Optogenetics: A Bright Future for Voltage Gated Ion Channels

Viviana Agus
Axxam S.p.A., Milano, Italy
Presentation Summary

- **Hamamatsu and AXXAM:** FDSS µCELL demo period

**Cav1.3 and ChR2 assay**

- **Optogenetics:** overview and advantages
- **Channelrhodopsin2** to modulate cell membrane voltage
- **Activation of Cav1.3 by ChR2:** recombinant assay setup
- Validation of “light protocol” at FDSS µCELL: test of reference compounds
- **Comparison with “K+ protocol” and patch-clamp** data
- **Conclusions** and future perspectives
FDSS μCELL DEMO @ AXXAM

**Ca^{2+}** assay (fluorescent dyes, luminescent photoprotein)
- CCKAR (GPCR)
- GLP1R (GPCR)
- ADORA1 (GPCR)
- DRD1-DRD2 (GPCR)
- Enzymatic assay

**Glow Luminescence assay:**
- PPARα, PPARδ (NHR)
- Promoter assay

**Genetically encoded sensor**
- TMEM16A (EYFP)

**Optogenetics**
- Cav1.3 (ChR2)

**Fluo8 LED (blue light)**
**MPdye LED (green light)**

**Hybrid Camera**
(Fluo & Lumi)
Optogenetics: overview

Optogenetics is a technology that combines:
1) A «genetic» component, able to target specific neuron types
2) An «optical» component, able to interact specifically with the genetic component to achieve fast control of well-defined events in specific cells of living tissue.

The starting point was the idea to have a system available to control the activity of specific neuron types in the brain in a better way.
Rhodopsins: Light-gated ion channels

**Opsins:**
- Seven-transmembrane, light-responsive proteins
- Rapidly translocate specific ions across the membranes of the cells in which they are expressed
- Contain the Vitamin-A derived chromophore all-trans-retinal as a light capture molecule
- Studied since the 1970s for their fascinating biophysical properties
- Used by several different life forms that use light as energy source or sensory cue

Structural simplicity, fast kinetics ► attractive tool for a rapid control of specific cellular processes, such as, for example, modulation of membrane voltage and neuronal action potentials propagation

Light as activating stimulus ► more physiological, compared to other hyperpolarizing or depolarizing stimulus (for example $K^+$ injection)

Possibility to target their expression to specific cell types ► genetically defined modulation of cellular processes
• **Seven transmembrane opsin** (eyespot of unicellular alga *Chlamydomonas reinhardtii*)

• **Activated by blue light (470 nm)**: the light causes a **conformational change** in the light sensitive molecule (retinal), which in turn causes a conformational change and the opening of the channelrhodopsin protein

• **Non-selective cation channel** (*Na*, *, K*, *, Ca*, *, H*): the **flow of ions** changes the electrical potential across the cell membrane which might, if sufficiently large, cause the neuron to fire

• **Widely used to depolarize neurons** and generate action potential firing: very good expression in different cell hosts
Optogenetic control of Cav1.3

- L-type calcium channel
- High Voltage Activated (HVA)
- α1 (pore) + α2δ, β, γ (accessory) subunits
- Therapeutic target: Cardiovascular, hormone secretion, CNS (Parkinson’s, Alzheimer’s disease)
- Drug need: Cav1.2 selectivity; state-dependent
Cav1.3 optogenetic assay

Human KIR2.3

ChR2 (D156A)

Human Cav1.3 (α1,β3,α2δ1)

HEK-293 cells

Resting

Depolarized

Fluo-8,NW
Channelrhodopsin-2 and cell based assays

**KEY QUESTIONS**

- Is it possible to adapt the assay to the FDSS µCELL optics for use in HTS?
- Can ChR2 be used to depolarize cells, such as HEK293, avoiding the artificial depolarization protocols such as KCl injection?
- Does the exposure of the cells to blue light of adequate intensity induce a ChR2 dependent cellular depolarization with subsequent activation of the transfected target?
- Does the ion flux through ChR2 alter the detection of the transfected target?

**POTENTIAL ISSUES**

- The light produced by the instrument LED system might not have the adequate intensity for ChR2 activation.
- The ion flux through the ChR2 might be not sufficient to induce membrane depolarization.
- The membrane depolarization induced might be not sufficient to drive the activation of transfected voltage gated channels.
- ChR2 is not permeable to Ca\(^{2+}\) in the presence of extracellular Na\(^{+}\); therefore Cav channels are ideal targets to be modulated with optogenetics, since their activity can be monitored by the use of a Ca\(^{2+}\) sensitive dye.

