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Natronomonas Halorhodopsin Enhanced for Diversifying and Extending Optogenetics

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Abstract

This report is concentrated on reviewing two essential papers on eNpHR (enhanced natronomonas Halorhodopsin), an inhibitor of optogenetics that were found and developed by team from Stanford, and briefly introduced related researches. The first paper, published in 2008 on *Brain Cell Biology*, illustrates the invention and advantages of the eNpHR. The second paper, published in 2010 on *HHMI*, illustrates further development of eNpHR and applications of eNpHR 3.0 in lab on diversifying and extending optogenetics. Besides literature reviews of eNpHR and Optogenetics, this report also discussed related new techniques.

Literature Review

What is inhibition and NpHR

As we have learnt in class, Optogenetics is a technique combining optics and genetics and can do fast control of defined cells within functioning tissues. Compared with genetic approaches and pharmacological control strategies, Optogenetics has advantages on precisely timed gain of specified events in targeted cells. It uses light to control neurons after genetically modification to express light-sensitive ion channels and the reagents used are light-sensitive proteins. Neuronal control is achieved using optogenetic actuators like channelrhodopsin, halorhodopsin, and archaerhodopsin.

The function of optogenetic actuators can be generally divided by into two types: excite neurons and inhibit neurons. Inhibiting neurons count on light-activated pumps to bring anions into neurons or remove protons from the neurons. (Annalisa M., 2014). The microbial halorhodopsin NpHR is a fast light-activated electrogenic Cl^- pump that can do temporally precise inhibition of distinct cell types in the intact nervous system. (Viviana G. et al, 2010)

Why enhanced NpHR

Although previous NpHR can make neurons optically hyperpolarized and inhibited from action potentials at moderate expression levels, it is of difficulty to make it to extremely high levels. Therefore, molecularly engineer NpHR is needed and developed to achieve strong expression. Enhanced NpHR (eNpHR) allows safe, high-level expression in mammalian neurons, without toxicity and with augmented inhibitory function, in vitro and in vivo.

Mechanism of eNpHR

The first paper searched for the limiting factor of high NpHR expression back to a membrane trafficking complication. They found high expression correlated with endoplasmic reticulum (ER) accumulation. The best strategy that could make NpHR properties greatly improve is by adding FCYENEV to the NpHR C-terminus as well as the signal peptide from the β subunit of the nAChR to the NpHR N-terminus prevented aggregate formation. (Viviana G. et al., 2008). They named the improved microbial opsin gene as “enhanced NpHR” or “eNpHR”.

eNpHR can totally abolished accumulations at very high expression levels by allowing normal export of NpHR from the ER and by driving increased surface membrane expression. It also displayed increased peak photocurrent in the absence of aggregations or toxicity, and po[tent optical inhibition was observed not only in vitro and in vivo.

Development of eNpHR

The original NpHR is extracted from different classes of microbes. From simpler organisms present clear opportunities, but may not express (or be tolerated) well by more complex cells. Deriving optogenetic tools from multiple classes of microbes promises substantial diversity of triggering and effector functions (Zhang et al., 2008).

Fusion of the FCYENEV ER export motif from a vertebrate inward rectifier

potassium channel to the NpHR C terminus prevented aggregate formation and greatly enhanced tolerability at high expression levels. Thus, eNpHR 2.0 greatly enhanced tolerability at high expression levels, successfully employed in vivo and in intact tissue.

The third generation, eNpHR3.0, contains new quantitative properties. Hyperpolarizations by greater than 100 mV with eNpHR3.0 provide a substantial step forward in the potency of optical inhibition. The inhibition provided with eNpHR3.0 is 20-fold stronger than the initial NpHR.

Extending Optogenetics

As eNpHR can be applied in high expression levels, it deduced molecular trafficking principles to expand optogenetic repertoire by molecular trafficking principles via three aspects:

(1) Optical regulation at the far-red/infrared border and across the entire visible spectrum;

(2) Increased potency of optical inhibition without increased light power requirement;

(3) Targeting cells based on not only genetic identity, but also morphology & tissue topology;

The massive photocurrents observed for eNpHR3.0 (~20-fold stronger than the initial NpHR), suggested optogenetic control with far-red light might be achieved. After experiments, researchers found that eNpHR3.0 photocurrents readily blocked action potentials induced by current injection, validating the extension of optogenetic control channels to far-red light.

After observation, eNpHR3.0 is not only a potent far-red optical control tool, but also the most potent known blue light-driven opsin-based inhibitor (>400 pA at 472 nm). The spectrum of optogenetics has included infrared, blue and green, which extended optical regulation to the entire visible spectrum.

Recent Techniques

Because there is a limitation of in vivo optogenetics that an animal must be tethered to an optical fiber for delivery of light and the process is dangerous and crucial.

In 2014, a new method is proposed for in vivo, optogenetic inhibition of neural activity using an internal, animal-generated light source based on firefly luciferase. Virally encoded luciferase is able to generate sufficient light to activate halorhodopsin and suppress neural activity and change behavior. This approach could be used to generate inhibition in response to activation of specific molecular pathways. (Land, B. B,2014)

Discussion

Previously, after presentation, the parameters' units that measure efficiency were asked. In the experiment, they use voltage and photocurrent difference of membrane to find that using the eNpHR, the optical inhibition potency was increased but required light power remains the same. Thus, the two parameters in the following figure Fig.01 is for photocurrent and voltage difference. pA is short for pico (10^-12) ampere and mV is short for milli (10^-3) volts.

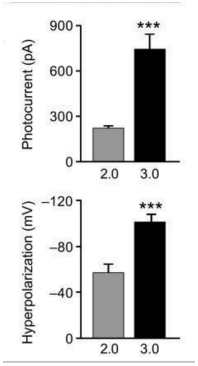

Fig.01 average levels in cells expressing eNpHR3.0 and eNpHR2.0

References

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