Optogenetics on FDSS7000EX: Light-stimulated membrane depolarization in cultured cells expressing channelrhodopsin using a kinetic plate reader.



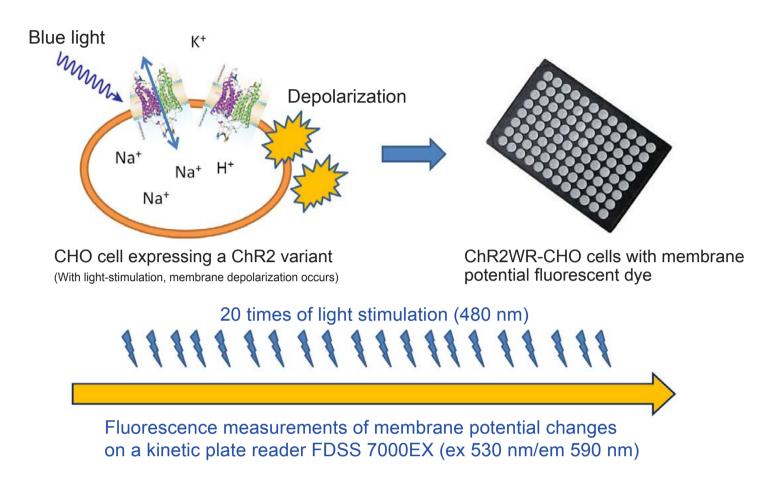


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Uverview

Established clonal cell lines stably expressing a channelrhodopsin variant. Measure membrane potential changes with a fluorescent dye induced by repetitive (20 times) light-stimulations in a 96-well plate on a kinetic plate reader FDSS 7000EX.



Introduction

Channelrhodopsin (ChR), an algal protein from *Chlamydomonas reinhardtti*, is a light-activated cation channel capable of inducing membrane depolarization and can trigger action potentials when it is expressed in excitable cells. Coupling of such optogentic tools with fluorescent-based high-throughput screening (HTS) assays is expected to play a major role in the neural and cardiovascular drug discovery and development. this study, we incorporated the blue-light stimulation capability to the FDSS 7000EX kinetic plate reader to establish the optogenetic-membrane potential dye assay system.

We developed clonal cell lines that stably express ChR2WR (a chimeric protein of channelrhodopsin 1 and 2; ChR2WR) and ChR2WR with a human voltage-dependent sodium channel (NaV1.5), and a human inward rectifier potassium channel (KCNJ2).

Our optogenetic-membrane potential dye assays using the FDSS 7000EX kinetic plate reader revealed that Depolarization initiated by light-(480 nm) induced ChR2WR activation resulted in Lidocaine-sensitive membrane depolarization. Also responses to 20 repetitive light stimuli were all captured by the FDSS 7000EX plate reader suggesting its applicability for use dependent drug screening. Our report suggests that fluorescence measurements using the FDSS 7000EX kinetic plate reader when it is combined with optogenetic is a useful tool for the HTS.

**** Materials and Methods**

Sample preparations

We established clonal cell lines stably expressing ChR2WR (channelrhodopsin/wide receiver; a chimeric protein of channelrhodopsin 1 and 2).

The ChR2WR-expressing cells were plated to the 96-well half-area plates (Corning) at 40,000 cells/well and incubated in 120 μ l of the MEM α culture medium with 10% FBS. The cells were loaded with a fluorescent dye. A single vial of the FMP red dye kit (Molecular Devices) was suspended in 10 ml of Hanks' balanced salt solution with 20 mM HEPES (Life Technologies) and 0.1% BSA warmed at 37°C according to the manufacturer's recommendation. 48 μ l of the dye solution was applied to the well and incubated for 45 min at 37°C in 5% CO₂. The measurements were performed without washing the dye solution.

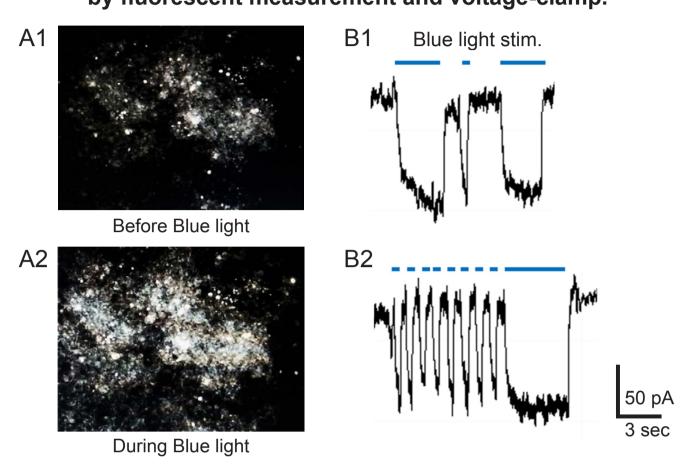
Light activation experiments on FDSS 7000EX

Light-stimulated membrane depolarization was measured on FDSS 7000EX (Hamamatsu). Twenty light pulses (480 nm, 1~10 s of pulse width, 16 s of pulse interval) from a Xe lamp were added to the cells in a 96-well plate repetitively and fluorescence intensities with 530 nm of excitation were measured at 590 nm of emission wavelength every 400 ms.

