

Optogenetic Modulation of GtACR1 on Myocardial Electromechanical Properties: A Computational Study

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Abstract

*Over the past decade, optogenetic tools have been proposed as powerful means to modulate cardiac physiological and pathological activities. Cation non-selective Channelrhodopsin (ChR) has been extensively corroborated that it can depolarize the membrane potential in cardiomyocytes (CMs) and elicit action potentials (APs). By contrast, *Guillardia theta* Anion Channelrhodopsin (GtACR1) shows efficient photoinhibition and is used for silencing CM activity. This study aimed to assess the effects of GtACR1 on electromechanical characteristics of CM by computational modeling, which combined a module of excitation-contraction coupling in the CM with a module of GtACR1 photocurrent kinetics. We simulated light sensitization of GtACR1. To analyze consistency of electrophysiological and mechanical effects, we varied light pulse timing (10–1000 ms) and intensity (0.001–10 mW/mm²). The simulation results showed that GtACR1-based optically paced CM displayed optogenetic voltage forcing and CaTnC kinetics weakening, and light intensity inhibited myocardial electromechanical activity more than light duration. Our findings suggest that GtACR1 plays an important role of optogenetic modulation on CM electromechanical properties. It should be considered in future pathological cardiac mathematical modeling.*

1. Introduction

Optogenetics, a central tool in neuroscience research, has been intensely applied in cardiology within the past decade. The translational applications of cardiac optogenetics mainly focus on light-mediated cardiac resynchronization therapy, cardioversion and painless defibrillation [1]. Compared to electrical stimulation, the main advantage of optogenetic stimulation is the high spatiotemporal accuracy and cell specificity providing the chance for precise modulation of membrane potential in a specific light-sensitive region, whereas the other one usually triggers extra excitation beyond the stimulated area,

such as surrounding skeletal muscle [2].

All-optical electrophysiology draws upon a comprehensive toolkit of optogenetic sensors and actuators to track and manipulate membrane potential and intracellular calcium dynamics. Commonly used optogenetic sensors are known as genetically encoded calcium and voltage indicators (GECIs and GEVIs): the former including the GCaMP, the R-CaMP, the R-GECO and the NIR-GECO family; and the latter, VSFP2.3, ArcLight, and near-infrared-absorbing GEVIs [3]. Relevant optogenetic actuators of voltage include excitatory opsins and inhibitory opsins [4]. The channelrhodopsin-2 (ChR2) based, depolarizing opsins have the complex capability to regulate the action potential (AP), the conduction properties of myocardial tissue, and cardiac rhythm, and thus are widely used as modulators of cardiac function [5, 6]. Despite these advantages, their efficacy is still limited, due to light absorption and scattering in myocardium and the demand for strong irradiance and long light duration [7]. These flaws can be compensated by the hyperpolarizing opsins. One class of them is anion channelrhodopsins (ACRs), such as *Guillardia theta* anion channelrhodopsin-1 (GtACR1), which have properties including fast response kinetics and bidirectional modulation of voltage that render them attractive optogenetic tools [8, 9].

Here, we conduct simulations in a preliminary computational model combining GtACR1 photocurrent kinetics and cardiomyocyte (CM) electromechanical function, to investigate contribution of GtACR1-based optogenetic factors to excitation-contraction coupling in myocardium.

2. Materials and methods

We conducted computational simulations using a combined model of GtACR1-transduced guinea-pig ventricular myocyte. CM mechanical activity and calcium regulation of contractile proteins, membrane kinetics, as well as GtACR1 photocurrent kinetics, were represented using the formulations derived by Sulman et al. [10], Noble et al. [11] and Ochs et al. [2], respectively. An overview of

the simulations was given as follows.

2.1. Single ventricular myocyte model

This model included an electrophysiology module and a mechanical module, which has been described in prior work [10, 11]. The kinetics of membrane potential, ionic currents, intracellular concentrations of ions, cytosolic calcium, as well as myocardium tension and length have been characterized in detail.

2.2. Mathematical Representation of GtACR1 photocurrent kinetics

The GtACR1 photocycles were simulated using a model as developed by Ochs et al. [2]. The authors developed a two-state Markov chain model with light-gated transitions between one open and one closed state. Equations of the current density of the channel (I_{GtACR1}) and the membrane potential of ventricular myocyte (V_m) were given by

$$I_{GtACR1} = P(O) * g_{GtACR1} * (V_m - E_{GtACR1}) \quad (1)$$

$$\frac{dV_m}{dt} = -\frac{1}{C_m} (I_m + I_{E_stim} + I_{GtACR1}) \quad (2)$$

where $P(O)$ is the open-state probability, g_{GtACR1} is the maximal GtACR1 channel conductance, E_{GtACR1} is the GtACR1 reversal potential (-40 mV), C_m is the total membrane capacitance, I_m is the sum of all transmembrane ionic currents, I_{E_stim} is the externally applied electrical stimulus current.

