Glucose Stimulates Glucagon Release in Single Rat α-Cells by Mechanisms that Mirror the Stimulus-Secretion Coupling in β-Cells

Hervør Lykke Olsen, Sten Theander, Kristen Bokvist, Karsten Buschard, Claes B. Wollheim and Jesper Gromada

Lilly Research Laboratories (H.L.O., K.Bo., J.G.), Hamburg, Germany; Bartholin Instituttet (K.B.), Rigshospitalet, Copenhagen, Denmark; and Department of Cell Physiology and Metabolism (S.T., C.B.W.), University Medical Center, Geneva 4, Switzerland

Endocrinology 146(11):4861-4870, 2006

Introduction

Homeostasis of blood glucose is maintained by hormone secretion from the pancreatic islets of Langerhans

Mechanism of insulin secretion in β-cell

Mechanism of glucagon secretion in α-cell

The ability of high glucose concentrations to suppress glucagon release

paracrine effects exerted by factors released by neighboring β-cells and α-cells

• Candidate paracrine inhibitors of glucagon secretion include insulin (Greenbaum et al 1991, Ishihara et al 2003 & Franklin et al 2005), Zn²⁺, which is cosecreted with insulin, and γ-amino butyric acid (GABA) (Rorsman et al 1989 & Braun et al 2004–11) as well as somatostatin (Gromada et al 2001 & Cejvan et al 2003)
Pancreatic \( \alpha \)-cells are electrically excitable and generate spontaneous \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) dependent action potentials (Barg et al. 2000, Gromada et al. 1997, Yoshimoto et al. 1999).

Pancreatic \( \alpha \)-cells are equipped with ATP-sensitive \( \text{K}^+ \) channels (\( \text{K}_{\text{ATP}} \) channels) of the same as \( \beta \)-cells (Bokvist et al. 1999, Ronner et al. 1993, Gromada et al. 2004).

Different complement of voltage-gated ion channels (Göpel et al. 2000, Gromada et al. 2004).

**Objective**

To study the effects of glucose on glucagon secretion from isolated pancreatic \( \alpha \)-cells and the mechanisms of those actions of glucose.

**Methods & Results**

**Preparation of islet & isolated \( \alpha \)-cells**

- Male Sprague-Dawley rats
- Pancreas
- Collagenase digestion
- Isolate islets
- Dispase
- Single cells
- Hormone secretion assay

Fluorescent-activated cell sorting (FACS)

- > 90% \( \alpha \)-cell & < 3% \( \beta \)-cell
- Commercial assay
- Hormone secretion

**Statistical analysis**

- Results are presented as mean ± S.E.
- Statistical significances were evaluated using Student’s \( t \) test for pair of data
- Dunnett’s test for multiple comparison with a control
- Tukey’s test when multiple comparisons between groups
Effects of glucose on glucagon secretion from isolated rat α-cells

Glucagon secretion in batches of isolated α-cells exposed for 30 min to KRBH buffer in the absence or presence of glucose concentrations (0–16.8 mM).

Glucose secretion in batches of isolated α-cells exposed for 30 min to KRBH buffer in the absence or presence of glucose concentrations (0–16.8 mM).

* p<0.05
** p<0.01

\[ ^{[125I]} \text{cAMP} \] cAMP antibodies

Isolated rat α-cell

\[ ^{[125I]} \text{cAMP} \] + cAMP antibodies + SPA beads

cAMP measurement

counted in a γ-scintillation counter

TABLE 1. Effects of glucose and epinephrine on cellular cAMP content in FACS-isolated rat α-cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>cAMP (pmol/cell)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM G</td>
<td>9.7 ± 2.5</td>
<td>6</td>
</tr>
<tr>
<td>0 mM G + epinephrine</td>
<td>45.8 ± 4.5</td>
<td>5</td>
</tr>
<tr>
<td>16.8 mM G</td>
<td>10.2 ± 5.1</td>
<td>6</td>
</tr>
<tr>
<td>16.8 mM G + epinephrine</td>
<td>61.3 ± 4.3</td>
<td>5</td>
</tr>
</tbody>
</table>

