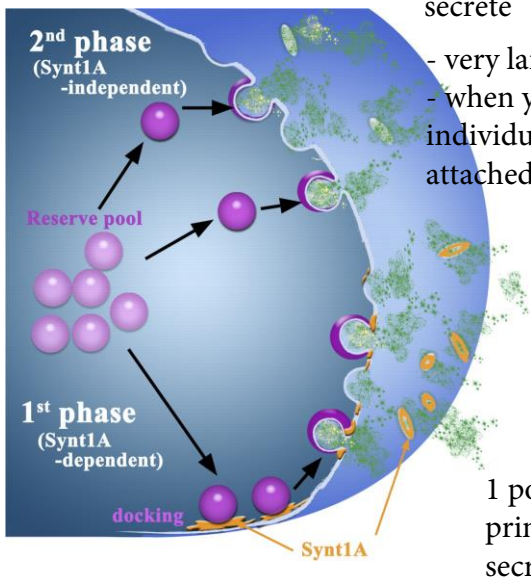
## Calcium signalling: relationship between the phases of glucose-induced secretion and the calcium signal

1st diagram doesnt explain the oscillation of Ca2+

oscillatory phase



## One model for the two phases in the glucose-induced response

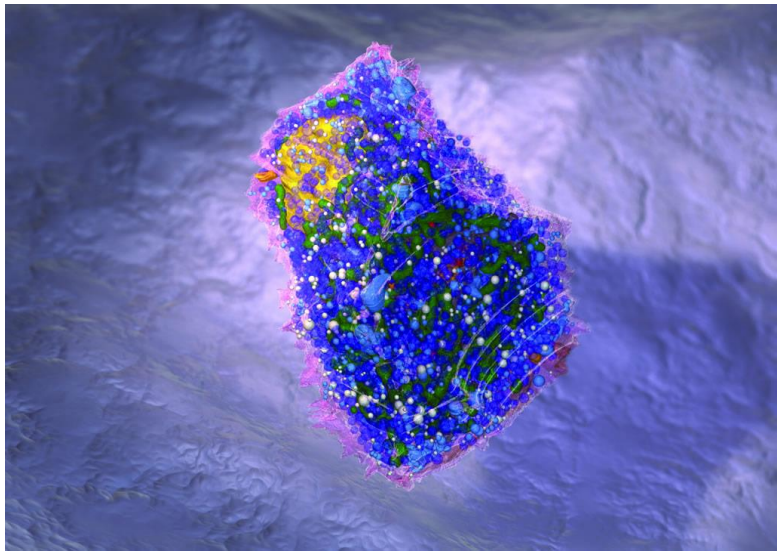



2nd phase - need to dock first before can secrete

- very large reserve pool
- when you stimulate the cells, you can see individual granules, the ones already attached/primed don't actually fuse...

1 population of insulin whch is primed - allows for 1st phase of secretion

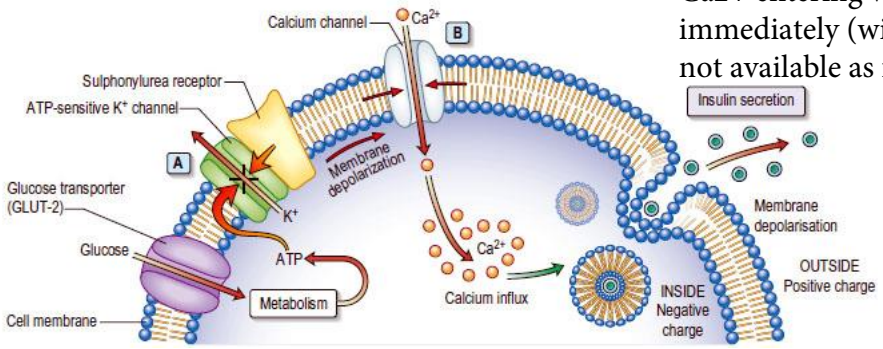
## Spatial:



Brad Marsh/Andrew Noske

## What do we know about association between the calcium signals and exocytosis?

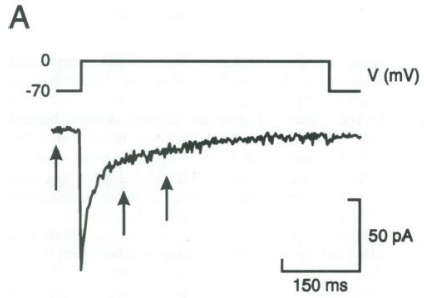
when Ca2+ entry occurs in neuron or beta cell, 99% of the Ca2+ entering will buffer almost immediately (within the cytosol - not available as free Ca2+ ions)

