Role of endogenous ROS generated by Src activation in impaired metabolism-secretion coupling in GK rat islets

Shimpei Fujimoto, Rieko Kominato, Eri Mukai, Nobuya Inagaki

Department of Diabetes and Clinical Nutrition, Graduate School of Medicine, Kyoto University
1. Mechanism of impaired glucose-induced insulin secretion in diabetes
   – Background

2. Endogenous ROS generating system that impairs mitochondrial carbohydrate metabolism

3. Role of endogenous ROS generating system in impaired metabolism-secretion coupling in diabetes

4. Effect of incretin on endogenous ROS generating system
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Insulin release from pancreatic β-cells

- Glucose
- K_{ATP} channel
- Ca^{2+}
- [Ca^{2+}]_{i}
- ATP/ADP
- Other signals
- ATP
- Insulin release

Metabolism
Insulin release from perfused pancreas

Glucose-induced insulin secretion is selectively impaired in diabetic condition

ATP content in islets

Increase in ATP content by high glucose is impaired in diabetes

Hughes SJ et al. Diabetes, 1998

Anello M et al. Diabetologia, 2005
Impaired glucose metabolism plays important role in impaired glucose-induced insulin secretion
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Na/K ATPase

Maintain intracellular Na\(^+\) and K\(^+\) homeostasis in all cells

Reduced activity in diabetic state

in neuron, myocyte, cardiomyocyte, and endothelial cell

linking to cellular damage

Specific inhibitor: Ouabain
Effect of ouabain on glucose-induced biphasic insulin secretion

16.7mM G

2.8mM G

1 mM Ouabain

Insulin release (ng/20 islets/min)

Time (min)

$0 < 0.05$ vs without Ouabain
Effect of ouabain on ATP contents

- 16.7 mM G
- 16.7 mM G + Ouabain
- 2.8 mM G

* p<0.05 vs 16.7 mM G
Glucose-induced mitochondrial membrane hyperpolarization

Cytosol Mitochondrion

hyperpolarization

\[ \Delta \Psi_m \]

ATP synthase

H+ H+ H+

ATP

O2 H2O NAD+ NADH

TCA cycle

CO2

Acetyl CoA

Adenine dinucleotide phosphate (ADP)

glucose

pyruvate

Inner membrane

Outer membrane
Effect of ouabain on mitochondrial membrane potential

1μM FCCP

- 16.7mM G
- 16.7mM G + Ouabain
- 2.8mM G

Time (min)

JC-1 fluorescence (arbitrary units)
Exogenous administration of H$_2$O$_2$, most abundant ROS, causes

1. Depolarization of glucose-induced hyperpolarization of inner membrane

2. Reduction of glucose-induced increase in ATP level

3. Reduction of glucose-induced insulin release

in INS-1 cells

Mechler P et al., 1999
Effect of ouabain on ROS production

2.8mM G 16.7mM G

†

1mM Ouabain

* P<0.05 vs. 16.7mM G
† P<0.01 vs. 16.7mM G
Scavenge effect of α-tocopherol on ouabain-induced inhibition

**ATP content**
(16.7 mM glucose)

**Insulin release**
(16.7 mM glucose)

- **ATP content**
  - Control: 10 pmol/islet
  - Ouaibain: 6 pmol/islet
  - Ouaibain + α-tocopherol: 8 pmol/islet

- **Insulin release**
  - Control: 2 ng/islet/60min
  - Ouaibain: 1 ng/islet/60min
  - Ouaibain + α-tocopherol: 2.5 ng/islet/60min

* * p<0.01 vs. control

Glucose, pyruvate enter the cell. TCA cycle, NADH, Acetyl CoA, ATP synthase, ATP, ADP+Pi, Insulin release.

Plasma membrane:
- Glucose
- Ouabain
- Na-K ATPase

Inner membrane:
- TCA cycle
- Acetyl CoA

Outer membrane:
- ATP synthase
- ΔΨ
- H+ (protons)
Src is involved in signal transducing function of Na-K ATPase to generate mitochondrial ROS in cardiomyocytes.

Xie Z et al., 2003

Src: membrane associated non-receptor tyrosine kinase

Specific inhibitor: PP2
Effect of Src inhibitors on ATP contents (60 min incubation)

<table>
<thead>
<tr>
<th>Glucose (mM)</th>
<th>ATP (pmol/islet)</th>
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<tr>
<td>2.8</td>
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<tr>
<td>16.7</td>
<td></td>
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<tr>
<td>2.8</td>
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<tr>
<td>16.7</td>
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- Ouabain
- PP2 (10μM)
Effect of Src inhibitor on ROS production (60min incubation)

- p<0.01
- n.s

For Glucose (mM): 16.7

- Ouabain
- Ouabain
- PP2
Regulation of Src activity

Src

SH3

SH2

kinase

N

416

527

C

Extracellular signal

Inactivation

activation

Csk (C-terminal Src Kinase)

Negative regulator of Src

Y527

Y416

phosphorylation

dephosphorylation

phosphorylation

dephosphorylation
Ouabain-induced Src phosphorylation

<table>
<thead>
<tr>
<th>IP Src</th>
<th>IB total Src</th>
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<th>IP Src</th>
<th>IB pY416 Src</th>
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<th>IP Src</th>
<th>IB pY527 Src</th>
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Control Ouabain
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Effect of PP2 on insulin release

Control

GK

n.s.

P<0.01

Insulin release (ng/islet/30min)

G16.7

G 2.8

n=10

PP2
Effect of ROS scavenger on insulin release

Control

GK

n.s.

P<0.01

Insulin release (ng/islet/30min)

VE+VC

G16.7

G 2.8

n=10
Effect of ouabain, PP2, and ROS scavenger on ATP content

Control

GK

P<0.01

P<0.01

N.S.

N.S.

n=8

n=10
ROS production (60min incubation)

Control vs. GK

Glucose 16.7 mM

Ou, PP2, VE+VC

CM-DCF fluorescence (relative units)

P<0.01

N.S.
Effect of overexpression of Src kinase-negative mutant on ROS production in GK islets

![Bar graph showing CM-DCF fluorescence (relative units) for Cont and SrcKD treatments.](image)

- Cont: 4.0 units (P<0.001 vs. SrcKD)
- SrcKD: 2.0 units

16.7 mM G

CM-DCF fluorescence (relative units)
GK β cell

Glucose → ROS → ATP → Insulin Secretion

Src activation → Mitochondrial Carbohydrate Metabolism
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Insulin release in the presence of forskolin (perfusion)

Why intracellular cAMP elevation causes prominent enhancement of impaired glucose-induced insulin secretion in diabetic state?

Accumulation of oxidative stress in islets in diabetes (8-OHdG staining)

**Human**

Non-DM

DM

**Rats**

GK

Wistar

5w 8w 12w 21w

Sakuraba H et al. Diabetologia, 2002

Ihara Y et al. Diabetes, 1999
Incretin improves impaired metabolism-secretion coupling in diabetic state by regulating endogenous ROS generation.
Colleague

Department of Diabetes and Clinical Nutrition, 
Kyoto University Graduate School of Medicine

Mariko Kajikawa
Rieko Kominato
Eri Mukai
Prof. Nobuya Inagaki

Prof. Yutaka Seino (Kansai Electric Power Hospital)

Department of Oncogene Research, 
Research institute for Microbial Diseases, Osaka University

Chitose Oneyama
Prof. Masato Okada