Optogenetic Modulation of Cardiac Membrane Potential as an Anti-arrhythmic Therapy

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Introduction

- Deadly disturbances of the heart's electrical activity ('arrhythmias') may be triggered by failed restoration of cellular membrane potential (V_m) to a resting state ('early after-depolarisation', EAD).
- Optogenetics, involving the use of genetically-expressed light-activated ion channels to modulate V_m of cardiac cells, may represent an anti-arrhythmic tool.

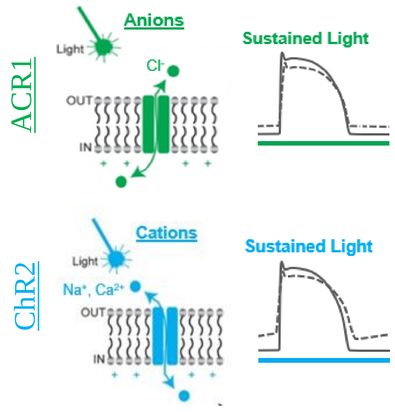
Aims and Objectives

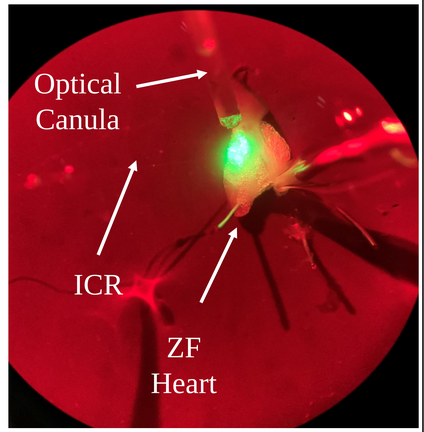
Our goal is to investigate the use of optogenetics for suppression of EAD-triggered arrhythmias as an anti-arrhythmic tool.

- **Aim 1:** Define effects of optogenetic tools on cellular V_m in the whole heart.
- **Aim 2:** Establish a whole heart EAD model.
- **Aim 3:** Devise strategies for the use of optogenetics for EAD suppression to prevent arrhythmias in the whole heart.

Methodology

- **Experimental Preparation:** Experiments were performed using live hearts isolated from adult zebrafish genetically expressing the light-activated anion-selective channelrhodopsin-1 (ACR1; n=6) or cation-nonselective channel, channelrhodopsin-2 (ChR2; n=6).
- **Measurement of Electrical Activity:** V_m and action potential (AP) characteristics were measured by intracellular microelectrode recordings.
- **Optogenetic Modulation:** ACR1 or ChR2 were activated by green (530nm) or blue (470nm) light of varying intensity (0.005-0.024mW/mm^2) focused on regions of the heart with a fibre-optic cannula.
- **Pharmacological Interventions:** EADs were induced by simultaneous pharmacological activation of a calcium current (L-type) and block of a potassium current (rapid delayed rectifier) important for restoration of resting V_m in cardiac cells.
- **Data Analysis:** AP parameters were analyzed with custom MATLAB routines and compared using paired or unpaired t-tests, as appropriate.





Results/Findings

- It was found that activation of ACR1 (Fig. 1) or ChR2 (Fig. 3) caused a similar change of resting V_m (+26±6% vs +21±8, with the greatest light intensity; p=0.61), rate of AP upstroke (-40±10% vs -32±12; p=0.62), and AP amplitude (-29±7% vs -24±9; p=0.67 – Figs. 2 and 4).
- ACR1 activation caused a shortening of the AP (-27±6%; p=0.01 – Fig. 2), however there was no change with ChR2 (-6±3%; p=0.11 – Fig. 4), suggesting that ACR1 may be useful for EAD suppression.
- Pharmacological manipulation of ion currents resulted in EAD-triggered arrhythmias in the whole heart (Fig. 5).
- ACR1 activation by sustained or timed light prevented EAD-triggered arrhythmias (Figs. 6 and 7).

