Spanning time scales and levels of organization of insulin secretion with mathematical modeling: From seconds to hours, from molecules to organ

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Glucose stimulated insulin secretion

- Insulin is secreted from the pancreatic beta-cells in response to (mainly) glucose.
- Insulin is stored in secretory granules.
- Released by $Ca^{2+}$ triggered exocytosis.
Phasic insulin secretion

- Insulin is secreted in typical biphasic pattern in response to glucose-step
- Seen \textit{in vivo}, from pancreases and from islets
- Pools of granules?

\textit{In vivo} glucose clamp 
(De Fronzo et al., 1979)

Perfused rat pancreas 
(Grodsky, 1972)
Minimal models of insulin secretion – estimated from in vivo data

- C-peptide minimal model allows estimation of dynamic and static beta-cell responsivity
  (Toffolo et al., Diabetes 1995, AJP 2001; Cobelli et al., AJP 2007)
Dynamic secretion term

- Derivative control: The pancreas responds not only to glucose concentration \( G \) ("static", with delay), but also to rate-of-change \( dG/dt \)

- Necessary to fit in vivo data
  (Graded up-down: Toffolo et al., AJP 2001; OGTT: Breda et al., Diabetes 2001)

• Where does it come from?
Staircase experiment

- Rat pancreas  
  (Grodsky, JCI 1972)

- Sum of peaks = peak at max concentration \(\rightarrow\) threshold hypothesis  
  (Grodsky, JCI 1972)
Grodsky's threshold hypothesis

- Two pools of "packages"
- "Labile packages" are heterogeneous, different glucose thresholds for release
Model development

- Previous attempts: Grodsky (JCI '72), Landahl & Grodsky (BMB '82)

- Recent granule models: Bertuzzi et al. (AJP '07), Chen et al. (BJ '08), Pedersen & Sherman (PNAS '09)

- Cells (not granules!) activate at different glucose concentrations → heterogeneous RRP

(Pedersen et al., Phil Trans Roy Soc A 2008)

(Jonkers & Henquin, Diabetes 2001)
It does the job...

- Other recent models do not reproduce staircase
- Heterogeneous RRP allows reproduction of staircase experiment
- In contrast to Grodsky, due not to threshold on granules but on cells (or islets?) as seen in experiments
Where does derivative control come from? (Pedersen et al., AJP 2010)

- Threshold distribution underlies derivative control (Grodsky, JCI 1972; Licko, Bull Math Biol 1973)

- Here:
  - $SR = mF$
  - $\frac{dF}{dt} = -(m+k)F + f H(G), \quad H(G) = \int_0^G h(g)dg$
  - $\frac{dH}{dt} = \int_0^G dh(g)/dt \, dg + h(G) \, dG/dt$
    \[ = - (f+p^{-})H(G) + p^{+} I \Phi(G) + h(G) \, dG/dt \]
  - Assume quasi steady-state

- $SR(t) = \text{const} \left[ p^{+} I(t, \tau) \Phi(G(t)) + h(G(t)) \, dG/dt(t) \right]$

Static responsitivity

Dynamic responsitivity
Relative contributions of dynamic vs. static secretion

- Glucose profile following a meal
- Model parameters adjusted to give reasonable C-peptide data

Legend:
- Full model
- Approximation
- Dynamic
- Static
Conclusions (part 1)

- Relatively simple model, but founded on biologically established principles (non-phenomenological)
- Can explain static and dynamic secretion terms
  - *Dynamic* due to recruitment of cells (or islets?)
  - *Static* due to refilling of RRP (introduces delay)
- The model could (should!?) be coupled to models of calcium dynamics
- Such models provide mechanistic underpinning of the assumptions of the minimal models
  - Granules → cells → pancreas
- … and could help in interpreting in vivo data (disturbances in diabetics?)
Distinct mechanisms account for 1\textsuperscript{st} and 2\textsuperscript{nd} phase secretion

Docked granules fuse at Synt1A clusters (~80%), newcomers fuse away from Synt1A clusters (~85%)

