Optogenetics: A Bright Future for Voltage Gated Ion Channels

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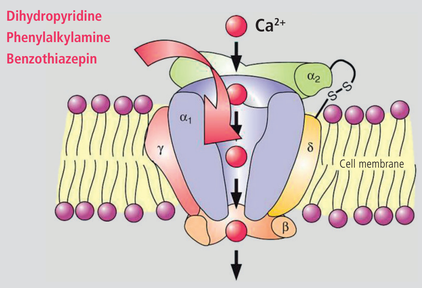
1. Introduction and scientific background

- Channelrhodopsin-2 (ChR2) is a light-activated microbial cation channel which can be used to depolarize neurons through the incidence of blue light (470 nm). The possibility to optically control the plasma membrane voltage opens new and interesting perspectives for the characterization of voltage-gated ion channels and the search for modulators.
- Proof of principle studies have been performed to verify the applicability of this tool for the development of cell based assays in High Throughput Screening (HTS) platforms as microplate readers.
- An HEK-293 cell line, which stably co-expresses the human voltage-gated calcium channel hCav1.3 and the inward rectifier hKir2.3 channel, was over-transfected with a ChR2 carrying a single amino acids mutation. This latter results in a prolonged lifetime of the conducting state of ChR2 and in a reduced light power requirement for its activation.
- A protocol of light stimulation of ChR2 and record of calcium ion influx through Cav1.3 with the use of a calcium-sensitive fluorescent dye (Fluo8) was tested in the Hamamatsu FDSSµcell. A well-known Cav1.3 blocker, Isradipine, tested in the resting and the partial inactivated Cav1.3 states, was used to confirm the pharmacological profile.
- Data obtained for the ChR2/hCav1.3 cell line by light stimulation have been also compared to the extracellular potassium stimulus and to patch-clamp to cross-check their reliability.

Key-questions of the study

- Is the blue light produced by the FDSSµcell LED intense enough to activate ChR2? → Can the light protocol be runned with the FDSSµcell?
- Is the activation of ChR2 sufficient to induce cell membrane depolarization? → Can the ChR2 be used to depolarize cells avoiding the irreversible depolarization protocols using KCl injection?
- Is the resulting membrane depolarization sufficient to activate the transfected voltage-gated channel Cav1.3?
- Can the cation flux through ChR2 disturbe the detction of Ca^{2+} flux through the transfected target Cav1.3?



2. Optogenetics: overview

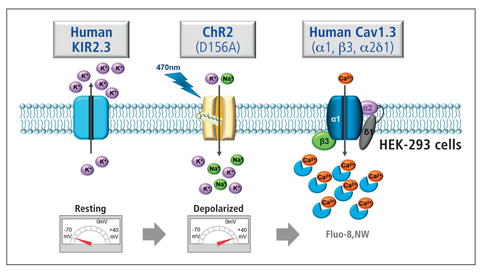
- Optogenetics is a technology that combines genetic and optical tools to achieve fast control of well-defined events in specific cells of living tissue.
- The genetic component is able to target specific neuron types, while the optical component is able to trigger an event. The optical component is also able to interact specifically with the genetic component.
- Key-players of optogenetics are light-sensitive proteins as opsins, seven-transmembrane proteins used by many different life forms that use light as energy source or sensory cue.
- Special type of opsin are the rhodopsins which are light-gated ion channels.
- Among rhodopsins, one of the most used to be an optogenetics actuator is the channelrhodopsin-2, a non selective cation channel (Na^+, K^+, Ca^{2+}, H^+) activated by blue light (470 nm).
- Optogenetis is a tool suitable to study ion channels activity and all the events related to this activity. Recordings can be made with the help of optogenetic sensors for calcium, chloride or membrane voltage.
- The advantages of optogenetics are: the fast kinetics (rapid control of specific cellular processes); a more physiological activating stimulus (compared to other hyperpolarizing or depolarizing stimulus as K^+ injection); the possibility to genetically define the target of their expression to specific cell types.