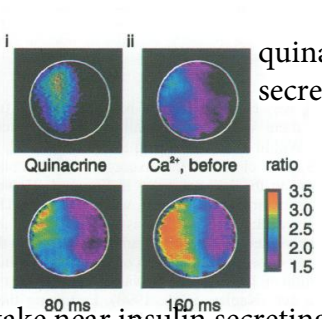



….almost nothing

## Evidence for colocalisation of caclium and exocytosis

possible association of ca2+ channel with exocytosis




quinacrine - insulin secreting granules

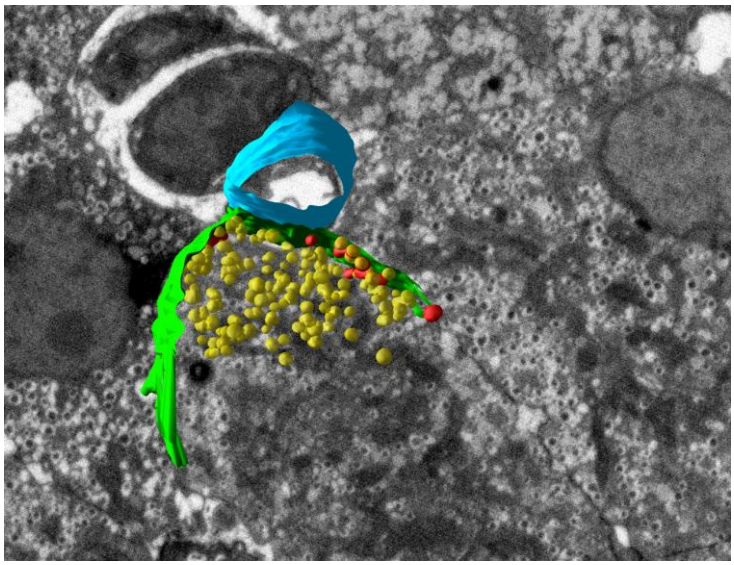
Ca2+ intake near insulin secreting granules -done with in vivo beta cell - not correctly positioned + incorrect vasculature

**Co-localization of L-type $Ca^{2+}$ channels and insulin-containing secretory granules and its significance for the initiation of exocytosis in mouse pancreatic B-cells**

**Krister Bokvist, Lena Eliasson, Carina Ämmälä, Erik Renström and Patrik Rorsman**

transients in the B-cell have been cor
insufficient temporal resolution and/or
the membrane potential. One study, I

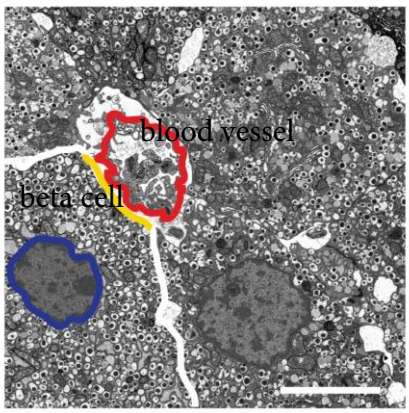
## Serial block section electron microscopy

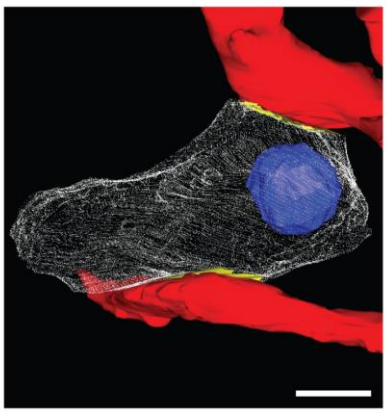


yellow = where beta cell touches blood vessel - discrete points of contact with beta cell ==> ~11% of area in contact with blood vessel

