Simultaneous electrical and fluorescence recording of HL-1 cells electrical activity in response to extracellular calcium stimulation

Ondrej Svoobo1,2, Larisa Baiazitova1, Vratislav Cmiel1, Josef Skopalik1, Zdenka Fohlerova2,3, Ivo Provaznik1, Jaromir Hubalek2,3,4

1 Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Technicka 12, Brno, Czech Republic
2 Central European Institute of Technology, Brno University of Technology, Purkynova 123, Brno, Czech Republic
Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Technicka 10, Brno, Czech Republic
3 SIX Centre, Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Technicka 10, Brno, Czech Republic

INTRODUCTION: The beating in vitro models serve as important tools for understanding propagation of action potentials in heart muscle. HL-1 cells, the mouse atrial cardiomyocytes, which express genes having pattern with adult atrial myocytes gives an important information about correlation of biochemical and electrophysiological aspect of the cardiac cells. The microelectrode arrays and fluorescence camera were used to simultaneous electrical and fluorescence recording of HL-1 electrical activity in response to extracellular calcium changes.

METHODS: The low passage HL-1 cells were plated at density of 5×104 cells/ml on gelatin/fibronectin coated microelectrode arrays and cultured for 24 hours at 37°C and 5% CO2. Thereafter, cells were transfected with Accelerated Sensor of Action Potentials 1 (ASAP-1) using PEI MAX 40K and cultured for further 24 hours. After reaching 100% confluence and at least 80% transfection efficiency cell response to extracellular calcium stimulation was simultaneously recorded using 120 channel MEA2100 and Andor Ixon3 860 camera. Intracellular calcium influxes were recorded using X-Rhod-1 AM dye as well. The final concentrations of 4.5, 9.0 and 22.5 µM Ca2+ were studied. Records were analysed in point of amplitude and frequency of action potentials, ASAP-1’s fluorescence response, calcium influxes and electrical/optical time constant (τe/o).

RESULTS: The HL-1 cells start to produce detectable action potentials (APs) when reached 90-100% confluency. These APs were perceptibly spontaneous and had -375±10 µV in the amplitude. Whereas after application of extracellular Ca2+, the APs became periodical with frequency 0.4±0.3 Hz and amplitude -434±45 µV. When reaching the final concentration 22.5 µM Ca2+, the APs's frequency reached 2.1±1.0 Hz and amplitude decreased to -501±14 µV. The ASAP-1 produce fluorescence response up to 21±5% ΔF/F which corresponds to APs propagation on the cell culture. Time constant (τe/o) between electrical and optical detection of AP was determined as 12±5 ms for our setup.