Regulation of Insulin Secretion II

Cellular Signaling in the Islet of Langerhans

Richard KP Benninger
Glucose stimulated insulin secretion (GSIS)

- Glucose enters the cell through GLUT2
- Glucose is phosphorylated by GK (Hexokinase 4)
- ATP is produced during glycolysis
- ATP activates potassium channels (KATP)
- ATP activates adenyl cyclase (AC)
- Cyclic AMP (cAMP) activates PKA and Epac
- Cyclic AMP and Epac activate glycogen synthase kinase (GSK)
- Cyclic AMP and Epac also activate protein kinase A (PKA)
- Insulin is secreted from the insulin granules

Defects in many genes that underlie GSIS increase risk of diabetes

- Kir6.2, Sur1 (K_ATP channel subunits)
  - Channel mutations can cause neonatal diabetes
  - SNPs associated with enhanced risk to type 2 diabetes
- GK (Hexokinase 4)
  - Heterozygous mutations cause MODY-2
  - Homozygous mutations cause neonatal diabetes
- Ins1, Ins2 (Insulin genes)
  - Mutations cause neonatal diabetes
- HNF1-α (transcription factor, regulates mitochondria)
  - Mutations cause MODY-3
- ZnT8 (Zn²⁺ Transporter)
  - SNPs associated with enhanced risk to type 2 diabetes
  - Other SNPs such as TCF7L2 also enhance risk to type 2 diabetes
Many points underlying GSIS are potential therapeutic targets

- Sulfonylureas close $K_{ATP}$ channels and trigger insulin secretion:
  - Glibenclamide (Glyburide)
- GLP1R agonists elevate cAMP and enhance insulin secretion and cell proliferation:
  - Exendin-4
- …
GLUT2 and GK

- GLUT2 is a high capacity glucose transporter
  - Intracellular [glucose] ≈ Extracellular [glucose]
- GK (Hexokinase 4) phosphorylates glucose
  - Produces glucose-6-phosphate
  - Enters glycolysis and then CAC metabolism
  - Elevates ATP/ADP ratio

GK is the rate limiting step in GSIS

- $K_D$ of GK is ~7mM glucose
  - Rate limiting step for ATP synthesis
  - Compared to <1mM for Hexokinase 1,2,3
  - Within physiological range of blood glucose levels
Glucose stimulated insulin secretion (GSIS)

- Glucose metabolism leads to membrane depolarization
  - $K_{ATP}$ channel closure
- Repeated APs fire following depolarization
  - Regulated by voltage gated $K^+$, $Na^+$, $Ca^{2+}$ channels

**Electrical activity in the β-cell**

- Glucose metabolism leads to membrane depolarization
  - $K_{ATP}$ channel closure
- Repeated APs fire following depolarization
  - Regulated by voltage gated $K^+$, $Na^+$, $Ca^{2+}$ channels
ATP-sensitive Potassium channel (K$_{\text{ATP}}$)

- K$_{\text{ATP}}$ is a hetero-octomer
  - Kir6.2 and Sur1 4:4
- ATP closes channel
- MgADP opens channel
- Channel mutations can shift the sensitivity to ATP

The K$_{\text{ATP}}$ channel and electrical activity play a key role in islet function

- Expression of ATP-insensitive K$_{\text{ATP}}$ channels leads to a loss of serum insulin

Targeted Overactivity of β Cell K$_{\text{ATP}}$ Channels Induces Profound Neonatal Diabetes

J. C. Koster, N. A. Marshall, N. E. Ennis, J. A. Cobbett, and C. G. Nichols

The Department of Cell Biology and Physiology
Washington University School of Medicine
St. Louis, Missouri 63110

C

Expression of ATP-insensitive K$_{\text{ATP}}$ channels leads to a loss of serum insulin
The $K_{\text{ATP}}$ channel and electrical activity play a key role in islet function

**ORIGINAL ARTICLE**

Activating Mutations in the Gene Encoding the ATP-Sensitive Potassium-Channel Subunit Kir6.2 and Permanent Neonatal Diabetes


From the Institute of Biomedical and Clinical Science, Peninsula Medical School, Exeter, United Kingdom

- Mutations that reduce the ATP sensitivity of $K_{\text{ATP}}$ channels cause neonatal diabetes in humans

The $K_{\text{ATP}}$ channel and electrical activity play a key role in islet function

**ORIGINAL ARTICLE**

Switching from Insulin to Oral Sulfonylureas in Patients with Diabetes Due to Kir6.2 Mutations


- Sulfonylureas are an effective treatment for patients with $K_{\text{ATP}}$ induced permanent neonatal diabetes
Other ion channels are also important

- Voltage gated $K^+$ channels ($K_v$)
  - Regulates AP repolarization
  - $K_{v2.1-/-}$ shows elevated insulin secretion

Glucose stimulated insulin secretion (GSIS)