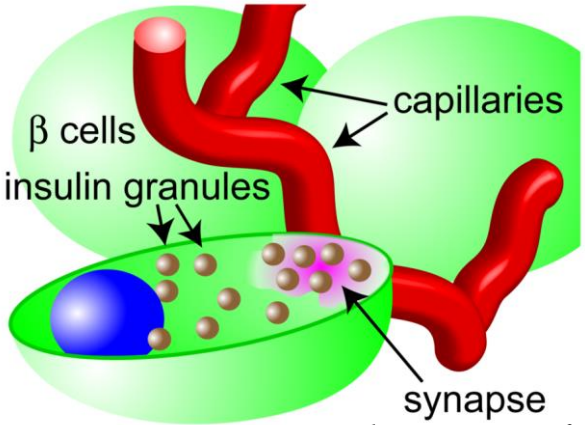
~~blue = nucleus of betea cell~~

## Beta cells make spatially discrete contact with the Vasculature.



## Evidence for a beta cell "synapse"

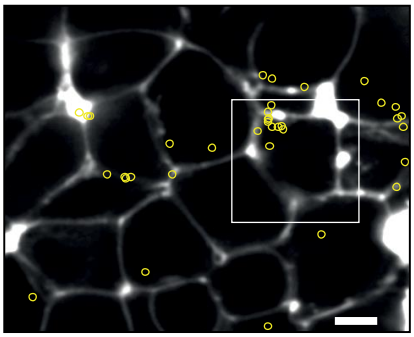


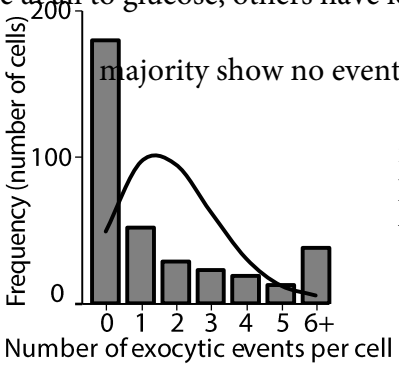

discrete point of contact with blood vessel

insulin secretion is targetted at the synapse

## Granule fusion is non-random

some beta cell shows no response at all to glucose, others have loads of response





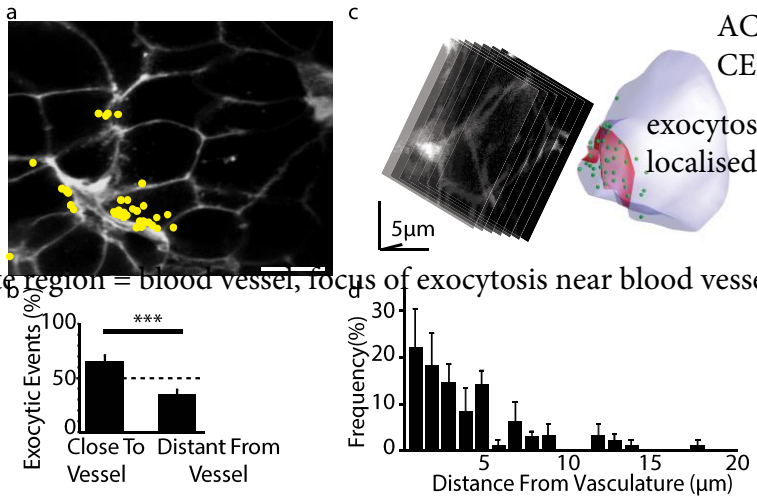

majority show no events at all

it is possible we are looking in the wrong **PLANE**

yellow = fusion events
white = dye that outlines cell

Low et al 2014 Diabetologia

## Granule fusion is biased towards the vasculature



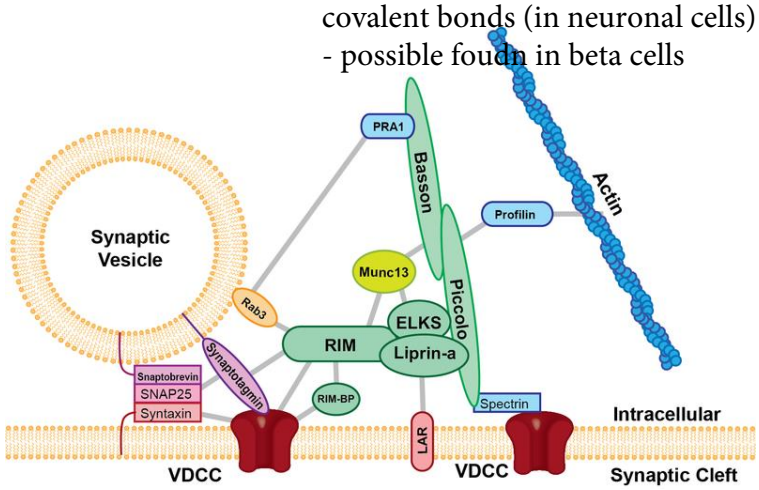

Low et al 2014 Diabetologia

MAPPING OUT POINTS OF EXOCYTOSIS ACROSS THE WHOLE CELL

exocytosis (green dots) closely localised to blood vessels

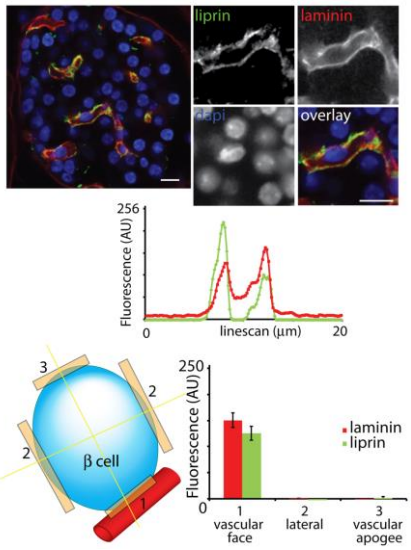
white region = blood vessel, focus of exocytosis near blood vessel

complex of multiple proteins held togehter by covalent bonds (in neuronal cells)
- possible foudn in beta cells