Results

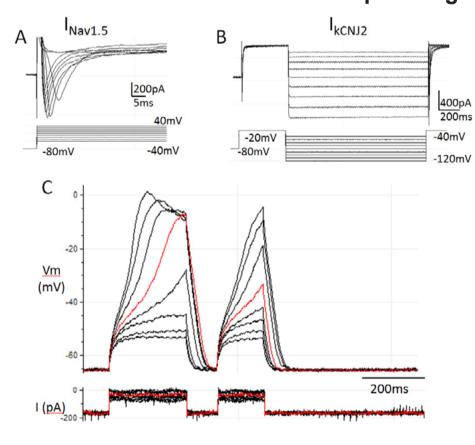
1. Characterization of a ChR2WR expressing cell line

Characterization of ChR2WR expressing HEK cell line by fluorescent measurement and voltage-clamp.



- A. Optical detection of the ChR2WR responses by the blue light stimuli recorded by MP dye. A1 before light control, A2. during Blue light stimulation.
- B. Blue light induced inward ChR2WR currents were recorded under the Patch-clamp method (Voltage-clamp mode, Hp=-60mV). As originally described by Wang *et al.* (2009), ChR2WR induced inward current does not desensitized (B1). The size of the inward ChR2WR current amplitude induced by repetitive short light pulse (<500ms.) was close to that of the steady state amplitude induced by following longer pulse.

Voltage- and Current-Clamp characterization of Nav1.5/KCNJ2 expressing HEK293 cell



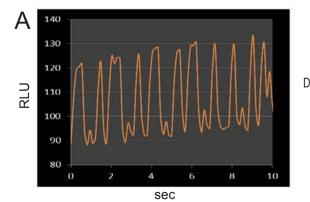
Expression of I_{Nav1.5} current (A) and I_{KCNJ2} current (B) were confirmed under the voltage-clamp in Nav1.5/KCNJ2 HEK 293 cell line.

C. Action potentials in Nav1.5/KCNJ2 HEK cell. Series of positive current injections resulted in graded (action) potentials. The responses by the 2nd pulses were smaller (red trace) suggesting that the Nav1.5 sodium channel that responded to the 1st stimulus was in the refractory period (inactivated).

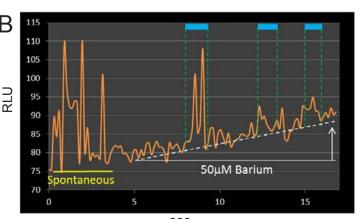
Solution

The extracellular buffer consisted of: NaCl, 126 mM; KCl, 4.0 mM; CaCl₂, 1 mM; MgCl₂, 1 mM; HEPES, 24 mM; Glucose, 10 mM; pH adjusted to 7.4 with NaOH. The intracellular solution consisted of: 130 mM KCl, 1.55 mM MgCl₂, 5 mM EGTA, 2 mM CaCl₂, 2 mM Na₂ATP, 10 mM HEPES, pH 7.2.

Spontaneous and light-induced depolarization in ChR2WR /Nav1.5/KCNJ2 HEK293 cells



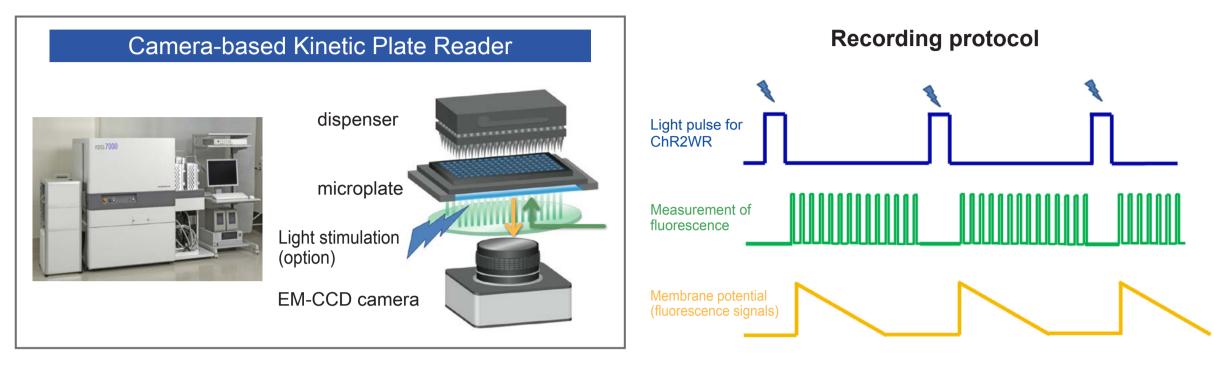
A. An example of the cell that exhibits spontaneous activity. The membrane potential was measured by MP dye. Fluorescent photos of the Baseline and depolarized states are shown in the right column.



