Cannabinoid 1 Receptors in Pancreatic β Cells: Function and Therapeutic Implication

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Endogenous Cannabinoid System (ECS)

- Endogenous Cannabinoids (ECs)

  - Lipid transmitters
  - Synthesized only ‘on demand’ by depolarization in brain and islet of Langerhans
  - Synthesized in liver, muscle and adipose tissue
  - Cannabinoid receptors (CB1R and CB2R)
Endogenous Cannabinoid System (ECS)

- **Cannabinoid 1 Receptor (CB1R)**
  - Inhibitory G protein (G\(\alpha\)i)-coupled receptor: [cAMP] ↓, [Ca\(^2+\)] ↓
  - Cannabinoid receptors (CB1R and CB2R)  (Amino acid sequence similarity: about 44%)
  - Several putative cannabinoid receptors have been put forward
## Impact of CB1R Agonism

<table>
<thead>
<tr>
<th>Site of Action</th>
<th>Mechanism(s)</th>
<th>Clinical Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain (Hypothalamus)</td>
<td>➕ Food intake</td>
<td>Body weight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abdominal obesity</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>➖ Adiponectin; ➕ Lipogenesis; ➖ Thermogenesis (BAT)</td>
<td>Dyslipidemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>Muscle</td>
<td>➖ Glucose uptake</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>Liver</td>
<td>➕ Lipogenesis; ➔ Fatty acid synthesis; ➔ Glucose production</td>
<td>Dyslipidemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>Pancreas (β cell)</td>
<td></td>
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</tbody>
</table>
Pancreatic β cells have a self-contained EC system

- CB1R
- CB2R

Kim et al., *Diabetes*, 2011
Pancreatic β cells have a self-contained EC system

- EC synthetic and degrading enzymes
  - Synthetic enzymes: NAPE-PLD and DAGLα
  - Degrading enzymes: FAAH and MAGL

- The capacity to generate ECs

Kim et al., Diabetes, 2011
Impact of CB1R Blockade on β-Cell Grow and Survival

Kim et al., *Diabetes*, 2011
Kim et al., *Science Signaling*, 2012
CB1R blockade improves blood glucose levels in streptozotocin-treated mice

**A**

Daily STZ injection

Daily AM251 injection for 3 weeks

Plasma collected

Euthanize: Pancreas, islets & plasma collected

*p < 0.05; **p < 0.01, n=5 per group

**B**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No STZ</td>
<td>100 ± 20</td>
</tr>
<tr>
<td>STZ only</td>
<td>500 ± 50</td>
</tr>
<tr>
<td>STZ DMSO</td>
<td>400 ± 40</td>
</tr>
<tr>
<td>STZ AM251</td>
<td>300 ± 30</td>
</tr>
</tbody>
</table>

**C**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Plasma insulin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No STZ</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>STZ DMSO</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>STZ AM251</td>
<td>1.5 ± 0.1</td>
</tr>
</tbody>
</table>

Kim et al., Science Signaling, 2012
CB1R blockade improves β-cell mass in STZ-treated mice

Kim et al., Science Signaling, 2012
CB1R blockade improves β-cell growth and survival in STZ-treated mice

Kim et al., *Science Signaling*, 2012
Increased β-cell mass due to enhanced β-cell proliferation in AM251-treated db/db mice

*db/db (Lepr−/−) mice

Kim et al., Diabetes, 2011
Schematic unifying the regulation of IR signaling by ECs upon cell growth and survival

Kim et al., Science Signaling, 2012
Impact of CB1R Blockade on β-Cell Function

Unpublished Data
Effects of CB1Rs on Ex-4-stimulated cAMP accumulation in MIN6 cells

**Scramble siRNA**

**CB1R siRNA**

ACEA (μM) 0 0 10

Ex-4 (25 nM) **n.s.**

---

**Scramble siRNA**

**CB1R siRNA**

cAMP (% of control) 0 100 200 300 400 500 600 700

**Scramble siRNA**

**CB1R siRNA**

ACEA (μM) 0 0 10

Ex-4 (25 nM) **n.s.**
Effects of CB1Rs on Ex-4-stimulated Insulin Secretion

- **ACEA (μM)**
  - 0
  - Ex-4 (25 nM)
  - KCl (30 mM)

- **Insulin secretion (% of control)**
  - 0
  - 10
  - 20

- **Blood glucose (mg/dl)**
  - 0
  - 10
  - 20

- **Plasma insulin (ng/ml)**
  - 0
  - 10

- **Time (min)**
  - 0
  - 10
  - 20
  - 30
  - 40
  - 50
  - 60
  - 70
  - 80
  - 90
  - 100

(GLP-1 (1.5 pmol/kg/min) SC infusion)