## Localisation of the synaptic scaffold protein, liprin

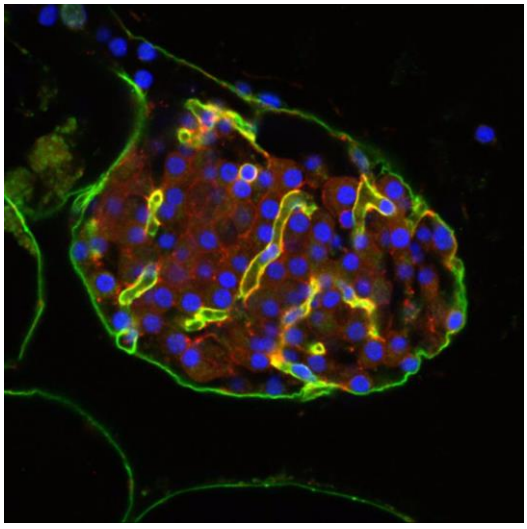
red = laminin for blood vessels
green = liprin, close overlay with blood vessel
should see green next to red, as they should be side by side and colocalised, but not overlayed



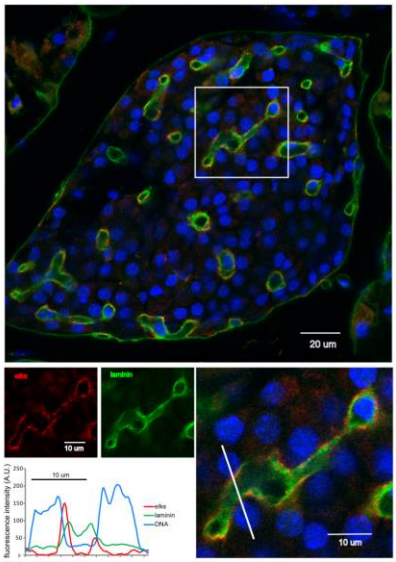

Low et al (2014) Diabetologia

Michael Zavortink

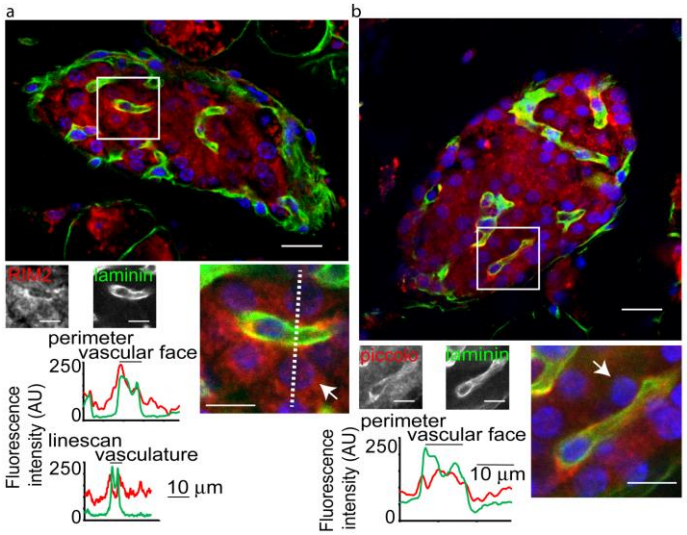
## Liprin: red  Laminin: green



## ELKS is enriched at the vascular face




## RIM2 and piccolo are also enriched at the vascular face

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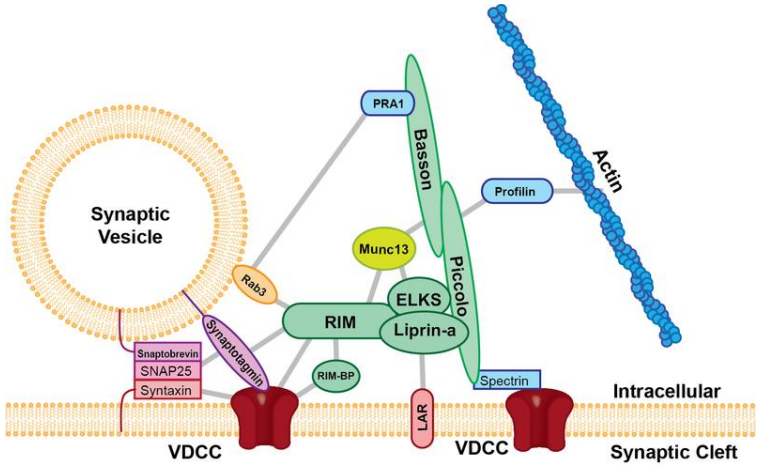