3. Material and methods

- Target: human Cav1.3 (α1/α2δ1/β3)
- Recipient cell line: HEK-293/kKir2.3
- Detection system: Ca^{2+} sensitive Fluo8 No Wash dye
- Stimulus: depolarization induced by ChR2 activation with blue light pulses
- Cav1.3 Blocker: Isradipine
- HTS instrumentation: Hamamatsu FDSSµcell

A stable HEK-293 cell line co-expressing the α1/α2δ1/β3 subunits of the hCav1.3 and the inward rectifier hKir2.3 channel was developed and then transfected with a ChR2 variant suitable to be activated by blue light. This allows the modulation of the Cav1.3 activity due to depolarization events induced by blue light pulses.

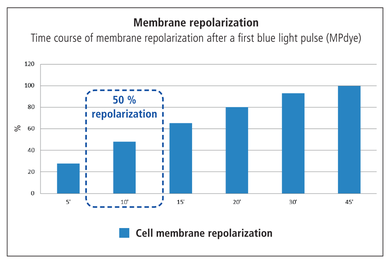
A protocol using blue light to modulate Cav1.3 activity was validated on the FDSSµcell to study the effect of the compound of interest on both the resting and the inactivated state of this calcium channel.



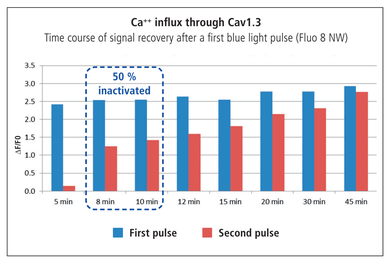
Time course of light-induced effects

The ChR2 activation evokes a cell membrane depolarization that in turns induces Cav1.3 activation/inactivation. The cell membrane depolarization and the recovery over time of the Cav1.3 from inactivation after exposure to blue light were measured.

% of cell membrane repolarization was assessed monitoring the fluorescence emitted by the Membrane Potential Dye at the indicated time points after the initial depolarization induced by blue light. Half-recovery from depolarization was achieved 10 min after the first blue light pulse.

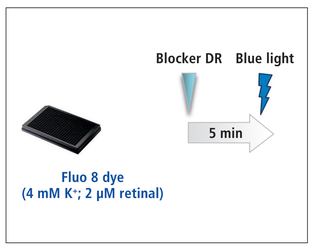


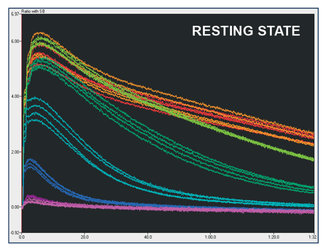
% of Cav1.3 recovery after a blue light pulse was assessed by detecting the Ca^{2+} influx (Fluo8 Ca^{2+}-sensitive dye) through repetitive light stimulation of the cells at different time points. Half-recovery from inactivation was achieved by re-pulsing the cells 8-10 min after the first blue light stimulation.

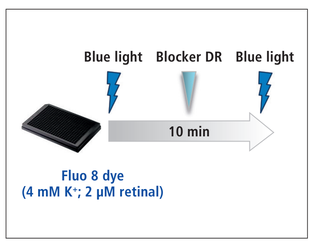


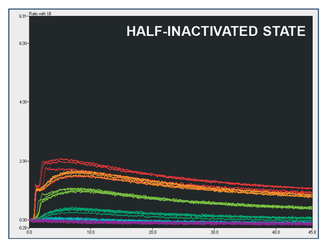
Validation of Light Protocol

Isradipine blocks (Cav1.3 blocker) dose-response was measured in both resting state and light half inactivated state. Fluo 8 dye was used to detect Ca^{2+} influx through Cav1.3 activated by the membrane depolarization induced by blue light activated ChR2.





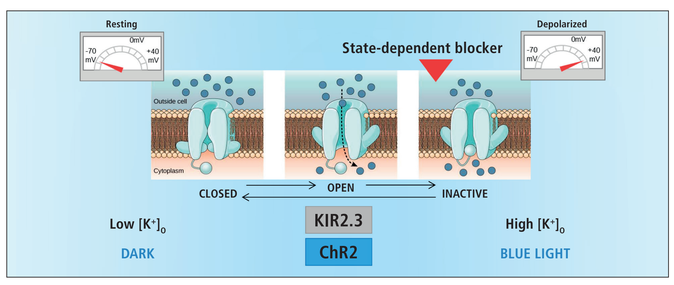




Comparison of Isradipine pharmacology in Light protocol, K^+ protocol and QPatch – 1

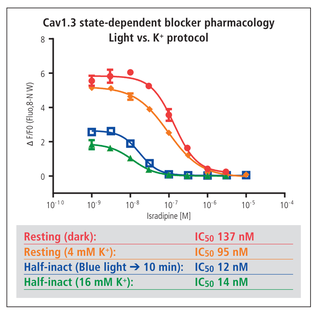
Pharmacology of Isradipine was tested using three different protocols to cross-check the reliability of data obtained with the "Light protocol".

