Simultaneous measurement of sarcomere shortening and calcium transients in primary rat cardiomyocytes exposed to electroporation 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). Basic knowledge of handling with cells is required for this laboratory practice.

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 and cell homeostasis disrupted. Voltage-gated channels can also get affected, although the consensus on the mechanisms and the consequences 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.

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. This can lead to undesired and unpredictable side-effects. For example, excitation-contraction coupling (pairing cardiomyocyte depolarization with mechanical contraction) depends largely on Ca^{2+} signalling 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.

The efficiency of electroporation is influenced by the parameters of the electric field, cell size, geometry, and 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 employed to investigate the influence of these parameters on electroporation and the same dyes can be used to monitor electroporation.

The aim of this laboratory practice is to monitor the effects of monophasic single electric pulse on Ca^{2+} homeostasis, contractility and recovery of adult rat ventricular cardiomyocytes.

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-2AM. As cardiomyocytes are paced, Ca^{2+} concentrations inside the cells change, due to its importance in action potentials, while supraphysiological electric pulses cause changes in Ca^{2+} concentrations due to perturbations in membrane permeability (due to electroporation). Once Ca^{2+} enters the sarcoplasm of cardiomyocytes, they bind to the dye and change its fluorescence intensity more than 100-fold (Figure 1).

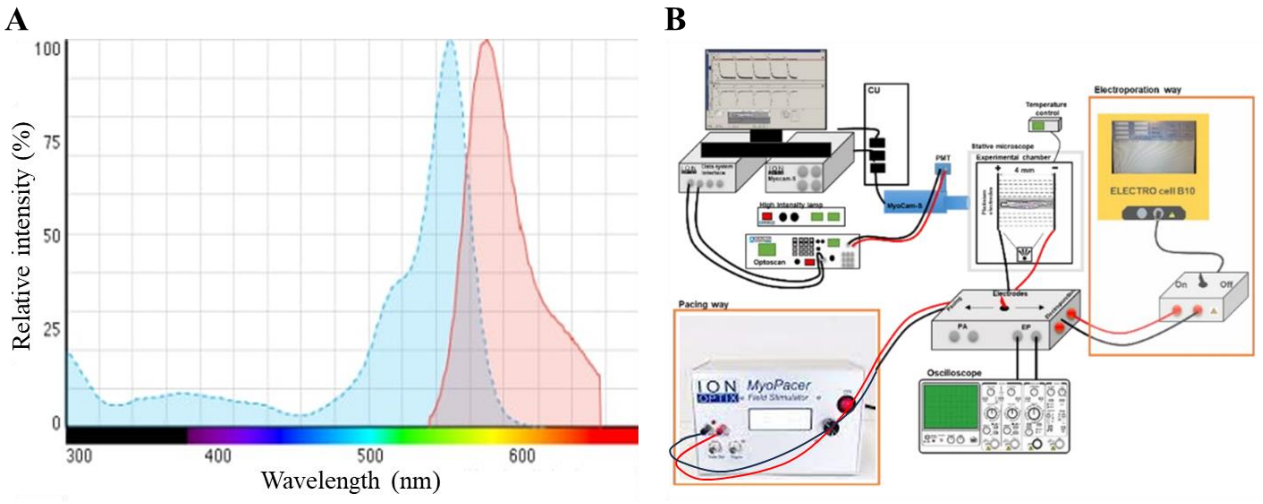

Figure 1: (A) Fluorescence excitation (blue) and emission (red) spectra of Rhod-2 for different concentrations of Ca^{2+} (image from *https://www.thermofisher.com/order/fluorescence-spectraviewer?SID=srch-svtool&UID=14220ca#!/*). (B) Schematic of the experimental setup (adapted from Chaigne, S. et al., 2022).

