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A Comparison of Drug-induced Changes in Electrical Excitability Using Optogenetic Sensors and Conventional Field Stimulation in hiPS-derived Cardiomyocytes

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Abstract

Drug induced changes in myocardial electrical excitability are difficult to assess in medium to high through-put assay systems. One practical difficulty is the use of solid-state electrodes to provide a stimulus to a number a large number (100-200/day) independent cardiac preparations assays while preventing cross contamination of the sample by the electrodes or the complex use of multiple independent electrodes. Optogenetic probes, e.g. Channel Rhodopsin type-2 offers a potential solution to this problem in that the presence of this ChR2 in excitable cell membranes allows the use of light (~470 nm) to initiate an action potential and subsequent contraction.

Methodology

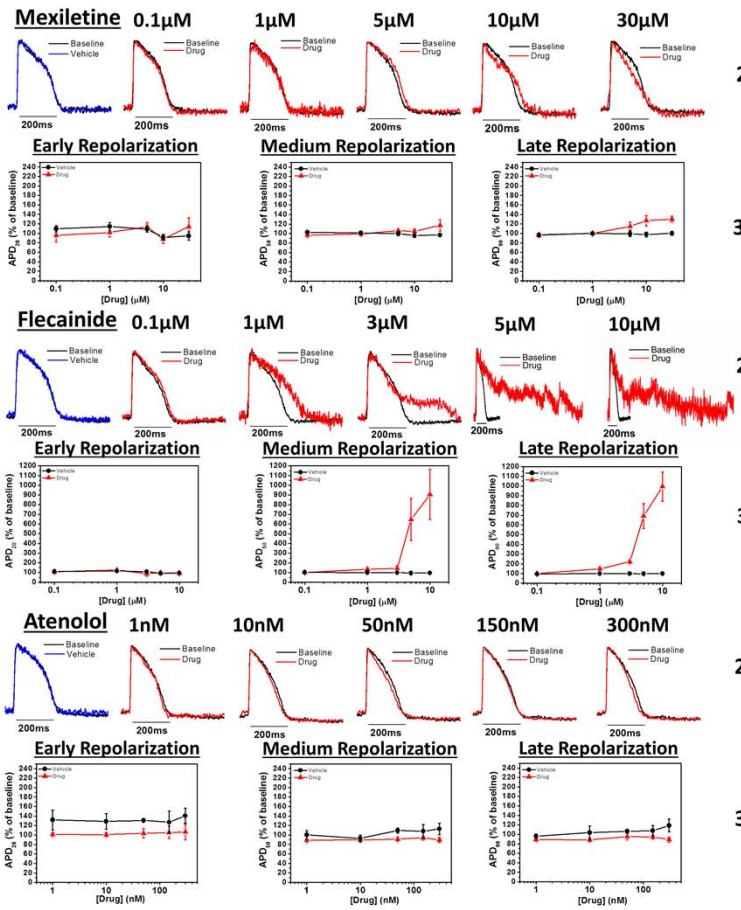
HiPS derived cardiomyocytes (Axiogenesis AG) were seeded onto fibronectin (1:100 DPBS) coated 35 mm glass bottomed plates. Cells were incubated overnight with an adeno-associated virus containing ChR2 (Addgene# 20938M) at MOI approx. 30,000. 48 hours post-transfection cells were stimulated to contract using a 2 ms pulse of light at 2 Hz and 3 Hz using a 470 nm LED. The intensity of light was gradually increased until the myocytes responded to stimulation. Data were compared with threshold measurements using graphite electrodes (no ChR2). Electrical activity was recorded from hiPS derived cardiomyocytes plated on 96-well plates and loaded with Di-4-ANEPPS using CellOPTIQ™ platform.

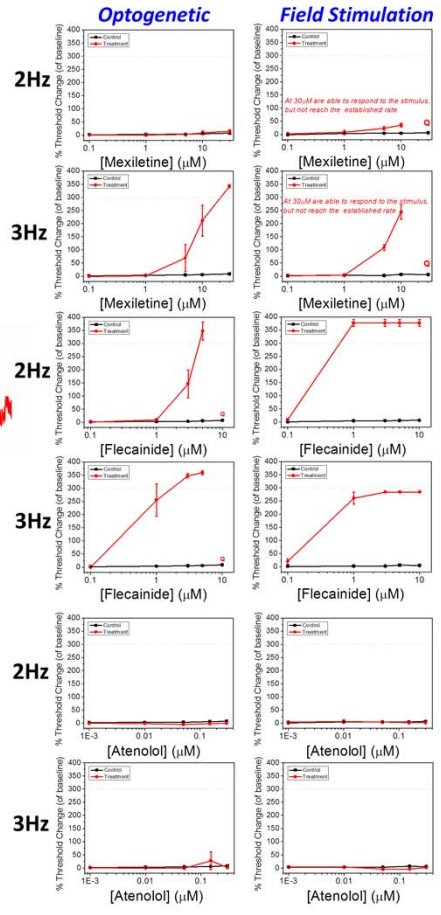
Purpose

We investigated changes in electrical excitability by adapting the CellOPTIQ™ platform (Clyde Biosciences Ltd) to stimulate hiPS-derived cardiomyocytes with either solid-state or optogenetic techniques and compare changes in excitability caused by a range of reference drugs

Results

The negative effect of Mexiletine and Flecainide over the excitability was proved with both techniques. Both drugs showed different response. Whereas Mexiletine up to 10 µM caused frequency dependent decreases in excitability as assessed by Optogenetic approach and Field Stimulation method, Flecainide up to 10 µM showed comparable changes in excitability at both 2 Hz and 3 Hz using both techniques. Drugs not expected to alter excitability as Atenolol was without effect. The reproducibility between field stimulation and optogenetic approach discard possible side effect on hiPSC-CMs electrophysiology due to ChR2 expression in those drugs.





Conclusions

- This work demonstrates that optogenetic techniques applied to hiPS-CMs offers the possibility of a convenient and sensitive method of detecting changes in cardiac excitability that can be applied to medium-to-high through-put assay systems.

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