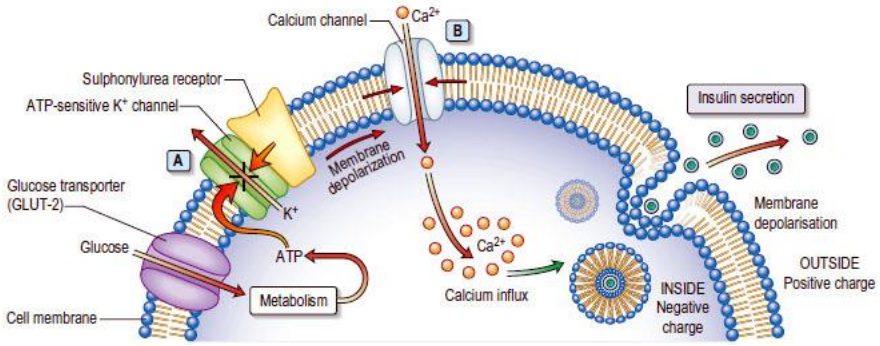
Conclusion: structural and functional evidence for a synapse




Stimulus-secretion coupling in normal β cell:

-temporal

-spatial special region of beta cell set up for exocytosis



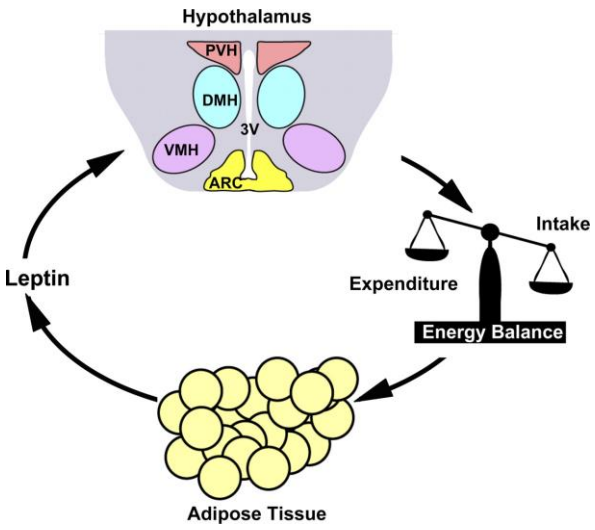
spontaneous mutation in the leptin receptor

# Db/db model of type 2 diabetes

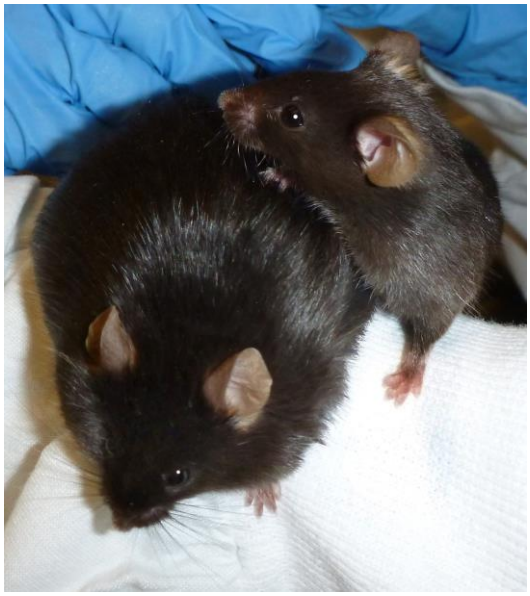
every single step breaks down in diabetes
done in mouse islets

normally what leptin does

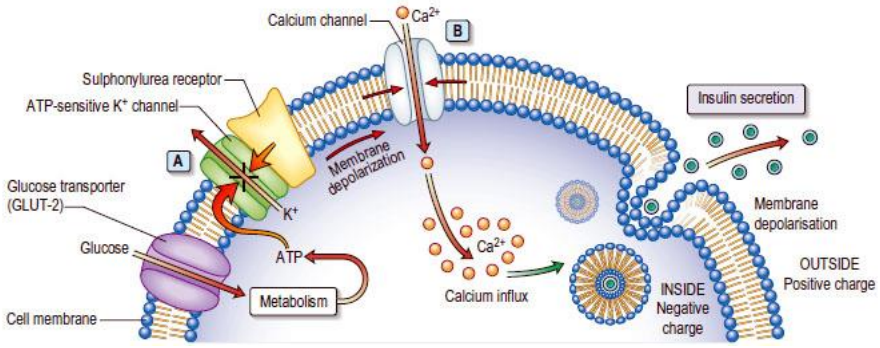
## Db/db model of type 2 diabetes: spontaneous Loss of the leptin receptor - controls appetite



