Defects in glucose utilization & GLP-1

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High glucose in normal person

β-cell

Glucose → GLUT 2 → Glucose-6-phosphate → Glucokinase → Oxidation → ATP

ATP + K⁺ channel (closed) → Depolarization → ER

Ca²⁺ channel (open)

Ca²⁺
High glucose in prediabetes

β-cell

Glucose → [GLUT 2] Glucose-6-phosphate → Oxidation → ATP → Mitochondria → Depolarization → [ATP + K⁺ channel (closed)]

Insulin → ROS → ER stress

Ca++ channel (open)
High glucose in diabetes

β-cell

Glucose → GLUT 2 → Glucose-6-phosphate → Glucokinase → Glucose oxidation → ATP → Mitochondria → ROS → K+ channel (closed) → Depolarization → Ca++ channel (open) → ER stress → Insulin
High glucose in diabetes

- β-cell death

Surviving β-cell
- Impaired glucose-stimulated insulin secretion

- Insufficient amount of insulin in the blood (vs. glucose)
- Decreased negative feedback toward glucagon secretion

Low I/G ratio

- Impaired glucose utilization in insulin-sensitive tissues
- Glucose toxicity in various tissues

Diabetic complications
Brain in diabetes

**Neurons rely on glucose metabolism for function and survival**

**Neurodegenerative disorders: impairments in the glucose availability**
[Adhihetty PJ & Beal MF, Neuromol Med, 2008]
[Browne SE et al, Neurobiol Dis, 2006]

: Parkinson’s disease, Huntington’s disease, Alzheimer’s disease, Amyotrophic lateral sclerosis

**Neurotransmitter release defects by low ATP**
**AMPK-mediated, ER stress-mediated cell death**

**In diabetes**, glucose availability much lower

**Exaggeration of the diseases**
In diabetic β-cells, Unlike gastric inhibitory peptide (GIP)

- **Acute effect**
  Potentiated glucose-dependent insulin secretion $\rightarrow$ glucagon $\downarrow$

- **Chronic effects**
  Increased insulin gene expression & biosynthesis
  Decreased apoptosis
  Increased proliferation and neogenesis (in animal experiments)

**High I/G ratio**

- Appetite $\downarrow$ $\quad$ **Lowers blood glucose levels in human DM** $\quad$ GI motility $\downarrow$
**GLP-1 synthesis**

Enteroendocrine L-cell  
α-cell  
Brain  
Taste buds

Exendin-4  
= GLP-1R agonist

Gila monster lizard
GLP-1 receptor

7-transmembrane G-protein coupled receptor (GPCR)

- cAMP
  - Protein kinase A (PKA) = cAMP-dependent PK
  - cAMP-GEF (Epac1, 2)
  - EGFR transactivation

- Class B (secretin family, glucagon-related subfamily (GLP-2, glucagon)
- Short arm of Chromosome (6p21 in human)
- 64-kDa protein
- Islets, brain, heart, kidney, GIT
Protein kinase A (PKA)
cAMP-GEF (Epac)

- cAMP-regulated Guanine nucleotide exchange factors
- Exchange protein directly activated by cAMP
- Two variants of Epac (Epac1 and Epac2)

Biological actions in beta cells

Protein-Epac complex (non-kinase effect)

Kinase effect

Protein

cAMP

Effector
EGFR transactivation
GLP-1 potentiates triggering mechanism of GSIS

- $K_{ATP}$: close (SUR1) → close (SUR1)
- $K_v$: close
- $Cav$: open
- CICR: open (epac2)
- IP3-ICR: open
- Secretory proteins: SNAP-25, Rim2, gSUR, piccolo
- Glucokinase: ?

GSIS → [Ca$^{2+}$]i → Mitochondrial metabolism → ATP → Cell survival
Amplication mechanisms of GLP-1 on GSIS

Holz GG, 2004, Diabetes
GLP-1 also potentiates glucokinase activity

β-cells
Motor neuron → ATP generation

Not
Hepatocytes → Glycogen synthesis
GLP-1 potentiates glucokinase activity in $\beta$-cell & neurons
Effect of GLP-1, exendin-4, and exendin-9 on 2-deoxy-[³H]-glucose uptake and cellular ATP levels
Effect of GLP-1 on glucose-stimulated inhibition of $K_{ATP}$ current, increase of $[Ca^{2+}]_c$ and insulin secretion.
Effect of GLP-1 on GLUT2 and GK expression

A

Total treatment time of GLP-1 (min)

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<tr>
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<td>10 mM GlcN</td>
<td>+</td>
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B

Total treatment time of GLP-1 (min)

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Effect of GLP-1 on GK activity

A

![Graph showing the effect of 2-deoxyglucose on GK activity. The x-axis represents the concentration of 2-deoxyglucose (mM) from 2 to 20. The y-axis represents GK activity (% of 15 mM 2DG control). Two conditions are shown: Control and 100 nM GLP-1. The graph shows a significant increase in GK activity with higher concentrations of 2-deoxyglucose.](image)