Protocol: The experiments will be performed on adult rat ventricular cardiomyocytes, isolated from Wistar rats, weighing 250-350 grams. Briefly, on the morning of the experiments adult Wistar rat will be euthanized by anaesthetic isoflurane overdose. After the onset of asphyxia heart will quickly be excised, submerged briefly in cold EGTA solution and then perfused on Langendorff set-up. It will be first perfused with EGTA solution for approximately 5 minutes and then with enzyme solution until the heart will be sufficiently digested. Atria will be removed, while ventricles will be cut in smaller pieces and triturated with a Pasteur pipette for 3 minutes. Cell suspension will then be filtered through a 200 µm nylon mesh filter and resuspended in $CaCl_2$ buffers of increasing Ca^{2+} concentrations until we reach final concentration of 1 mM. Isolated cardiomyocytes will then be pelleted, supernatant will be removed, and the pellet will be resuspended in 199 medium, supplemented with Earle's salts, L-glutamine, 25 mM HEPES, 2.2 g/L $NaHCO_3$, FBS (5 % v/v) and PenStrep. After 60 min of incubation in 199 medium, cardiomyocytes will be washed twice with Tyrode solution (134 mM NaCl, 5 mM KCl, 12 mM HEPES, 1.2 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 11 mM Glucose, 1 M $CaCl_2$, and 5 mM Sodium Pyruvate), after which they will be ready for our measurements. Before measurements, cardiomyocytes will be incubated in Tyrode buffer with 2 µM Rhod-2AM for 20 min at 37 °C (Thermo Fisher Scientific). After the incubation time, cardiomyocytes will be washed with fresh Tyrode to remove excess dye and then seeded on 25 mm square coverslips for imaging.

Insert laminin-coated glass coverslips into IonOptix stimulation chamber on fluorescence microscope (Zeiss Axiovert 200) and use 40xLD objective. Wait 5 min and then start the perfusion of Tyrode 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.

Using a IonOptix MyoPacer Field Stimulator pace cardiomyocytes on the coverslip with bipolar pulses at 1 Hz and 4 ms. IonOptix stimulation chamber is equipped with 2 platinum electrodes (4 mm spacing) and our setup also includes a switch box to alternate between pulses from the IonOptix MyoPacer Field Stimulator for physiological pacing and ELECTRO cell B10 (BetaTech, France) for monopolar

supraphysiological pulses. Using a framing adapter, frame a cardiomyocyte that is perpendicular to the electric field (90±10°) and contracts. Measure basal changes in sarcomere length and Ca^{2+} transients (capture at least 10 contractions). Afterwards use ELECTRO cell B10 device and deliver one electric pulse of 100 µs with voltages either 80 or 140 V (200 and 350 V/cm, respectively) while measuring sarcomere length and Ca^{2+} transients. 1 min after 80 or 140 V supraphysiological pulse delivery start pacing the cell again and record sarcomere length and Ca^{2+} transients for the last time (10 contractions). Capture all measurements for 3 cardiomyocytes on one coverslip for 80 V and one coverslip for 140 V. Compare values for sarcomere shortening and Ca^{2+} transients for both 80 V and 140 V (as shown in Figure 2).

