L13 Simultaneous measurement of sarcomere shortening and calcium transients in primary rat cardiomyocytes exposed to electrical pulses

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Duration of the experiments: 90 min
Max. number of participants: 3
Location: Cell Culture Laboratory 1
Level: Advanced

PREREQUISITES

Participants should be familiar with Laboratory safety (S1) and Electroporation hardware safety (S2). Basic knowledge of handling with cells is required for this laboratory practice.

The aim of this laboratory practice is to observe the effects of electroporation – using monopolar single electric pulse or a burst of bipolar high frequency irreversible electroporation pulses (H-FIRE) on Ca^{2+} homeostasis, contractility and recovery of adult rat ventricular cardiomyocytes.

THEORETICAL BACKGROUND

Despite a long history of using electric pulses on the cardiac tissue for defibrillation and pacing, effects of high-voltage (supraphysiological) electric pulses at the level of a single cardiomyocyte (or even neurons and excitable cells in general, for that matter), remain relatively unexplored. It is known that during electroporation, cell membrane is damaged, its conductivity is increased, and cell homeostasis disrupted [1]. Voltage-gated channels can also be affected, although the consensus on the mechanisms and the consequences of electroporation on voltage-gated channels is not yet reached. Interestingly, in a recent molecular dynamics study, the voltage sensor area of the voltage-gated channels was shown to be permanently damaged under the influence of high electric fields [2]. During electroporation, Ca^{2+} enters the cell in an uncontrolled way through the permeabilized membrane. Thus, the precise calcium balance, needed for normal cardiomyocyte function is disrupted [3]. This can lead to undesired and unpredictable behaviors. For example, excitation-contraction coupling (pairing cardiomyocyte depolarization with mechanical contraction) depends largely on Ca^{2+} signaling and disturbances of Ca^{2+} homeostasis can severely impact muscle physiology and give rise to various myopathies and cardiac disorders. Under physiological conditions, cardiomyocytes can efficiently remove the excess intracellular Ca^{2+}, which enters during the action potential plateau phase to prepare for the next cycle. However, with electroporation, the uptake of Ca^{2+} can be much higher than in normal working conditions; moreover, the cell membrane is damaged and does not reseal for minutes after the treatment. Furthermore, ATP, necessary for membrane resealing and operation of pumps, following electroporation leaks out of the cell [4].

The efficiency of electroporation depends on the parameters of the electric field, cell size, geometry, position, and physiological characteristics of the medium surrounding the cell. Different fluorescent dyes (e.g. Rhod-2, Fluo-4, Fura-2, Fura-3,...) can be used to investigate the influence of these

parameters on electroporation and the same dyes can be used to monitor how electroporation affects Ca^{2+} transients [5].

EXPERIMENT

We will simultaneously monitor sarcomere shortenings, i.e., contraction and Ca^{2+} transients of rat cardiomyocytes with IonOptix-upgraded Zeiss Axiovert microscope which applies Fast Fourier Transform to measure sarcomere length while at the same time measuring signal from fluorescent Ca^{2+} sensitive indicator Rhod-2 AM (excitation and emission spectra presented in Figure 1A). As cardiomyocytes are paced, Ca^{2+} concentrations inside the cells change, due to its importance in action potentials, while supraphysiological electric pulses, i.e. electroporation, cause changes in Ca^{2+} concentrations due to perturbations in membrane permeability (due to electroporation). Once Ca^{2+} enters the sarcoplasm of cardiomyocytes, it binds to the dye and changes its fluorescence intensity more than 100-fold.