## Db/db model of type 2 diabetes

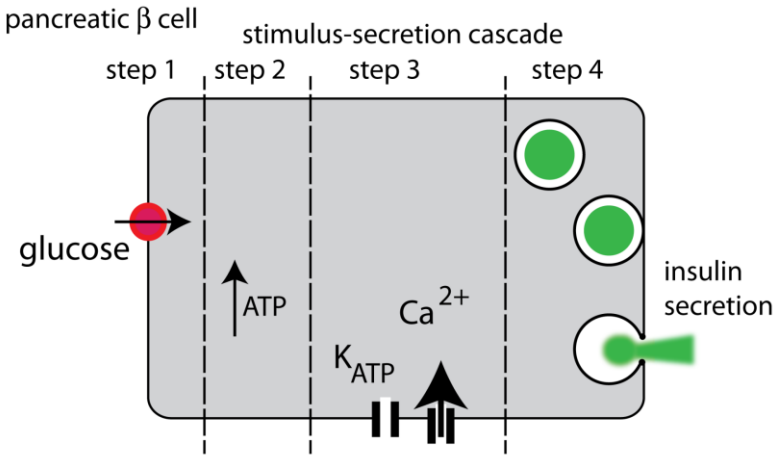


## Stimulus-secretion coupling in the β cell



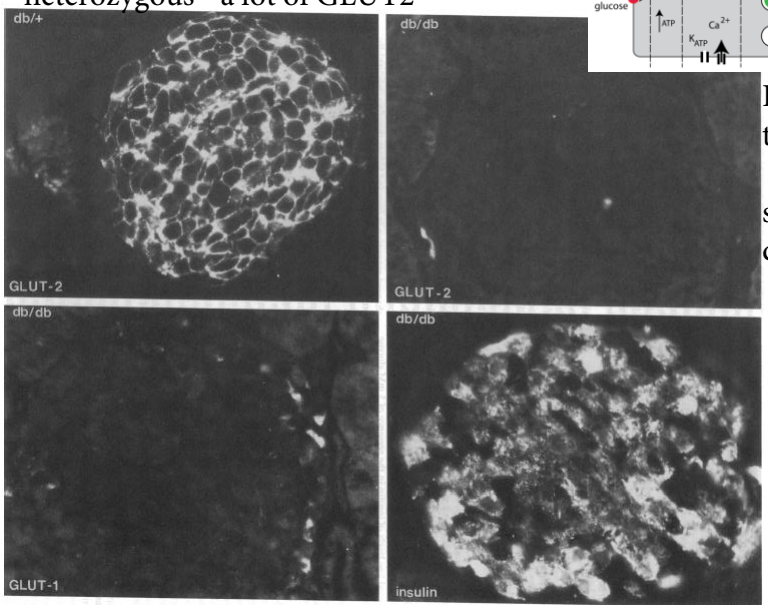

http://www.youtube.com/watch?v=yavhcGGxB5E

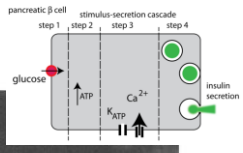
## Db/db Loss of insulin secretion




## 1. GLUT 2 decrease

heterozygous - a lot of GLUT2

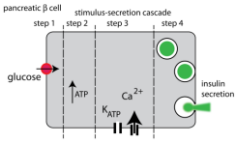


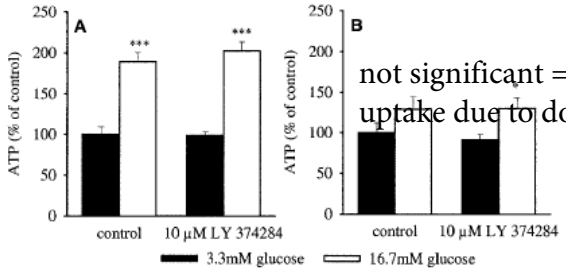

KO mice - no glut2 transporter

specifically downregulated?