* p < 0.01 against control at same glucose level

Glucagon secretion from batches of isolated α-cells in the absence or presence of 16.8 mM glucose

G = Glucose
Dia = Diazoxide: opener of KATP channels
Azide = Sodium azide: inhibitor of mitochondrial cytochrome c oxidase
Man = Mannoheptulose: inhibition of glycolysis

* p<0.05
** p<0.01
Glucagon secretion from batches of isolated \( \alpha \)-cells in the absence or presence of 16.8 mM glucose

\[ G = \text{Glucose} \]

Tolb = Sulfonylurea tolbutamide : closure of \( K_{ATP} \) channels

Arginine : depolarization of the \( \alpha \)-cell

Epi = Epinephrine : induce glucagon release

Glucagon secretion from batches of isolated \( \alpha \)-cells in the absence or presence of 16.8 mM glucose

\[ G = \text{Glucose} \]

Ins = Insulin

\[ n = 5 \]

* \( p < 0.05 \)

** \( p < 0.01 \)

Effects of glucose on whole-cell \( K_{ATP} \) channel activity in single \( \alpha \)-cells

Extracellular solution (mM)

- 138 NaCl
- 5.6 KCl
- 2.8 CaCl\(_2\)
- 1.2 MgCl\(_2\)
- 5 HEPES
- pH 7.4 with NaOH

Pipette solution (mM)

- 76 K\(_2\)SO\(_4\)
- 10 KCl
- 1 NaCl
- 1 MgCl\(_2\)
- 5 HEPES
- pH 7.35 with KOH
- 0.24 mg/ml amphotericin B

Effects of glucose on whole-cell \( K_{ATP} \)-channel activity in single \( \alpha \)-cells

Perforated-patch whole-cell configuration

For 0 mM Glucose (\( n = 6 \))

For 20 mM Glucose (\( n = 6 \))
Whole-cell $K_{\text{ATP}}$ conductance normalized to cell capacitance ($G/C$)

$$G/C = \frac{\text{conductance}}{\text{capacitance}}$$

$C = \text{capacitance} \rightarrow \text{surface area}$

** $p<0.01$

Tolb = Sulfonylurea tolbutamide: closure of $K_{\text{ATP}}$ channels

G = conductance

$n = 6$

Effects of sodium azide on single $K_{\text{ATP}}$ channels in cell-attached recordings

** Effects of sodium azide on single $K_{\text{ATP}}$-channel activity in single rat $\alpha$-cells

2 mM NaN$_3$ (inhibitor of mitochondrial cytochrome c oxidase)

200 μM Tolbutamide (closure of $K_{\text{ATP}}$ channels)

KATP channel in $\beta$-cell = KATP channel in $\alpha$-cell

Effects of glucose on $[Ca^{2+}]_i$ in $\alpha$-cells

Effects of glucose on $[Ca^{2+}]_i$ in $\alpha$-cells
Measurements of $[\text{Ca}^{2+}]_i$

**Ratiometric measurement**

$340 \, \text{nm}$

**Excitation** $380 \, \text{nm}$

Fura-2

340 nm 380 nm

Emission at 340 nm

Emission at 380 nm

Calcium

$[\text{Ca}^{2+}]_i$

Compared with calibration that known free $\text{Ca}^{2+}$ concentration

**[Ca$^{2+}$], measured in a single rat $\alpha$-cell**

A. (n = 9)

20 mM Glucose

$[\text{Ca}^{2+}]_i$

increased $[\text{Ca}^{2+}]_i$

ATP

stimulate $\text{Ca}^{2+}$ pump

( Arkhammar 1987 & Merglen 2004)

transient hyperpolarization

membrane hyperpolarization

B. (n = 14)

20 mM Glucose

+10 mM Mannoheptulose

$[\text{Ca}^{2+}]_i$

$\text{K}_{\text{ATP}}$ channel open

Tolb = Sulfonylurea tolbutamide: closure of $\text{K}_{\text{ATP}}$ channel
Effects of ion channel modulators on glucose-induced glucagon secretion in isolated rat α-cells