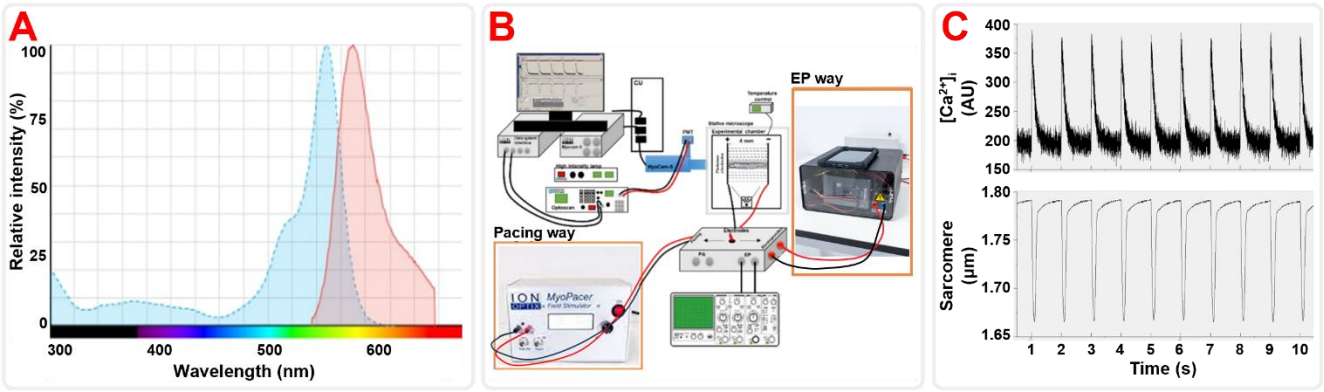

Figure 1: (A) Fluorescence excitation (blue) and emission (red) spectra of Rhod-2 AM for different concentrations of Ca2+ (image from https://www.thermofisher.com/order/fluorescence-spectraviewer?SID=srch-svtool&UID=14220ca#!/). (B) Schematic of the experimental setup (adapted from Chaigne, S. et al., 2022 [3]). (C) Simultaneous acquisition of calcium transients and sarcomere shortenings with IonOptix system.

Protocol: The experiments will be performed on adult rat ventricular cardiomyocytes, isolated from female Wistar rats, weighing 250-350 grams [6,7]. Before measurements, cardiomyocytes will be incubated in Tyrode's buffer with 2 µM Rhod-2, AM for 20 min at 37 °C (Thermo Fisher Scientific). After the incubation time, cardiomyocytes will be washed with fresh Tyrode's to remove excess dye and then seeded on 25 mm square coverslips for imaging.

Insert fibronectin/gelatin-coated glass coverslips into IonOptix stimulation chamber on fluorescence microscope (Zeiss Axiovert 200) and use 63×LD objective (Figure 1B shows the schematic of the whole experimental setup). Wait 5 min and then start the perfusion of Tyrode's buffer at 37 °C. IonOptix MyoCam-S3™ Fast CMOS camera will be used to capture sarcomere shortening while IonOptix photomultiplier tube with be used to capture signals for Rhod-2AM. Measurements will be recorded and processed using IonOptix IonWizard software (example of measurements shown in Figure 1C).

Using an IonOptix MyoPacer Field Stimulator pace cardiomyocytes on the coverslip with bipolar pulses at 2 Hz and 2 ms (2 ms positive phase followed immediately by 2 ms negative phase). IonOptix

stimulation chamber is equipped with 2 platinum electrodes (4 mm spacing edge to edge) and our setup also includes a switch box to alternate between pulses from the IonOptix MyoPacer Field Stimulator for physiological pacing and HF-IRE pulse generator (University of Ljubljana) for monopolar or bipolar supraphysiological pulses. Using a framing adapter, frame a cardiomyocyte that contracts in sync with delivered pacing pulses. Measure basal changes in sarcomere length and Ca^{2+} transients (capture at least 10 contractions). Afterwards use HF-IRE pulse generator and deliver one monopolar electric pulse of 100 μs or a burst of H-FIRE pulses (25 bipolar pulses, 2 μs positive and negative phase, 2 μs interpulse and interphase delay), which will represent an electroporation (EP) pulse. Start with 100 V/cm for monopolar pulses, and with 300 V/cm for H-FIRE pulses. 1 minute after delivered pulse, start pacing the cell again with 2 ms bipolar pulses. If cell still contracts, deliver another pulse with higher electric field (with increments of 50 V/cm). Continue in the same fashion until cell hyper contracts and does not respond to pacing 1 min after EP pulse delivery. With this approach we expect to observe different types of responses to EP protocol at different electric field strengths (from no response as shown in Figure 2A, to irregular contractions that lead to cell being nonexcitable 1 min after EP protocol as shown in Figure 2D). Compare results for sarcomere shortening and Ca^{2+} transients of cells treated with monopolar or H-FIRE pulses.