2.3. Simulation protocol

This study aimed to analyse effects of GtACR1-based optogenetic factors on electrophysiology and mechanics of CM with various light durations and irradiances. We evaluated the response of the GtACR1 photocurrent model to light in the ventricular myocyte electromechanical model, and simulated kinetics of V_m , complexes of calcium with troponin C (CaTnC), force and sarcomere length (SL) under different light conditions. The model was implemented in MATLAB. Simulation results were recorded after 100 optical stimuli to ensure the model stable. A variable-step integral algorithm (ode15s) with absolute and relative error tolerances of 10^{-6} was used.

3. Results

3.1. GtACR1-based model characterization

As shown in Figure 1, I_{GtACR1} played an inhibitory role in CM excitation and contraction in both cases of isometric and isotonic twitches. After a normal excitation evoked by electrical stimulation (first orange star), subsequent illumination (green bar) elicited a rapid depolarization and early repolarization, and then V_m was forced to -45 mV

(close to E_{GtACR1}). AP was not triggered by electrical stimulation (second orange star) during illumination (Figure 1a). When V_m was above (or below) the GtACR1 reversal potential, I_{GtACR1} became an outward (or inward) current, showing the dual role of the current in cell polarization. V_m went back to the resting state and I_{GtACR1} slowly returned to 0 with the end of illumination (Figure 1b). The electrical excitation was accompanied by the changes in tension due to electromechanical coupling role. The force during illumination in both twitches was significantly lower than that generated during the first electrical excitation, which indicated that the illumination had a suppression effect on cell contraction (Figure 1c).

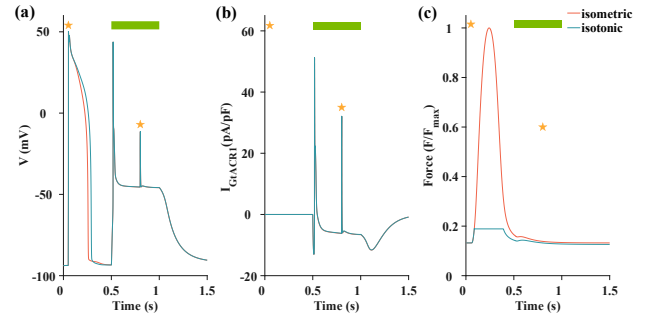


Figure 1. Schematic diagram of I_{GtACR1} -induced inhibition of AP and force at both isometric and isotonic afterloaded twitches. Orange stars represent timing of electrical stimuli and green bars show the interval of illumination (green light, 515 nm, 500 ms, 5.0 mW/mm²). (a) V_m , (b) I_{GtACR1} , and (c) Force (expressed in fractions of the peak isometric force F_{max}).

3.2. Optogenetic actuation in myocardial electromechanical model

Figure 2 was the simulation of CM electrophysiological and mechanical properties obtained at various light durations (increased from 10 to 1000 ms). The irradiance was fixed to 5.0 mW/mm². The top panel represented the case of isometric twitches, and the bottom represented isotonic twitches. For both twitches, ten milliseconds illumination was too late to evoke APs, and the very low concentration of CaTnC in this case also could not generate contraction. When the illumination was prolonged to trigger an AP, the action potential duration (APD) corresponded to the light duration (Figure 2a and 2e), while the concentration of CaTnC (Figure 2b and 2f), as well as tension (Figure 2c and 2g) and SL (Figure 2d and 2h) were hardly affected.

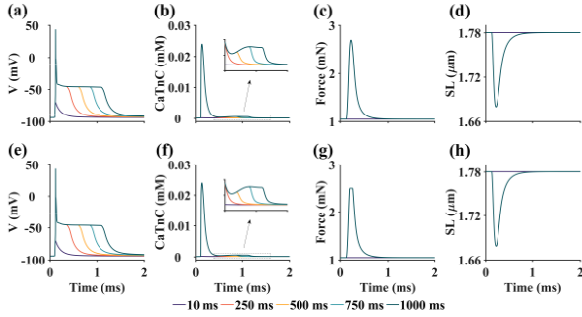


Figure 2. Simulation of a series of electromechanical properties during illumination with a fixed irradiance (5.0 mW/mm^2) and light duration varying from 10 to 1000 ms. Insets show magnified images of the regions indicated by the dashed boxes. (a-d) V_m , concentration of CaTnC, Force and SL in isometric twitches, (e-h) V_m , concentration of CaTnC, Force and SL in isotonic twitches applied an afterload of 2.5 mN.

Figure 3 showed the effects of illumination on CM electromechanical properties obtained at various irradiances (increased from 0.001 to 10 mW/mm^2). The light duration was fixed to 500 ms. Twitches of the top and bottom panels were same as Figure 2. When the irradiance was enough to elicit CM excitation and contraction, the larger the irradiance, the faster the membrane potential reached its maximum value, and the slower the repolarization (insets in Figure 3a and 3e). The effects of irradiance were more marked in concentration of CaTnC and mechanical indexes. For both twitches, time to peak concentration of CaTnC as well as peak concentration decreased as irradiance increased (Figure 3b and 3f). In isometric twitches, the virtual CM responded with decreases in peak force, end-systolic lengths of CM and twitch duration when irradiance was enhanced (Figure 3c and 3d). In isotonic twitches, both durations of plateau force and twitches shortened as irradiance increased (Figure 3g). Variations in SL (Figure 3h) showed the same trend as those obtained in isometric twitches.

