Photonic control of TRPC3 via Opto-GSK in HEK293 cells

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I. INTRODUCTION

Optogenetics is a biophysical field focused on luminous regulation of specific compounds in genetically-engineered cells. The genetic modifications allow for expression of light-sensitive bio structures in living tissue and non invasive activation and inhibition of the target substances. Eventually a new branch, called optopharmacology, researching light-switchable compounds in drug design, arose.

Ion channels fulfill various essential tasks in cells, ranging from homeostatis to excitability and cell signaling ("Biophysic II Practical Course" 2019.) Human mutations of various Ca^{2+} proteins have been linked to diseases. These mutations impair specific components of the Ca^{2+}-machinery and consequently leads to the disruption of Ca^{2+} homeostasis (Bagur and Hajnóczky 2017). Therefore ion channels are a major interest for bio-medical research, where optogenetic methods allow for inoffensive studies on ion channel behavior. Excitable molecules which undergo confomational changes upon activation can modulate cellular processes in nature. Several substances like bacteriorhodopsin, halorhodopsin and channelrhodposin were found in nature, with the commonality of a rhodopsin domain. If associated with a specific ion channel engineered rhodopsin-hybrids can switch them from a closed state to a open state and vice versa.

This powerful tool is applied to TRPC3, a Ca^{2+} channel which is activated by the presence of diacylglycerol (receptor-operated). Opto-GSK, a photsosensitive compound associates with TRPC3 and induces a conformational change in the channel and thus can potentially regulate Ca^{2+} homeostasis in the cell. To visualize the flux of Ca^{2+} ions, a genetically encoded calcium indicators for optical imaging (R-GECO) was used ("Biophysic II Practical Course" 2019).

II. METHODS AND MATERIALS

A. MATERIALS

1) Tools:

- tweezers
- fluorescence microscope

2) Chemicals:

- R-GECO (Exc. 570nm, Em: 600nm)
- YFP-TRPC3
- dimethyl sulfoxide (DMSO)
- Opto-GSK (Act. 365nm Inhib. 430nm)
- HEK 293 cells (YFP-TRPC3 / R-GECO mutants)
- 2 mM Ca^{2+} extracellular solution
- 0 mM Ca^{2+} free extracellular solution

B. METHODS

In this experiment, the effect of the photoswitchable compound Opto- GSK TRPC3 was observed by chnges in the R- GECO fluorescence. Two measurements were carried out, one including OptoGSK as well as a control group containing DMSO. Human embryonic kidney cells (HEK) were transfected with YFP-TRPC3 and R-GECO. Opto-GSK acts as an activator for TRPC3 channels upon excitation 365nm and inactive upon excitation at 430nm. Thereby Ca^{2+} entry via TRPC3 is proposed to be enhanced upon stimulation with 365nm and reduced upon application of 430nm. The transfected HEK 293 cells were washed (Ca^{2+}-free extracellular solution) and placed into a cell chamber. After resuspension in Ca^{2+}-free buffer they were put on an oil immersion inverted microscope. Suitable cells that contained enough YFP (513 nm) and R-GECO (570 nm) for the measurement were identified (Control n = 2, Experiment n = 4). The cell sizes were mapped onto the microscope software. R-GECO emission was recorded at 570nm. After 1 minute of basal level (0mM Ca^{2+}) the solution was exchanged for a 2 mM Ca^{2+} solution and incubated for 2 minutes, followed by addition of DMSO respectively Opto-GSK (10 μM each). After 1 minute, Opto- GSK was activated with an excitation wavelength of 365 nm for 10 seconds. The recording was continued for 20 seconds before Opto-GSK was inactivated by an excitation wavelength of 430 nm for 30 seconds. The measurement was continued for 2 minutes. The activation of Opto-GSK was carried out 3 times, after the last activation no inactivation was executed.

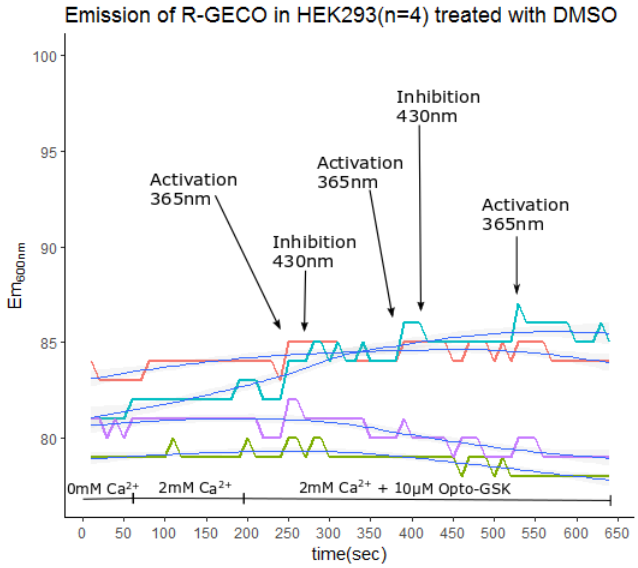
III. RESULTS

The first experiment was performed as a control and included four cells with varying basal emission rates. Upon excitation (365 nm) the cells showed no clear peak in signal, consequently no response after the stimulation with the inactivation wavelength of 430 nm (Fig. 1a).

