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

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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 gelatine/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% confluency 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.