## 2. Decrease in ATP production

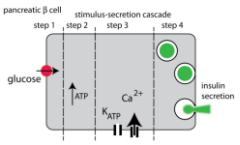


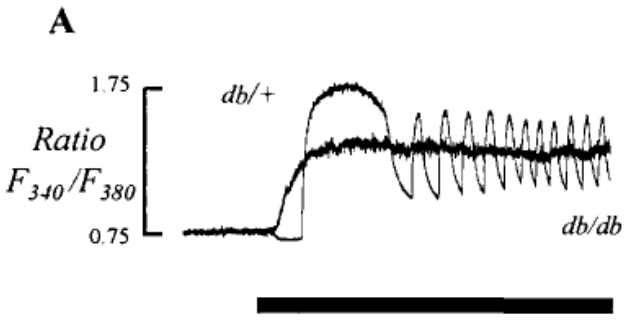

control db/db




not significant = makes sense as no glucose uptake due to downregulated GLUT2

## 3. Change in calcium signalling




dbdb = ca2+ levels increase slowly and doesn't oscillate

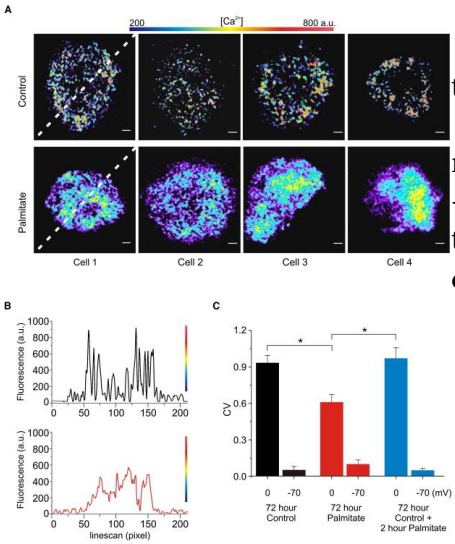
=more than ATP levels changing, GLUT2 damage, something happens to the Ca2+ as well, possible achange in Ca2+ distribution

**Defective Glucose-dependent Endoplasmic Reticulum $Ca^{2+}$ Sequestration in Diabetic Mouse Islets of Langerhans***

(Received for publication, April 22, 1994)

Michael W. Roe‡, Louis H. Philipson§, Crist J. Frangakis, Andrey Kuznetsov‖, Robert J. Mertz, Mary E. Lancaster, Ben Spencer, Jennings F. Worley III, and Iain D. Dukes¶

## Loss of association between calcium and sites of exocytosis

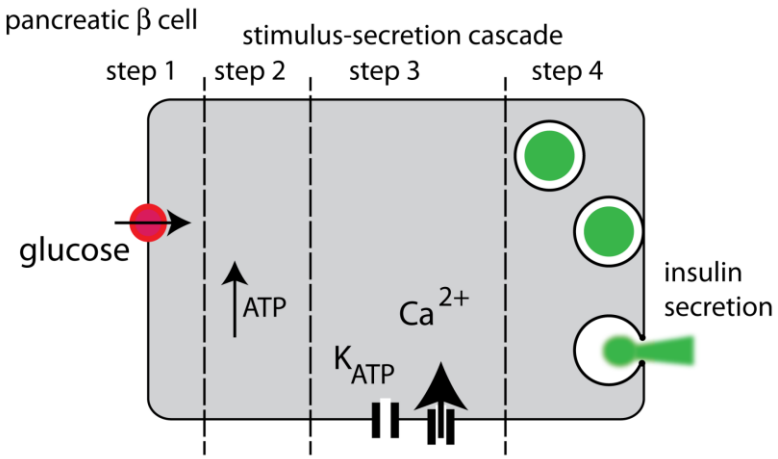



**Chronic Palmitate Exposure Inhibits Insulin Secretion by Dissociation of $Ca^{2+}$ Channels from Secretory Granules**

Michael B. Hoppa,[1] Stephan Collins,[1] Reshma Ramracheya,[1] Leanne Hodson,[1] Stefan Amisten,[1] Quan Zhang,[1] Paul Johnson,[2] Frances M. Ashcroft,[3] and Patrik Rorsman[1,*]
[1]The Oxford Centre for Diabetes, Endocrinology, and Metabolism, Churchill Hospital, Oxford OX3 7LJ, UK
[2]Nuffield Department of Surgery, John Radcliffe Hospital, Oxford OX3 9DU, UK
[3]Department of Physiology, Anatomy, and Genetics, Oxford University, Oxford OX1 3PT, UK
*Correspondence: patrik.rorsman@dpag.ox.ac.uk
DOI 10.1016/j.cmet.2009.09.011

treated with palmitate - greater Ca2+ response, possible ca2+ receptors originally too spread out in db/db mice