While in the light protocol the target (Cav1.3) activation/half-inactivation was achieved with blue light stimulation of ChR2, in the K^+ protocol Cav1.3 activity was modulated by K^+ concentration. The injection of K^+ in the extracellular medium leads to K^+ influx through the inward rectifier KIR2.3 channel and depolarizes the cell membrane modulating Cav1.3 state.



In both protocols, Cav1.3 activity was assessed by detecting the Ca^{2+} influx with Fluo 8.

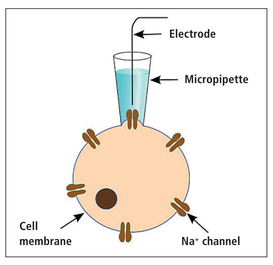
The two protocols gave comparable results in terms of IC_{50} parameters.



Comparison of Isradipine pharmacology in Light protocol, K^+ protocol and QPatch – 2

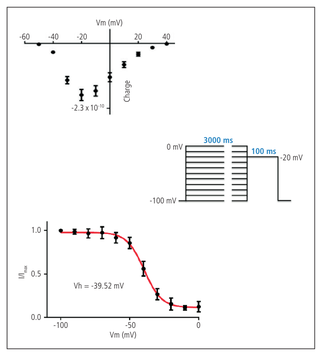
The **patch clamp** is the gold standard technique in Electrophysiology. It allows the study of single or multiple ion channels depending of the configuration used.

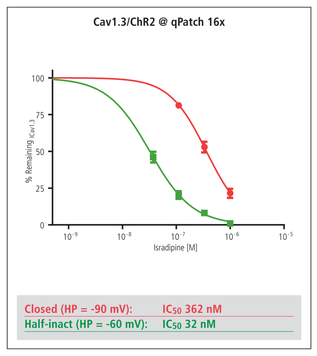
In the voltage-clamp mode/whole cell configuration, the amplifier controls the plasma membrane potential of the entire cell and measures the current resulting from the activity of the entire population of ion channels present in the cell.



The patch clamp experiments wer performed using the QPatch 16X, an automated patch clamp device from Sophion, Biolin Scientific.

An I/V protocol was used to construct both the I/V curve and the inactivation curve; in a another series of experiments, isradipine was tested at increasing concentrations clamping the plasma membrane at -90 or -60 mV. The concentrations / inhibition curves were fitted and the IC50s determined.





Comparison of Isradipine pharmacology in Light protocol, K^+ protocol and QPatch – 3

Good correlation of Light Protocol with K^+, qPatch protocol and literature data has been observed: calculated IC_{50} parameters in both resting and half-inactivated Cav1.3 state are comparable among the four different data source.

Isradipine IC_{50}	"K^+ protocol"	"Light protocol"	qPatch 16x	Literature
Resting	95 nM	137 nM	362 nM	300 nM (-90 mV)
Half-inactivated	14 nM	12 nM	32 nM	30 nM (-50 mV)
RATIO	**6.8**	**11.4**	**11.3**	**10**

4. Conclusions and future developments

- FDSSµcell optics is suitable for ChR2 activation (FDSSµcell blue LED light intensity effective to activate ChR2)
- The "light protocol" set up on the FDSSmcell allows to study the Cav1.3 channel in both its resting and partially inactivated states
- The pharmachology of Isradipine, a known state dependent Cav1.3 blocker, has been validated showing good agreement with the classical K^+ protocol, Patch clamp and literature data.
- This study shows for the first time the applicability of the ChR2 optogenetic tool to Hamamatsu FDSSµcell, to perform light-modulation of cell membrane voltage and voltage-gated ion channel activity.
- This tool could be useful to run screening on voltage-gated ion channels, in different channel states and in physiological extracellular potassium concentration.
- Future perspectives could be the light modulation of other voltage-gated ion channel.

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