Glucose (1 g/kg) IP

Collect plasma samples
Effects of CB1Rs on Glucose-stimulated Insulin Secretion

[Human islet]

**ACEA**

[Normal]

Glucose 4 mM 15 mM

Insulin secretion (% of control)

ACEA 2-AG

[T2DM]

**DMSO** AM251 DMSO AM251

Ex-4

Insulin secretion (% of control)

Effects of CB1Rs on Glucose-stimulated Insulin Secretion

Blood glucose (mg/dl)

Plasma insulin (ng/ml)

CB1R+/+ CB1R-/-

Time (min)

0 10 20 30 40 50 60 70

0 50 100 150 200 250 300 350

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0

0 10 20 30 40 50 60 70
Effects of CB1Rs on Gene Expression

<table>
<thead>
<tr>
<th>Islet</th>
<th>CB1R</th>
<th>p-IR</th>
<th>IRβ</th>
<th>p-IRS1/2</th>
<th>IRS2</th>
<th>p-AKT</th>
<th>AKT</th>
<th>GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-/-</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Intra-islet insulin (ng/islet)

Relative mRNA level (fold of control)

- Ins1
- Ins2

Whole pancreas

<table>
<thead>
<tr>
<th>Preproinsulin</th>
<th>CB1R+/+</th>
<th>CB1R-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td></td>
</tr>
</tbody>
</table>

GCK
GLUT2
β-actin

CB1R+/+
CB1R-/-

Insulin
GCK
GLUT2
Peripherally-Restricted CB1R Antagonists for the Treatment of T2DM

Unpublished Data
Impact of CB1R Antagonism

CB1R Antagonist

[Brain]

Appetite ↓, Hunger ↓

Decreased food intake and body weight

[Peripheral tissue]

Improved glucose homeostasis and insulin action

“How to minimize CNS psychiatric side effects”

“Anti-obesity drug”
Peripherally-Restricted CB1R Antagonist

The second generation/peripherally restricted CB1R antagonist (TM38837, etc.)

Central nervous system
- Hippocampus, hippocampus, Cerebral cortex, etc.

Peripheral tissues
- Lipid mobilization
- TAG storage
- Glucose production
- Glucose uptake
- Improve secretion of adipikines
- Anti-obesity
- Ameliorating insulin intolerance
- Improving dyslipidemia
- Modulating islet hormone (insulin/glucagon) secretion

Sustained pharmacologic effects (Intolerance)

Appetite and food intake: ↓
Body weight: ↓
Tachyphylaxis: within 1~2 weeks
Neuropsychiatric disorders (Anxiety, depression, suicide, etc.): ↓
Neuropsychiatric side effects: 

Peripheral tissues

BBB

Sustained pharmacologic effects (Intolerance)
Peripherally-Acting CB1R inverse agonists being developed

- TM-38837: Inverse agonist by 7TM Pharma.
  **Phase I clinical trial**

- JD2114, JD5006 and JD5037: Inverse agonist by Jenrin Discovery Inc.
  **Preclin**

- Compound 11: Inverse agonist by Sanofi-aventis
**In vitro Pharmacological Profile**

- CB1R IC₅₀ 0.3 nM
- > 1000x selectivity vs. CB2R
- Low brain presence potential
- Acceptable metabolic stability

Tam J et al., *Cell Metabolism*, 2012
**MATERIALS & METHODS**

- 7-week-old db/db mice were injected daily with either DMSO, JD5037 (1 mg/kg), or Rimonabant (1mg/kg) for two weeks.

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**Graphs**

- **Fasting Blood Glucose (mg/dL)**
  - Vehicle
  - Rimonabant
  - JD-5037

- **Total Islet Area (pixels x 10^3)**
  - Vehicle
  - Rimonabant
  - JD-5037

- **Insulin Intensity (arbitrary units x 10^5)**
  - Vehicle
  - Rimonabant
  - JD-5037

- **Glucagon Intensity (arbitrary units x 10^4)**
  - Vehicle
  - Rimonabant
  - JD-5037

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**Images**

- **Immunofluorescence Microscopy**
  - Insulin
  - Glucagon
  - TO-PRO-3
  - Merge

- **Western Blot**
  - JD-5037 (nM)
    - Glucose (mM)
    - p-IRβ
    - Total IRβ
    - p-AKT
    - Total AKT
    - β-actin
JD-5037 on Glucose-Stimulated Insulin Secretion
Peripheral CB1R Isoforms
as Targets for the Treatment of T2DM

Unpublished Data
Peripheral CB1R Isoforms

[hCB1R vs. mCB1]

[hCB1R isoforms]
Summary

* Peripherally-Restricted CB1R Antagonists that target only peripheral CB1R isoform, CB1b???
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노준기

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