REFERENCES:

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EXPECTED RESULTS

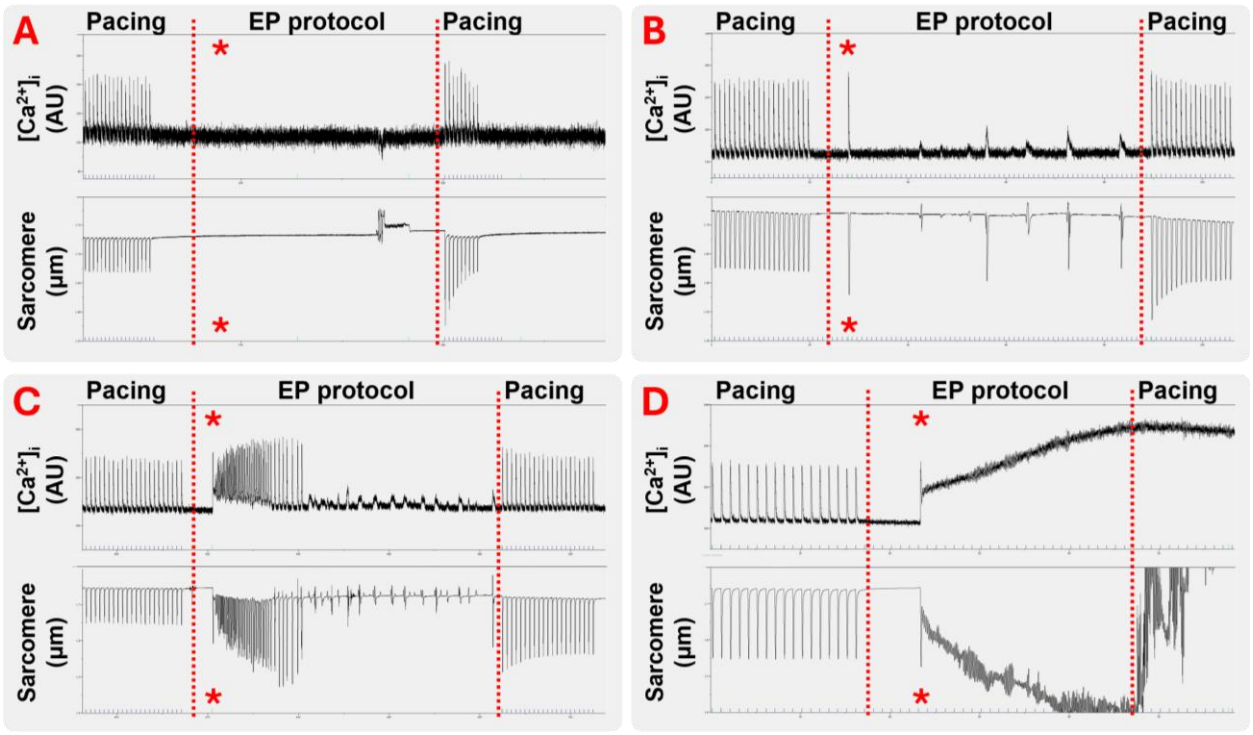

Figure 2: (A-D) Simultaneous measurements of calcium transients ([Ca^{2+}]i), and sarcomere shortening. Primary rat cardiomyocyte was firstly paced at 2 Hz with 8 V bipolar pulses of 2 ms (Pacing). After that the cell will be exposed to either one 100 µs monopolar pulse or one burst of bipolar H-FIRE pulses (25 bipolar pulses, 2 µs positive and negative phase, 2 µs interpulse and interphase delay) (EP protocol). Each pulse or burst of pulses is marked with an asterisk (EP protocol). 1 min after EP protocols 2 ms pacing continued (Pacing after EP protocol). Normally we see different types of responses to electroporation, which correlate with the intensity of electric field at which EP protocol is performed. (A) At lowest electric fields there is no response to EP protocols. (B) When electric field strength is increased EP protocol induces a contraction of a treated cardiomyocyte. (C) When electric field strength is increased even further an EP pulse induces several spontaneous contractions and oscillations in calcium, but the cell can still be paced 1 min after EP pulse delivery. (D) At highest electric fields spontaneous contractions and high levels of calcium are observed and the cell becomes hypercontracted after EP pulse delivery. Cell is unresponsive to pacing 1 min after EP pulse delivery.

NOTES & RESULTS