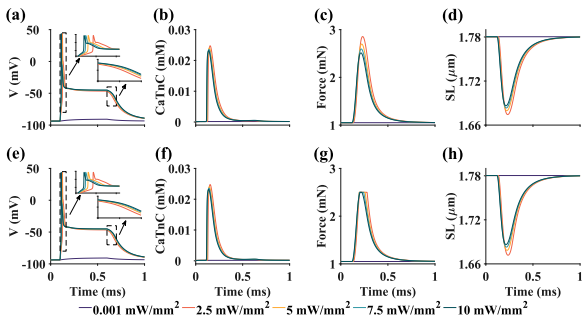


Figure 3. Simulation of a series of electromechanical properties during illumination with a fixed light duration (500 ms) and irradiance varying from 0.001 to 10 mW/mm^2 . Insets show magnified images of the regions indicated by the dashed boxes. (a-d) V_m , concentration of

CaTnC, Force and SL in isometric twitches, (e-h) V_m , concentration of CaTnC, Force and SL in isotonic twitches applied an afterload of 2.5 mN.

4. Discussion

In this study, we presented a combined computational model which coupled ventricular myocyte membrane kinetics and cross-bridge mechanics with GtACR1 photocurrent kinetics, to assess the roles of light in myocardial electromechanical properties via GtACR1-mediated optogenetic stimulation. Our main findings were: (1) GtACR1 photocurrent showed certain inhibitory effects on CM excitation and contraction, compared to electrical stimulation; (2) light duration had noticeable effects on APD while had a negligible impact on mechanical indexes; and (3) light intensity had a stronger influence on myocardial electromechanical properties than light duration.

Prior studies have identified GtACR1 as a key opsin for bidirectionally modulating APs in CMs [8, 9, 12]. The reason was that I_{GtACR1} relied on the chloride reversal potential (nearly -40 mV in CMs). Therefore, it could depolarize CMs when they were in the resting state, while decelerated early repolarization when they experienced excitation. During illumination, GtACR1-mediated photocurrent forced V_m to remain at the GtACR1 reversal potential and prevented CMs from exciting by subsequent electrical stimuli. This “light-induced voltage forcing” mechanism mediated by GtACR1 was quite different from depolarization-based ways to terminate abnormal excitation represented by ChR2-expressing models. Prior studies have identified that steady state inactivation of fast Na^+ channels and elimination of excitable gaps by creation of ChR2-stimulated wavefronts were two main factors for fibrillating termination [13, 14].

Kopton et al. validated experimentally that GtACR1-mediated photo-pacing and photoinhibition of CMs, and also measured the effect of light duration on contractility when CMs were under medium light intensity ($4\text{-}6 \text{ mW/mm}^2$) [15]. As shown in Figure 6 and 7 from reference [15], force amplitudes induced by a short-time light (10 ms) were comparable to the electrically elicited ones, while they were significantly suppressed by a long-time illumination (64 s). Our simulation (Figure 1) fitted well with the experimental results. It should be noted that the light duration in our simulations was assumed to be no more than 1 s. At this setting, medium irradiance still elicited visible changes in contraction and SL (Figure 2). These findings seemed different but actually were consistent with the observations of Kopton et al. that long-time illumination could almost fully inhibit contraction and deformation. The generated force and deformation were produced primarily by CaTnC kinetics during the fast depolarization, which remained an extremely low level during the plateau and repolarization phases of the APs

forced by a continual irradiation, and thus did not continuously activate contractions.

Our simulation study demonstrated that the impact of light intensity was more pronounced for AP and force dynamics compared to light duration, and the higher irradiance, the stronger inhibition. To date, this result has not yet been fully verified by experiments. Prior works were relatively more focused on testing energy requirements of GtACR1-mediated optogenetic stimulation and suggested that energy for GtACR1-based optogenetic defibrillation might be 2-3 orders of magnitude less than Chr2 [2]. However, our simulations implied that increasing light intensity could reinforce the suppression of GtACR1-based optogenetic stimulation on electromechanical activities of CMs. In future we would like to perform simulations to test potential of various optogenetic tools to inhibit cardiac electromechanical activity at the tissue and organ levels.

5. Summary

We have demonstrated the roles of GtACR1-based optogenetic stimulation in electromechanical activities via a detailed coupled model of CM and GtACR1. The inhibition mechanism was identified as optogenetic voltage forcing and CaTnC kinetics weakening. It was suggested that light intensity might inhibit myocardial electromechanical activity more than light duration.

Acknowledgments

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