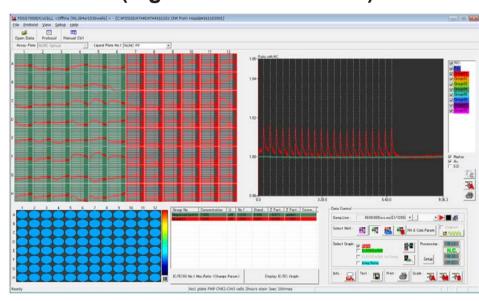
B. Blocking KCNJ2 resulted in slow membrane depolarization that terminated Spontaneous membrane oscillation.

KCNJ2 was blocked by the addition of Barium. Light stimuli were applied where indicated by thick blue lines. Voltage spikes were induced by the 1st light stimulus, but the following light stimuli failed to induce membrane spikes, suggesting that membrane depolarization by KCNJ2 block inactivated available Nav1.5. (Signals were captured every 150 msec.)

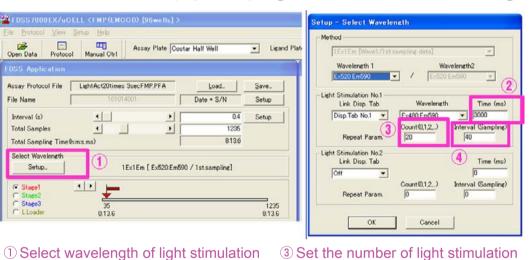
2. Measurement of light-stimulated membrane depolarization using FDSS 7000EX



Fluorescence measurements of membrane potential changes in ChR2WR-CHO cells (Light stimulation 3sec)

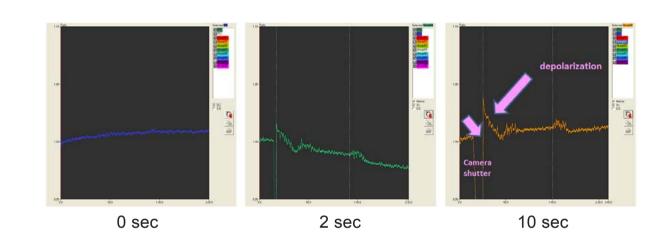


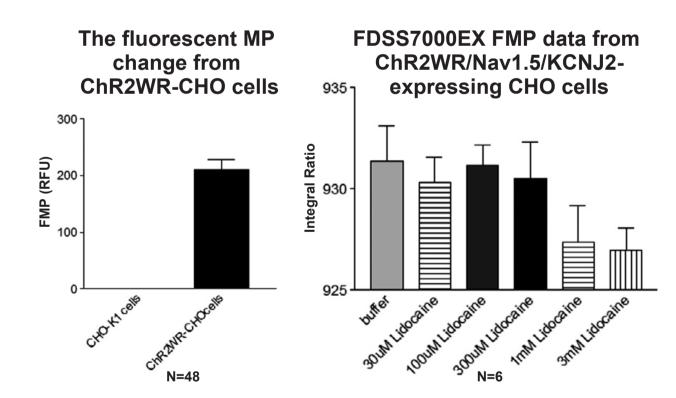
FDSS software (option) light stimulation setting



② Set exposure time of light stimulation ④ Set interval of each light stimulation

ChR2WR induce the membrane depolarization on FDSS





Conclusion

The FDSS 7000EX kinetic plate reader has gained the reputation as one of the best HTS screening systems suitable for the cell-based assays. We incorporated the blue-light stimulation capability to the FDSS 7000EX kinetic plate reader. In this report, we show that combining optogenetics and the fluorescent-based applications can be a powerful tool for ion channel HTS assays.

- 1. The stable CHO and HEK296 cell lines that co-express ChR2WR (a desensitization reduced version of channelrhodopsin, Wang et al. 2009), Nav1.5 and KCNJ2 were developed. Their functional properties were characterized using the patch-clamp method and the membrane potential dye.
- 2. Using the cell lines described above, the optogenetic-membrane potential dye assays were carried out using the FDSS 7000EX kinetic plate reader. Depolarization initiated by light-(480 nm) induced ChR2WR activation resulted in Lidocaine-sensitive membrane depolarization.
- 3. Responses to 20 repetitive light stimuli were all captured by the FDSS 7000EX plate reader suggesting its applicability for use dependent drug screening.
- 4. Our report suggests that fluorescence measurements using the FDSS 7000EX kinetic plate reader when it is combined with optogenetic is a useful tool for the HTS

Our report suggests that the combination of optogenetic tools and fluorescence measurements is an an excellent method to use in order to investigate pharmacological effects on ion channels in excitable cells, such as myocytes and neurons.

References

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Patent

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