Synt1A clusters are co-located with L-type Ca$^{2+}$ channels (Yang et al., PNAS 1999)

1\textsuperscript{st} (resp. 2\textsuperscript{nd}) phase secretion occurs mainly near (resp. away from) Ca$^{2+}$ channels

Ohara-Imaizumi et al. (J Cell Biol 2007)
... and docking is not a prerequisite for the 2\textsuperscript{nd} phase

Syntaxin (Synt)-1A knock-out cells show impaired docking and 1\textsuperscript{st} phase, but not 2\textsuperscript{nd} phase, secretion (Ohara-Imaizumi et al., J Cell Biol 2007)

Due to fusion of newcomer granules
Highly calcium sensitive pool (HCSP) of granules

- Wan et al. + Yang & Gillis (JGP 2004)
- Affinity ~ 2 μM (~ 20 μM for RRP)
- The HCSP resides away from calcium channels since depolarizations do not empty it
- Newcomers also fuse away from calcium channels
Including the HCSP
... in the model by Chen et al. (Biophys J 2008)

- Distinction between global, cytosolic and local, microdomain calcium, and between L- and R-type calcium channels

- HCSP assumed to reside away from Ca$^{2+}$ channels

- HCSP assumed to be independent of syntaxin-1A, and to consist of granules that are tethered, but still not completely docked

Pedersen & Sherman (PNAS 2009)
Simulations: Yang & Gillis protocol
Simulations:
Newcomer granules fuse from the HCSP

IRP secretion
HCSP secretion
Total secretion

Secretion rate [pg/islet/min]

2nd phase

Almost docked pool
Actin network

HCSP

DP  PP  IRP

Ca\(^{2+}\)_i  Ca\(^{2+}\)_{md}

Number of fusion events

Time after stimulus (s)
Simulations: Calcium channel KO

L-type KO/block
(Schulla et al., EMBO J 2003)

R-type KO/block
(Jing et al., JCI 2005)

10 mM gluc

D
NMRI + SNX482

16.7 mM glucose
Simulations: Synt1A KO cells by assuming reduced docking rate

Prediction and crucial test of the model:
The HCSP is intact and possibly increased in Synt1A cells
Different calcium sensors?

- Synaptotagmins (Syt's) are believed to be the sensors of calcium
  - Syt-9 is a low affinity (tens of $\mu$M) sensor present in beta-cells, and is likely the IRP sensor
  - Syt-7 and Syt-3 are high affinity (few $\mu$M) sensors, and have been suggested to be involved in insulin secretion (Syt-3 controversial). Could be the HCSP sensors
Simulations: KO of the HCSP sensor

... as expected

Prediction: Second phase secretion is impaired
Is Syt-7 the HCSP sensor?

Gustavsson et al. (PNAS 2008)

Both reduced 1\textsuperscript{st} and 2\textsuperscript{nd} phase – but there might be Syt-7/Syt-9 interactions changing the properties of IRP release (Schonn et al., PNAS 2008)
Summary (part 2)

- Part 1 bridges levels of organization (*granules* → *organ*)
- Part 2 spans timescales by coupling secretion (*minutes*) to capacitance measurements (*milliseconds*) for various perturbed situations
  - \(\text{Ca}^{2+}\) channels KO/blocking
  - Syntaxin-1A KO
  - \(\text{Ca}^{2+}\) sensor/synaptotagmin KO
- Including a HCSP as a transient state away from L-type calcium channels, naturally identified the HCSP with newcomer granules.
- Mathematical modeling was used to test the plausibility of the biological hypothesis
Conclusions

- Mathematical models are used to integrate separate experiments in a structured, coherent way
- Can be used to span timescales and levels of organization
- Two classes:
  - “Models to simulate”
    (test hypotheses, predict outcome of experiments; can include different levels of detail depending on the scope of the model)
  - “Models to measure”
    (extract information from data; must be simple/minimal to allow parameter estimation)
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Thank you!
Pools?

Henquin, 2002

Henquin, 2009
Results

Biphasic

Potentiation