B

![Bar graph showing the effect of 10 mM GlcN and 100 nM GLP-1 on GK activity at 15 mM 2DG. The x-axis represents different treatments: 10 mM GlcN and 100 nM GLP-1. The y-axis represents GK activity (% of control). The graph shows a significant decrease in GK activity with the combination of 10 mM GlcN and 100 nM GLP-1.](image)
Involvement of cAMP and Epac in the restorative effects of GLP-1 on 2-deoxyglucose uptake
Restorative effect of GLP-1 in Epac2-knockdown INS-1 cells

A

2-Deoxyglucose uptake (% of control)

120
100
80
60
40
20
0

10 mM GlcN
100nM GLP-1

+ + + + + +

Negative control Epac 2 siRNA

B

GK activity at 15 mM 2DG

120
100
80
60
40
20
0

10 mM GlcN
100nM GLP-1

+ + + + + +

Negative control Epac2 siRNA

Epac2 siRNA

Epac2
Actin

siRNA (nM) C 10 50
Restorative effect of GLP-1 in Rim2- or Rab3A-knockdown INS-1 cells

A

B

2-Deoxyglucose uptake

(% of control)

120
100
80
60
40
20
0

10 mM GlcN + + + + + + 8-pCPT-2'-O-Me-cAMP-AM + + + + + +

Rim2 siRNA Rim2 siRNA

Rab3A siRNA Rab3A siRNA

120
100
80
60
40
20
0

10 mM GlcN + + + + + + 8-pCPT-2'-O-Me-cAMP-AM + + + + + +

GK activity at 15 mM 2DG
(% of control)

SiRNA (nM) C 30 50 C 30 50

Rim2 siRNA Rab3A siRNA

Rim2 Actin

Rab3A Actin

** **

*** ***
Proposed mechanism to explain the effects of GLP-1 on GK activity
**Motor neuron cell line**

Effect of GLP-1 on 2-deoxy-[^3]H]-glucose uptake, glucokinase (GK) activity and intracellular ATP levels against glucosamine (GlcN)

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<th>GLP-1 (100 nM)</th>
<th>MP (1 mM)</th>
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<td>GLP-1 (100 nM)</td>
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<td>Glucose uptake (%)</td>
<td>100.0 ± 2.3</td>
<td>103.6 ± 3.5</td>
<td>63.4 ± 2.6***</td>
<td>85.2 ± 2.9***</td>
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<td>GK activity (%)</td>
<td>100.0 ± 4.0</td>
<td>104.0 ± 2.8</td>
<td>76.3 ± 6.4**</td>
<td>103.7 ± 4.0**</td>
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<td>Cellular ATP level (%)</td>
<td>100.0 ± 2.5</td>
<td>103.3 ± 4.2</td>
<td>78.6 ± 1.6***</td>
<td>92.0 ± 2.7#</td>
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*Neurosci Lett, 2010*
**muscle and adipocytes**

- **β-cell GLP-1R?**
- Unknown signaling mechanism
- Long-term Tx → GLUT2 expression, glycogen synthesis enzyme

**hepatocytes**

- **β-cell GLP-1R?**
- Unknown signaling mechanism
- Long-term Tx → GLUT2 expression, glycogen synthesis enzyme

GLP-1 or its analogues effects → long-term effect only via non-GLP-1R
Greater glucose-metabolism dependency of β-cells & neurons on action

Function as glucose sensor:
- Glucose-stimulated insulin secretion
- Insulin biosynthesis
- Cell survival and proliferation

Function by glucose:
- Neurotransmitter release
- Action potential
- Cell survival and proliferation

Exocytosis of secretory granules → GTP, ATP
Protein or neurotransmitter synthesis → ATP

Glucose-stimulated insulin secretion
Insulin biosynthesis
Cell survival and proliferation

Neurotransmitter release
Action potential
Cell survival and proliferation

Exocytosis of secretory granules
Protein or neurotransmitter synthesis

Glucose-6-p

Glucose

Mitochondrial metabolism
**Summary**

- **Diabetic Beta cell**
  - Via PKA & Epac: Ca\(^{2+}\) → ATP → *Function & survival*
  - Via Epac: GK activity → ATP

- **Energy-deficient Neuron**
  - Via Epac: GK activity

- **Insulin**
  - Glucose uptake in muscle and adipocytes
  - Glucagon in α-cell
  - Glycogen synthesis in hepatocytes
  - *prevent from hyperglycemia*

- **GLP-1**
  - GLP-1R
  - GLP-1 → *Function & survival*
  - ATP

- **GLP-1R**
  - *Function & survival*
Conclusions with our observations and previous reports

● GLP-1 may **insulin-independently potentiate glucose sensitivity** in diabetic β-cells and neurons.

● Effect of GLP-1 on the **other insulin-sensitive tissues** may be due to ameliorated I/G ratio by GLP-1.
감사합니다