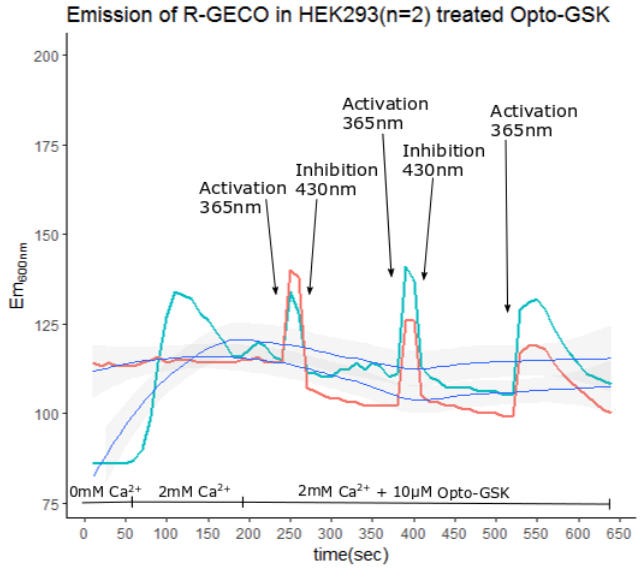
Em_{600nm}	c1	c2	c3	c4	c5	c6
Min	78.00	83.00	81.00	79.00	86.0	99.0
1st Qu.	79.00	84.00	82.00	80.00	107.0	102.8
Median	79.00	84.00	84.00	80.00	112.5	113.0
Mean	78.86	84.22	83.88	80.28	113.5	110.2
3rd Qu.	79.00	85.00	85.00	81.00	120.8	114.2
Max	80.00	85.00	87.00	82.00	141.0	140.0

TABLE I: Summary of the datapoints of the various cells (c1:c4 = control/ c5:c6 = treated).

The analysis of Opto-GSK was performed secondly, with the same parameters as in the first examination. There are clear peaks distinguishable upon excitation with 365 nm and



(a) R-GECO emission measured at 600 nm over a Ca^{2+} gradient and DMSO exposure. Four different cells were observed and plotted with the respective color: blue, green, red and purple.



(b) R-GECO emission measured at 600 nm in two cells (red, blue) over a Ca^{2+} gradient followed by Opto-GSK incubation.

Fig. 1: Control and treated R-GECO emission displays differences in the measured Ca^{2+} quantity of transfected HEK293 cells.

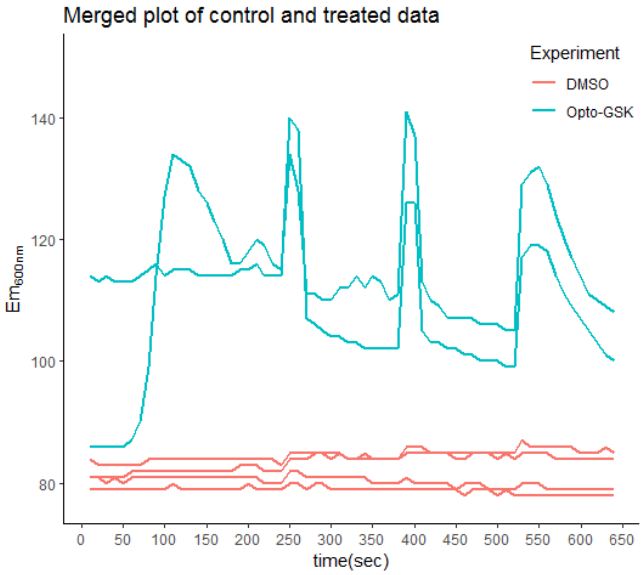


Fig. 2: Observation of the experiments in a mutual environment for a enhanced visual comparability of the data. The treated cells show very distinct intensity peaks, while the control cells are stable in their emission.

relatively stable valleys after excitation with 430 nm. Opto-GSK appears to undergo slow conformational re-conversion to the ground state without optical inhibition (Fig. 1b, t = 520 sec) There is an increase in R-GECO signal in one cell (blue) when changing the medium to 2mM Ca^{2+} which stabilizes before the first excitation of Opto-GSK (Fig. 1b, Δt = 50-230 sec). For enhanced visual comprehensibility of the experiments, the data was merged and plotted on the same scale, which results in clear distinction of the two measurements. Even though the ground state emission of the treated cells (blue) is higher than the control cells (red), the data gives clear indication on the switch-function of Opto-GSK (Fig. 2, Tab. I).

IV. Discussion

The data shows strong evidence for the conformational inductability of TRPC3 via the photosensitive compound Opto-GSK. The control cells show small deviations in their $\frac{max}{min}$ of $\approx$ 2,6%, 2,4%, 7,4% and 3,8%. The treated cells show deviations of $\approx$ 64% and 41,4% (Tab. I). The timestamps also map precisely to the respective activation and inactivation events of the cells. This research should provide enough evidence to conduct further studies with TRPC3 and Opto-GSK. To further validate this experiment, reproduction of this protocol with higher amount of cells can solidify the data and eliminate noise cause by different expression levels of the genetic constructs.

V. Appendix

The experiment was performed at Gruberstrae 40, Linz, Austria on 17.05.19 under the supervision of Sarah Weiss. The experiment was carried out by Caroline Rieser, Lukas Schartel and Stephan Drothler.

References

[19] "Biophysic II Practical Course". 2019.

[BH17] Rafaela Bagur and György Hajnóczky. "Intracellular Ca2+ sensing: its role in calcium homeostasis and signaling". In: *Molecular cell* 66.6 (2017), pp. 780–788.