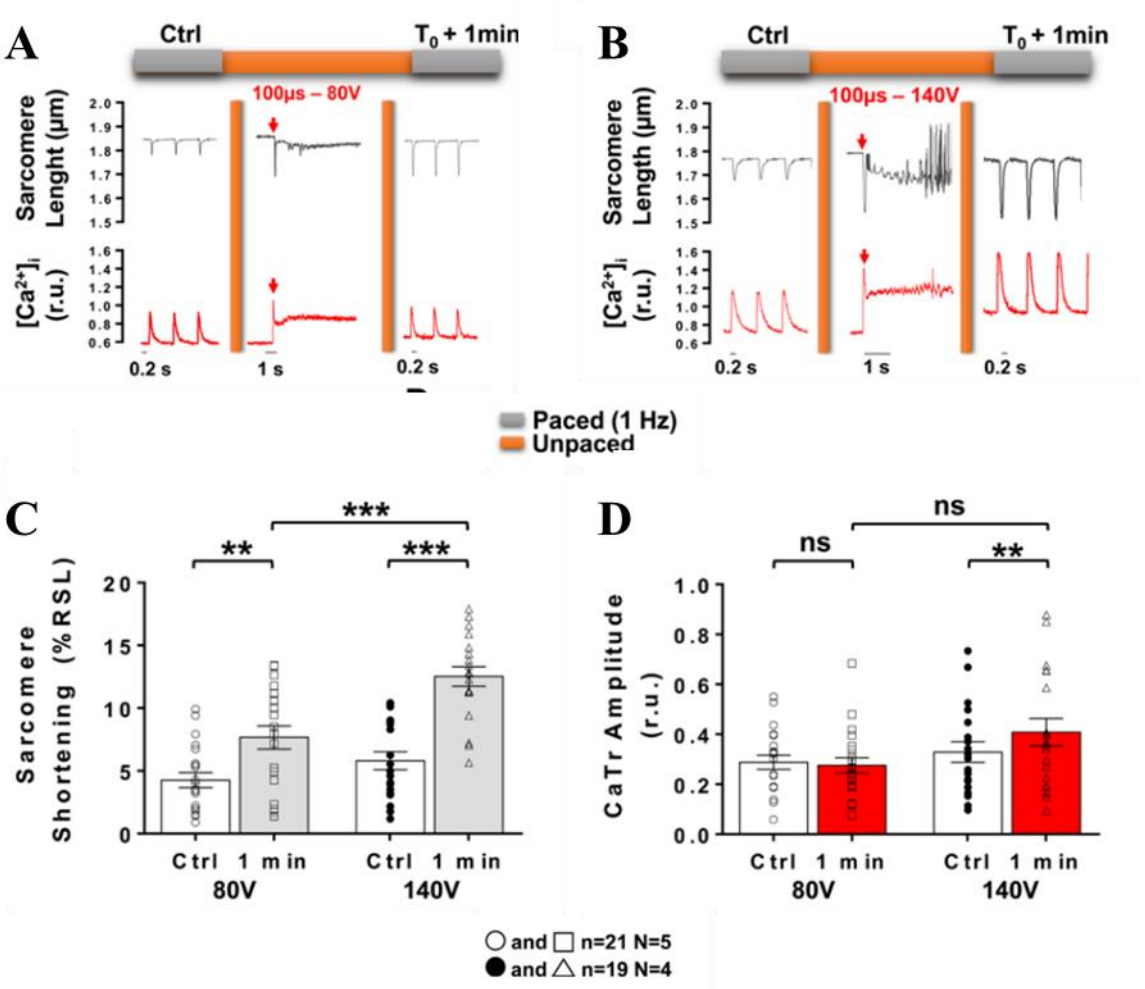

Figure 2: (A-D) Examples of measurements for the effects of 80 and 140 V pulsed electric fields on rat ventricular cardiomyocyte shortening and intracellular Ca^{2+} ($[Ca^{2+}]i$) at 1 minute after application. Representative sarcomere lengths and $[Ca^{2+}]i$ traces obtained from an isolated left ventricular myocytes during baseline (control [Ctrl]) 1 Hz pacing and 1 minute after (T0+1 minute) a 100 µs electric pulse (EP) delivered at (A) low- (80 V) and (B) intermediate-voltage (140 V). (C) Sarcomere shortening, expressed as a percentage of the resting sarcomere length (%RSL) was increased 1 minute after EP with a low- and an intermediate-voltage pulses. (D) Ca^{2+} transient (CaTr) amplitudes expressed in 340:380 ratio units (r.u.), were increased 1 minute after EP with an intermediate but not with a low-voltage pulse. Data are represented as mean±SE of the mean with individual values for each cell. N indicates number of animals; n, number of cells; and ns, not significant. Paired t test: ns, **P<0.01, ***P<0.001. (image from Chaigne, S. et al., 2022).

FURTHER READING:

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NOTES & RESULTS

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