- $K_{ATP}$
- ATP
- ADP
- $[Ca^{2+}]_{i}$
- $V_m$
- $Ca_V$
- $GK$
- glycolysis
- $G_s$ GPCR
- glucagon
- GLP1
- AC
- cAMP
- PKA
- Epac
- Insulin granule exocytosis

Jacobson et al. *Cell Metabolism* 2007
Ca\textsuperscript{2+} and insulin secretion

- Membrane depolarization activates L-type Ca\textsuperscript{2+} channels
  - Influx of Ca\textsuperscript{2+} into cell
  - Triggers insulin granule exocytosis

Measuring Ca\textsuperscript{2+} in cells

- Label cells with Ca\textsuperscript{2+} sensitive fluorescent dye

Fura-3 Calcium Response
Measuring Ca\(^{2+}\) in cells

- Image the fluorescence from the Ca\(^{2+}\) dye on a confocal microscope

Calcium oscillations in the islet
Pulsatile Insulin secretion

• Oscillations in plasma insulin are found in humans, dogs and mice
  – 3-8 minute period
• Pulsatile insulin has been shown to exert a greater glucose lowering action than continuous levels of insulin
• Insulin oscillations are disrupted obese individuals and in patients with type2 diabetes

Matthews et al. Diabetes 1983
Bratuschmarrain et al. Diabetes 1986
Porksen et al, Diabetes 2002

Pulsatile insulin secretion and Ca\textsuperscript{2+}

• Pulsatile insulin levels are related to Ca\textsuperscript{2+} oscillations in isolated islets

Nunemaker et al. Diabetes 2005
Summary I

- Electrical activity is critical for normal insulin secretion
  - Defects underlie many aspects of diabetes
- Many regulators of electrical activity can be potential therapeutic targets
  - e.g. $K_{ATP}$, $K_V$, $Ca_V$, channels, and more
- Oscillations in electrical activity leads to pulsatile insulin secretion
  - Insulin oscillations have a greater hypoglycemic effect and are disrupted in type2 diabetes

Multi-cellular properties of the islet

- High dynamic range of secretion
- Low dynamic range of secretion
- Coordinated pulsatile secretion
- Continuous irregular secretion
- Islet transplant: ✔
- β-cell transplant: ✗
Cell-cell communication in the islet

- Cells in the islet express several factors for cell to cell communication
  - Gap junction channels
  - Receptors for hormones and neurotransmitters
  - Cell adhesion molecules
  - Islets are vascularized and innervated

Role of gap junction in β cells

- Gap junctions are made of 2 connexon hemichannels
- Each connexon is made of 6 connexin subunits
- Gap junction channels allow ionic currents, and small molecules to pass between cells
Kir6.2[AAA] loss-of-function mutation

Koster et al. *Cell* 2000

Critical role of gap junction coupling

Inhibiting gap junction coupling leads to spontaneous Ca\textsuperscript{2+} bursts in the islet

Gap junction coupling overcoming the Kir6.2[AAA] induced excitability

Cx36 gap junctions couple β-cells


Ca²⁺ dynamics in Cx36 knockout

Cx36+/-: ~50%

Cx36-/-: ~0%

T=+5 sec  T=-5 sec
Oscillations in Cx36-/- islets

- Total loss in synchronized $[Ca^{2+}]_i$ activity

Played at x15 normal speed

Regulation of pulsatile insulin secretion?

- Loss of Cx36 leads to an absence of pulsatile insulin secretion from single isolated islets

Ravier et al. (2005) Diabetes
GSIS vs gap junctions in intact islets

No significant change in GSIS upon a loss of gap junction coupling

Cx36-/- mice are glucose intolerant

Male, 16 weeks  Female, 16 weeks
Summary II

- An absence of gap junction coupling leads to reduced glucose tolerance
- Gap junction coupling is necessary to coordinate oscillations in electrical activity
  - Necessary for pulsatile insulin secretion from isolated islets
- Gap junction coupling does not play a significant role in regulating the levels of insulin secretion and insulin sensitivity
- A loss of gap junction coupling leads to a loss of plasma insulin oscillations which in turn leads to reduced insulin action?

Glucose stimulated insulin secretion (GSIS)
Incretins and insulin secretion

- Incretins GLP1 and GIP amplify insulin secretion
- GLP1R activation stimulated cAMP synthesis
- cAMP acts on 2 main targets
  - PKA
  - Epac2
- Both PKA and Epac2 promote insulin granule recruitment and docking to PM

Measure other variables in the β-cell

- Use fluorescent protein biosensors
  - Fluorescent protein(s) plus a binding domain
  - Monitor change of fluorescence

Nikolaev et al. (2004) JBC
Overall summary

• Understanding the variables underlying GSIS are to understanding and treating diabetes
• GK is the rate limiting step in GSIS
• Electrical activity is critical for the regulation of insulin secretion
  – $K_{ATP}$ channel
  – Other ion channels
  – Gap junctions
• Oscillations in $Ca^{2+}$ underlie pulsatile insulin secretion
  – Important for insulin action
• GLP1 and GIP stimulate cAMP synthesis which further elevate insulin secretion