**GOAL**

Generate stable cell lines co-expressing a Voltage Gated ion channel of interest and ChR2 without altering the ion channel pharmacology.
ChR2 induced membrane depolarization

- FDSS µCELL LED efficiently activates ChR2\textsubscript{D156A}

(minimum light intensity required for wild-type ChR2 activation: 1mW/mm\textsuperscript{2}; Aravanis, 2007)

Blue light (\(\lambda = 480\) nm; 0.013 mW/mm\textsuperscript{2})

- Membrane depolarization half-recovered after \(\approx 10\) min
Cav1.3 half-inactivation protocol

**LIGHT**

- Cav1.3 efficiently activated by ChR2
- 50% recovery from inactivation after 10 min

**K+**

- Cav1.3 efficiently activated by K+
- 50% Cav1.3 inactivation in 16mM K+

**Ca++ influx through Cav1.3**

Time course of signal recovery after a first blue light pulse (Fluo 8 NW)

- 50% inactivated

**Fluo 8 dye**

- (4 mM K⁺; 2µM retinal)

**50% inactivated**

- Blue light
- 5 - 45 min

**INACTIVE**

**RESTING**

**Ca++ influx through Cav1.3**

- Different K⁺ concentration (Fluo 8 NW)

- 50% inactivated

**[K⁺] m M**

- 0mM - 75 mM K⁺
State-dependent blockers with «Light protocol»

ISRADIPINE dose-response @ µCELL

RESTING STATE

HALF-INACTIVATED STATE

• Very nice Cav1.3 activation by ChR2
• State dependency well detected by Light inactivation protocol

Read interval 0.1s
Exp.: 0.03s; Sens.: 3
Light protocol vs. K⁺ protocol vs. qPatch

- “Light protocol” well suitable for state-dependent blockers studies
- Good correlation with classical “K⁺ protocol” (less physiological)
- Good correlation with patch-clamp

**Cav1.3 state-dependent blocker pharmacology**

Light vs. K⁺ protocol

- Residing (dark): IC₅₀ 137nM
- Residing (4mM K⁺): IC₅₀ 95nM
- Half-inact (Blue light ➔ 10 min): IC₅₀ 12nM
- Half-inact (16mM K⁺): IC₅₀ 14nM

Cav1.3/ChR2 @ qPatch 16x

- Closed (HP = -90 mV): IC₅₀ 362 nM
- Half-inact (HP = -60 mV): IC₅₀ 32nM
Summary and conclusions

<table>
<thead>
<tr>
<th></th>
<th>Isradipine IC$_{50}$</th>
<th>“K$^+$ protocol”</th>
<th>“Light protocol”</th>
<th>qPatch 16x</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting</td>
<td>95 nM</td>
<td>137 nM</td>
<td>362 nM</td>
<td></td>
<td>300 nM (-90mV)</td>
</tr>
<tr>
<td>Half-inactivated</td>
<td>14 nM</td>
<td>12 nM</td>
<td>32 nM</td>
<td></td>
<td>30 nM (-50mV)</td>
</tr>
<tr>
<td>RATIO</td>
<td>6.8</td>
<td>11.4</td>
<td>11.3</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

**MAIN ACHIEVEMENTS:**
- FDSS µCELL optics is well suitable for ChR2 activation
- A “Light protocol” was set up at the FDSS µCELL to study the Cav1.3 channel either in resting or inactivated state
- The pharmacology of known state dependent blockers has been successfully validated, showing a good agreement with the classical “K$^+$ protocol”, patch clamp experiments and literature data

**HIGHLIGHTS:**
- First time ChR2 used for optical control of recombinant voltage-gated calcium channel assay
- Physiological, robust, precise activation of Cav1.3 channel

**FUTURE PERSPECTIVES:**
- Light modulation of other voltage-gated ion channel target is ongoing
Aknowledgments

**AXXAM:**
Alberto di Silvio cell line generation  
Sara Tremolada cell line validation  
Jean-Francois Rolland patch-clamp  
Katharina Montag clonings  
Loredana Redaelli cell biology head  
Lia Scarabottolo discovery services director  
Stefan Lohmer overall strategies

**Hamamatsu team:**
Jean Marc d'Angelo  
Annamaria Mauro  
Laura Confalonieri

Via Meucci 3  
20091, Bresso (Milan, Italy)  
phone + 39 02 210561  
fax + 39 02 2105602  
www.axxam.com