## Db/db Loss of insulin secretion

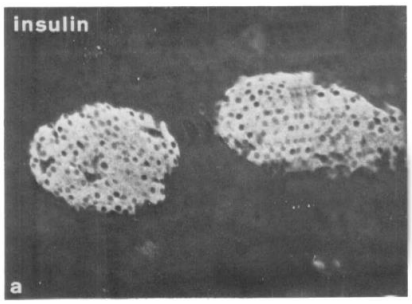


# 4. Decrease in insulin content

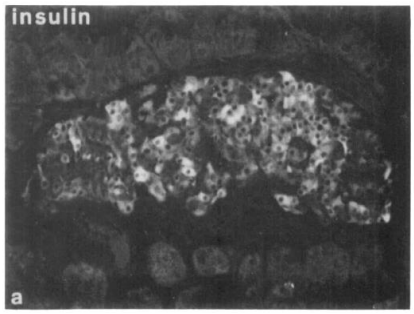
## Db/db loss of beta cells

immunostaining, a lot mor einsulin in control cell vs. in a diabetic insulin cell.

control



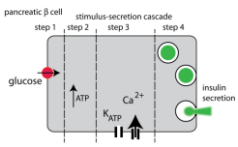

db/db




**Alteration of Islet Cell Populations in Spontaneously Diabetic Mice**

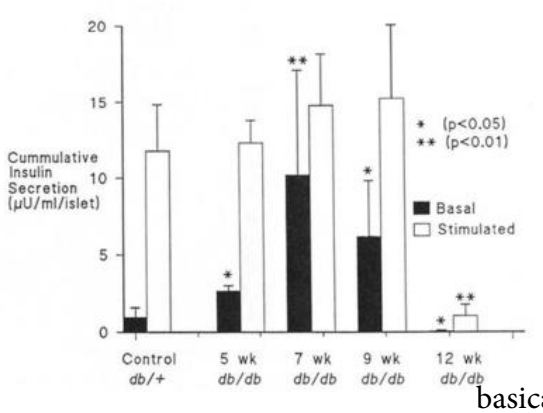
*D. Baetens, M.D., Y. Stefan, Ph.D., M. Ravazzola, Ph.D., F. Malaisse-Lagae, M.D., D. L. Coleman, Ph.D.,* and L. Orci, M.D., Geneva, Switzerland, and Bar Harbor, Maine**

# 4. Decrease in insulin content



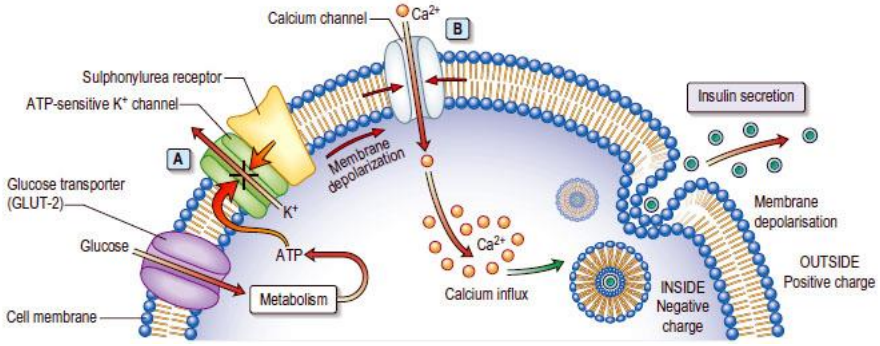

| | | Pancreatic weight (g) | Pancreatic insulin content (mg) |
|---|---|---|---|
| control | Control mice | | |
| | 5 wk | 0.16 ± 0.01 (5) | 14.7 ± 2.1 (5) |
| | 10 wk | 0.24 ± 0.01 (5) | 24.9 ± 3.4 (5) |
| | 13 wk | 0.30 ± 0.01 (5) | 34.6 ± 4.8 (5) |
| db/db | Unrestricted diabetic mice | | |
| | 5 wk | 0.18 ± 0.01 (5) | 8.9 ± 0.5 (5)† |
| | 10 wk | 0.28 ± 0.01 (5)* | 4.4 ± 1.0 (5)* |
| | 13 wk | 0.27, 0.23† | 1.4, 4.3* |

# 4. Db/db decreased insulin secretion




basically no response to glucose, glut2 transporter, ability to respond to ATP, reduced number of granules, defects in exocytosis

# Stimulus-secretion coupling in the β cell




http://www.youtube.com/watch?v=yavhcGGxB5E