**TABLE 2. Effects of K⁺, Ca²⁺, and Na⁺-channel modulators on glucagon secretion from FACS-isolated α-cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Glucagon release (pg/1000 cells.h)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM G</td>
<td>31±5</td>
<td>6</td>
</tr>
<tr>
<td>0 mM G + CuCl₂ (block R-type Ca²⁺ channel)</td>
<td>19±4*</td>
<td>5</td>
</tr>
<tr>
<td>0 mM G + α-conotoxin (block N-type Ca²⁺ channel)</td>
<td>23±5*</td>
<td>5</td>
</tr>
<tr>
<td>0 mM G + nifedipine (block L-type Ca²⁺ channel)</td>
<td>33±3*</td>
<td>6</td>
</tr>
<tr>
<td>0 mM G + SNX402 (block R-type Ca²⁺ channel)</td>
<td>32±5*</td>
<td>5</td>
</tr>
<tr>
<td>16.8 mM G</td>
<td>58±1*</td>
<td>6</td>
</tr>
<tr>
<td>16.8 mM G + CuCl₂</td>
<td>20±4*</td>
<td>5</td>
</tr>
<tr>
<td>16.8 mM G + α-conotoxin</td>
<td>29±5*</td>
<td>5</td>
</tr>
<tr>
<td>16.8 mM G + nifedipine</td>
<td>53±6*</td>
<td>6</td>
</tr>
<tr>
<td>16.8 mM G + SNX402</td>
<td>58±7*</td>
<td>5</td>
</tr>
</tbody>
</table>

* p < 0.05 against control at 0 mM glucose
** p < 0.01 against control at 0 mM glucose
*** p < 0.01 against control at 16.8 mM glucose

**Set of ion channel in α-cell**

- Voltage-gated Na⁺ channel
- Delayed rectifier K⁺ channel
- N-type Ca²⁺ channel

**Effects of thapsigargin on glucose-induced glucagon secretion**

In previous study
(Liu et al 2004)

store-operated Ca²⁺ channel

plays a pivotal role in the regulation of glucagon secretion
**TABLE 3. Effects of thapsigargin on glucagon secretion from FACS-isolated α-cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Glucagon release (pg/1000 cells/h)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM Glucose + 0 μM Thapsigargin</td>
<td>31 ± 6</td>
<td>6</td>
</tr>
<tr>
<td>0 mM Glucose + 5 μM Thapsigargin</td>
<td>33 ± 4</td>
<td>5</td>
</tr>
<tr>
<td>16.8 mM Glucose + 0 μM Thapsigargin</td>
<td>52 ± 6*</td>
<td>6</td>
</tr>
<tr>
<td>16.8 mM Glucose + 5 μM Thapsigargin</td>
<td>54 ± 4*</td>
<td>5</td>
</tr>
</tbody>
</table>

*P < 0.05 against 0 mM glucose

**In previous study**
(Gromada et al 2004)

**Effects of K⁺ on glucagon release**

Extracellular K⁺ concentration to 15 mM → inhibition of glucagon release

Extracellular K⁺ concentration > 25 mM → stimulation of glucagon release
Effects of K⁺ on glucagon release in single rat α-cells

n = 5

* p<0.05
** p<0.01

Effects of ω-conotoxin and nifedipine on high K⁺-induced glucagon release in single rat α-cells

n = 5

* p<0.05
** p<0.01

Effects of glucose on glucagon release from intact islets

n = 10

* p<0.05
** p<0.01

Effects of glucose on glucagon secretion from clamped intact rat islets

n = 10

* p<0.05
** p<0.01

Experimental B
**Effects of glucose on insulin secretion from clamped intact rat islets**