Figure 1. Effects of ACR1 Activation on Action Potential

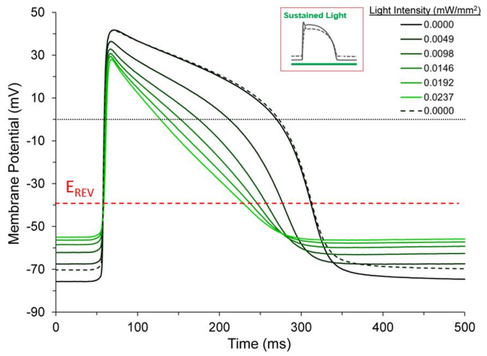


Figure 3. Effects of ChR2 Activation on Action Potential

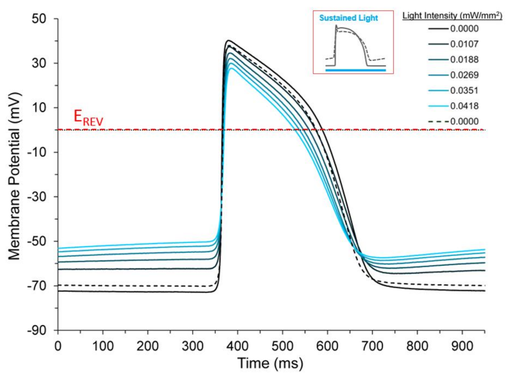


Figure 2. Effects of ACR1 Activation on Action Potential Parameters

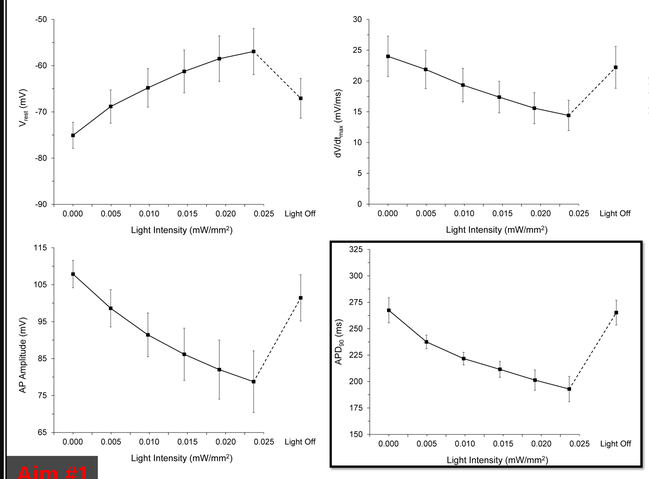
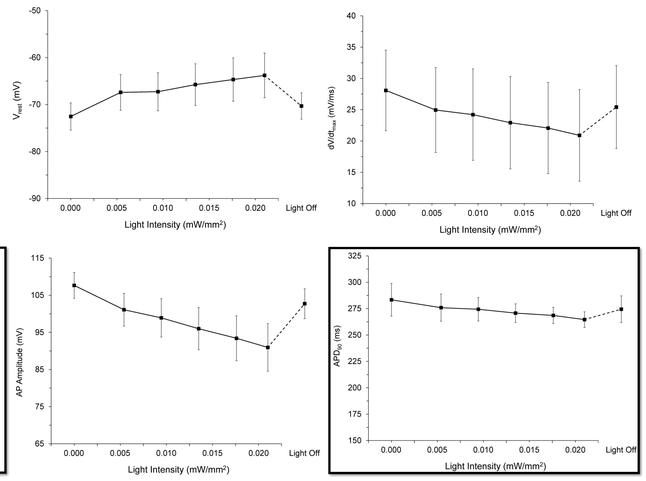
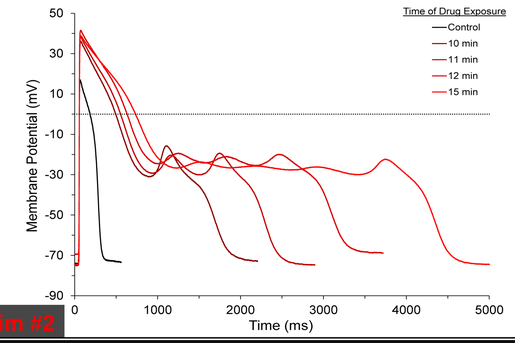


Figure 4. Effects of ChR2 Activation on Action Potential Parameters



Aim #1

Figure 5. EAD Progression in Zebrafish Action Potential



Aim #2

Figure 6. Sustained Light Suppression of Drug-induced EAD

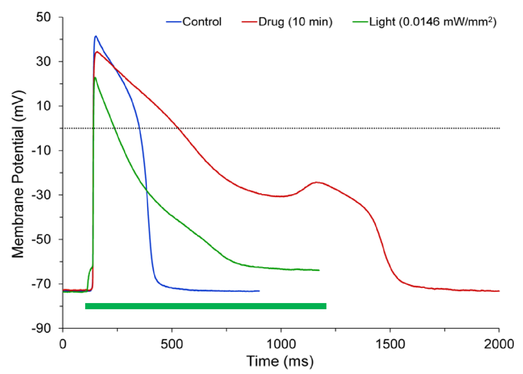
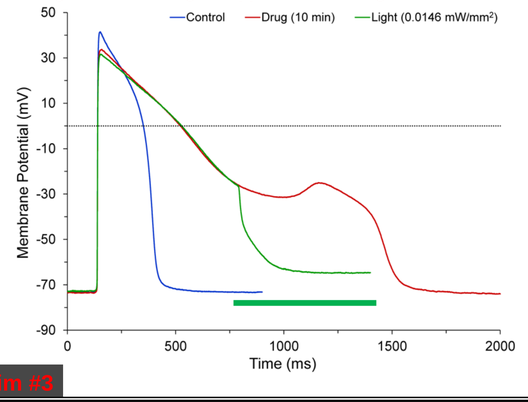


Figure 7. Timed Light Suppression of Drug-induced EAD



Aim #3

Analysis/Discussion

Our data shows the feasibility of using optogenetics to prevent arrhythmias in the whole heart. Here we have characterised the optogenetic tools available to select an appropriate light-activated channel for EAD suppression (ACR1), established a whole heart EAD model, and used ACR1 to suppress EAD-triggered arrhythmias.

Previous studies have demonstrated the use of optogenetics to abolish established sustained arrhythmias by blocking re-entrant pathways at the tissue-level. Here we have prevented the initiation of an arrhythmia by preventing the cell-level trigger (i.e., EAD). We have also established a whole heart model of EAD-triggered arrhythmias. It is questioned whether arrhythmias can be triggered by EAD in the whole heart. Our results reveal conditions under which this can occur, which has been an elusive experimental target.

- **Limitations: Results demonstrating optogenetic EAD suppression are preliminary; methodology requires further optimisation.**

Conclusions

Overall, we showed that optogenetics may be a useful means to prevent arrhythmias in the whole heart, warranting further exploration of its clinical utility as an anti-arrhythmic tool. The next steps are to optimise the temporal application of light by exploring the effects of light pulse duration and timing during the AP, and then build a device for 'smart' light application to reshape the AP back to the physiological morphology. Further, thus far measurements of V_m have only be made at the cellular level. In future experiments we will also make measurements of electrical activity using macroscopic dye-based fluorescence imaging (see below Figure), to understand what is occurring at the whole heart level and allow spatial targeting of light application.