Role of endogenous ROS in pancreatic β-cell dysfunction

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The effect of endogenous ROS on metabolism-secretion coupling
Src inhibition ameliorates impaired IS and ATP production in GK islets

**Wistar**

![Insulin release (ng/islet/30min)]

**GK**

![Insulin release (ng/islet/30min)]

**ATP content (pmol/islet)**

VE+VC: ROS scavenger  
PP2 (10 µM): Src inhibitor
Src inhibition decreases ROS production in GK islet cells.

Src activity is endogenously up-regulated in GK islets

Mukai E et al. Diabetes, 2011

[Images of Western blots showing increased band intensity of Src pY416 and Src pY416/Src in GK compared to Wistar islets.]

Caption: "Src activity is endogenously up-regulated in GK islets."
Exendin-4 suppresses Src activity in GK islets

**GK**
- IP Src
  - Control
  - Ex
- Src pY416
- Src

**Wistar**
- IP Src
  - Control
  - Ex
- Src pY416
- Src

**Graphs**
- Src pY416/Src
  - Band intensity (ratio)
  - 16.7 mM G
  - Cont
  - Ex
  - *p<0.05
  - n=4

**Legend**

**Notes**
- Exendin84 suppresses Src activity in GK islets
- *p<0.05
- n=4
Exendin-4 decreases ROS production in GK islet cells

**Graphs:**
- **Upper left graph:** CM-D CF fluorescence (fold increases) over time (min) for 16.7 mM G. Cont, Ex (100 mM), with statistical significance marked as *p<0.05, n=5~7.
- **Upper right graph:** CM-D CF fluorescence (fold increases) for 16.7 mM G. Cont, Ex, PP2, Ex+PP2, with statistical significance marked as *p<0.05, †p<0.01, ns, n=4~6.
- **Lower left graph:** *in vivo* gene transfection with Celiac artery, Liver, Spleen, Pancreas, Transgene: GFP, Confopectral, Optic.

**Referenced Studies:**
Exendin-4 increases ATP production in GK islets

**Wistar**

![Bar graph showing ATP content in Wistar islets](image1)

**GK**

![Bar graph showing ATP content in GK islets](image2)

Mukai E et al. Diabetes, 2011
Src activity is endogenously up-regulated in GK islets, which contributes to ROS production and impaired ATP production. GLP-1 signal ameliorates ROS production and ATP production through suppression of Src activation.
The decrease in ROS production by exendin-4 is dependent on Epac

Mukai E et al. Diabetes, 2011
PI3-K/Akt signaling is involved in the downstream pathway of Src.

LY294002 (50μM), Wortmanin (0.5μM): PI3-K inhibitor

Mukai E et al. Diabetes, 2011
The effects of exendin-4, suppression of Src activity and decrease in ROS production, are dependent on not PKA but Epac.
PI3K/Akt signaling, inhibited by exendin-4 or Src inhibitor, is involved in the downstream pathway of Src and regulates ROS production.

GLP-1 signaling improve β-cell function in the diabetic state because it ameliorates impaired metabolism-secretion coupling
The effect of a longer suppression of ROS on metabolism-secretion coupling

Antioxidant system

- Tempol
- SOD
- CAT
- Gpx1
- Ebselen

Glucose → Metabolism → ATP → Insulin secretion → GKβcell

ROS ↑ → Src ↑ → ATP ↓ → Insulin secretion ↓
TE treatment ameliorates impaired IS and ATP production in GK islets

Sasaki M et al. Diabetes, 2013
The effect of *in vivo* TE treatment on β-cell function in GK

**IPGTT**

- Glucose (mg/dl)
  - Control
  - TE

- Insulin secretion (ng/30min/islet)
  - 16.7mM G
  - 2.8mM G

* *p<0.05, **p<0.01 vs control

Sasaki M et al. Diabetes, 2013
Lactate overproduction uncouples between glycolysis and mitochondrial oxidation in GK islets

Glucose → Glycolysis → Pyruvate → Lactate → Oxidation

Krebs cycle

Sasaki M et al. Diabetes, 2013
TE treatment decreases the expression levels of LDH-A and HIF1α in GK islets

LDH-A

HIF1α

β-actin

C  TE  C  TE

Wistar  GK  Wistar  GK

† p<0.01

Sasaki M et al. Diabetes, 2013
HIF1α inhibition improves lactate overproduction and IS in GK islets

Sasaki M et al. Diabetes, 2013
ROS reduction ameliorates metabolism-secretion coupling by suppressing lactate overproduction through the inhibition of HIF1α stabilization. The Warburg-like effect, which is characteristic of aerobic metabolism in cancer cells by which lactate is overproduced with reduced linking to mitochondrial metabolism, plays an important role in impaired metabolism-secretion coupling in diabetic β-cells.
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