**A**

- Glucose → ATP → Insulin 
- ATP 
- K<sub>ATP</sub> channel 

**B**

- 30 mM K+ 
- 4.7 mM K+ 

* p<0.05 
** p<0.01 n = 10

**Effects of glucose on Ca<sup>2+</sup>-dependent exocytosis**

- Exocytosis was monitored in single α-cells as changes in cell capacitance
Effects of glucose on Ca\textsuperscript{2+}-induced exocytosis in single rat \( \alpha \)-cells

\begin{align*}
\text{Pipette solution (mM)} & \quad 76 \text{Cs}_2\text{SO}_4 \\
& \quad 10 \text{KCl} \\
& \quad 10 \text{NaCl} \\
& \quad 1 \text{MgCl}_2 \\
& \quad 5 \text{HEPES} \\
\text{pH} & \quad 7.35 \text{ with KOH} \\
& \quad 0.24 \text{mg/ml amphotericin B}
\end{align*}

Extracellular solution

\begin{align*}
\text{118 NaCl} & \\
& \quad 20 \text{tetracyclammonium (TEA)-Cl} \\
& \quad 2.0 \text{KCl} \\
& \quad 1.2 \text{MgCl}_2 \\
& \quad 2.6 \text{CaCl}_2 \\
& \quad 5 \text{HEPES} \\
\text{pH} & \quad 7.40 \text{ with NaOH}
\end{align*}

\( n = 5 \) * \( p < 0.05 \)

Effects of glucose & adenine nucleotides on exocytosis evoked by trains of depolarizations

Glucagon release

\begin{align*}
\text{Glucose} & \quad \text{ATP} \\
\text{K\textsubscript{ATP} channel} & \\
\text{[Ca\textsuperscript{2+}]} & \quad \text{Ca\textsuperscript{2+} channel}
\end{align*}

\begin{align*}
\text{Glucose} & \quad \text{ATP} \\
\text{K\textsubscript{ATP} channel} & \\
\text{glycolysis} & \quad \text{ATP}
\end{align*}

Effects of glucose & adenine nucleotides on exocytosis evoked by trains of depolarizations
Readily releasable pool (RRP)

Reserve pool

Readily releasable pool (RRP)

Standard whole-cell configuration

Pipette solution (mM)
- 125 Cs-glutamate
- 10 CsCl
- 10 NaCl
- 1 MgCl₂
- 5 CaCl₂
- 5 HEPES
- 0.05 EGTA
- 0.01 GTP & MgATP
- pH 7.15 using CsOH

Extracellular solution
- 118 NaCl
- 20 tetraethylammonium (TEA)-Cl
- 5.6 KCl
- 1.2 MgCl₂
- 2.6 CaCl₂
- 5 HEPES
- pH 7.40 with NaOH

Effects of glucose on exocytosis evoked by a train of depolarizations in isolated α-cells

A \( n = 6 \)
- \( V_m \) (mV)
- \( \Delta C_m \) (200 pF)

B
- \( \Delta C_m \) (200 pF)

Isolated α-cell

Glucose Glucagon secretion vesicle in readily releasable pool (RRP)

Glucose

Glucagon release

Effects of adenine nucleotides on exocytosis evoked by a train of depolarizations in single rat α-cells

A
- \( V_m \) (mV)
- \( \Delta C_m \) (200 pF)

B
- \( V_m \) (mV)
- \( \Delta C_m \) (200 pF)
Glucose stimulates glucagon release by mechanism that mirrors of the β-cell.

Discussion
**Isolated rat α-cell & single rat α-cell**

- Glucose stimulates glucagon release in isolated α-cells.
- ATP closure KATP channel.
- Increased RRP.

**Intact rat islets**

- Glucose suppresses glucagon release.
- Paracrine effect.

**Conclusion**

- Glucose stimulates glucagon secretion in isolated α-cells mirrors that of the β-cell.
- Glucose also increases secretion of glucagon by mean of increasing RRP via ATP action.

**Intact rat islets**

- Insulin, Zn²⁺ & GABA.

**Conclusion**

- Islet paracrine signaling is the primary regulatory mechanism governing glucagon secretion in intact islets.
- Loss of β-cell function.
- Reduced paracrine signaling.
- Hyperactivity of α-cells.
- Hyperglucagonemia.

**Conclusion**

- ATP closure KATP channel.
- Increased RRP.