Cardiac Electrophysiology II

2721-Pos

Light-Induced Depolarization to Stimulate Cardiomyocytes with High Spatio-Temporal Resolution and to Modulate their Differentiation *in Vitro*

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Electrical pacing of cells stimulates only excitable cells by evoking free-running action potentials and has limitations in long term application because of electrolysis. Here we report the use of the light-gated cation channel channelrhodopsin 2 (ChR2) to depolarize non-excitable embryonic stem (ES) cells, to modulate action potentials, and to stimulate long-term cell cultures.

ES cells were transfected with the ChR2-EYFP fusion protein under control of the chicken β-actin promoter. Both, ES cells and differentiated cardiomyocytes showed membrane bound EYFP fluorescence. Application of light (450nm) evoked a non-selective current and depolarization. Constant application of low-intensity light enhanced spontaneous beating frequencies of cardiomyocytes, whereas brief (5ms) high-intensity light stimulation triggered free-running action potentials. Longer illumination led to prolonged action potentials and to an increase of absolute refractory periods.

Monolayers of ChR2-expressing cardiomyocytes plated on multi-electrode-arrays could be locally stimulated by application of spatially confined (~400μm diameter) brief (5ms) illumination whereas constant stimulation induced electrical uncoupling of illuminated areas. Further experiments with different spatio-temporal stimulation patterns can be used to investigate the development of arrhythmias *in vitro*.

To study the impact of depolarization on cardiac development, ES cells were differentiated within embryoid bodies (EBs) on transparent fluorocarbon film on top of light-emitting-diodes. Pulsed light (450nm, 0.6mW/mm^2) was applied starting at day 4 of the *in vitro* differentiation with different stimulation patterns. At day 9 all EBs contracted upon light stimulation. Initial experiments showed higher EYFP fluorescence within beating, cardiac-α-actinin positive areas in illuminated EBs compared to non-stimulated control EBs, suggesting enhanced differentiation and/or hypertrophy of cardiomyocytes. Further long-term experiments with ChrR2 expressing ES cells and different stimulation patterns will be used to investigate the effect of excitation-transcription coupling during ES cell differentiation.

2722-Pos

Differential Conditions for EAD and Triggered Activity in Cardiomyocytes Derived from Transgenic LQT1 and LQT2 Rabbits

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Introduction: LQT1 and LQT2 syndromes, the two most common inherited long QT syndromes, are associated with sudden cardiac death (SCD) due to torsade de pointes (TdP). We investigated the role of EADs and triggered activity in the pathogenesis of arrhythmia in myocytes derived from transgenic rabbit models of LQT1 and LQT2.

Method: We used classic whole cell patch-clamp techniques to study EAD and triggered activity in single myocytes in Tyrode's solution with 3.6 mM K^+. Cardiomyocytes were derived from LQT1, LQT2 and littermate control (LMC) rabbits.

Results: Under extracellular K^+ concentration of 3.6 mM, EADs were observed in 13 out of 16 LQT2 myocytes, and spontaneous TdP-like membrane potential oscillations with a frequency of ~2-3 Hz were observed in one myocyte following the appearance of an EAD (see figure). By contrast, no EADs were observed in 11 LQT1 myocytes and 17 LMC myocytes under these conditions. Incubation with 50 nM of isoproterenol (ISO), however, induced EADs in 3 out of 11 LQT1 myocytes but in none of the 17 LMC myocytes. Spontaneous TdP-like membrane potential oscillation with similar frequency was also observed in one LQT1 myocyte with ISO incubation following the appearance of an EAD. The average action potential duration at 90% repolarization was 536.05 ± 32.54 ms (n=17) in LMC cells, 1013.18 ± 98.88 ms (n=11, $p<0.05$ *vs.* LMC) in LQT1 cells, and 1292.09 ± 189.52 ms at 34 ± 1°C (n=16, $p<0.05$ *vs.* LMC).

Conclusion: EADs and triggered activity tend to occur in LQT2 myocytes under basic conditions, while triggered activity in LQT1 myocytes requires sympathetic stimulation. Both LQT1 and LQT2 myocytes demonstrate the potential for continuous membrane oscillation once EADs are induced.

2723-Pos

Accelerated Heart Rate in Pregnant Mice is not Attributable to Responses to Sympathetic Stimulation

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Pregnancy is associated with an increase in heart rate (HR) which is a risk factor for the development of cardiac arrhythmias. It is unclear whether pregnancy related alterations in heart rate are mediated by changes in cardiac autonomic stimulation and/or alterations in the cardiac conduction system. Therefore the objective of this study was to compare heart rate between pregnant (P) and non-pregnant (NP) mice and determine whether heart rate is influenced by the autonomic nervous system. Initially, surface ECG were recorded from anaesthetized NP, P and post-partum (PP) mice under control conditions. The heart rate was significantly increased in P compared to NP and PP mice (P: 530.8 ± 14.0 bpm, n=15; NP: 469.6 ± 26.5 bpm, n=10; PP: 476.8 ± 16.3 bpm, n=6; $p<0.05$ P vs NP and PP). Moreover, the response to isoproterenol and propranolol was not significantly different between P and NP mice. In fact, in presence of isoproterenol (100 nM), heart rate increased by 15 ± 4% (n=7) and 27 ± 7% (n=7) in P and NP mice, respectively (p=NS). Whereas, propranolol (250 μM) reduced heart rate by 22 ± 2% (n=7) and 30 ± 4% (n=7) in P and NP mice, respectively (p=NS). This suggests that sympathetic stimulation is not responsible for the increased heart rate observed in pregnant mice. Furthermore, in Langendorff-perfused hearts, heart rate was faster in P compared to NP mice (HR: P 385.83 ± 18.11 bpm, n=11; NP 327.3 ± 15.75 bpm, n=10). Taken together, these results suggest that changes in heart rate in P mice are not attributable to pregnancy-related alterations in the sympathetic innervation of the heart. Thus, it is possible that changes in heart rate in pregnant mice may be mediated by alterations in the cardiac conduction system.

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Effect of Regional Mitochondrial Uncoupling on Electrical Propagation in Cardiac Cell Monolayers

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The collapse of mitochondrial inner-membrane potential ($\Delta\Psi_m$) is a key determinant of cell injury and arrhythmogenesis associated with ischemia-reperfusion or oxidative stress. We have previously demonstrated that mitochondrial uncoupling resulting from the activation of energy-dissipating mitochondrial channels can activate sarcolemmal K_{ATP} channels, which can profoundly alter cellular electrical excitability. We proposed that these regions could serve as "metabolic sinks" of current that could be a substrate for reentry. Here, we directly test whether local $\Delta\Psi_m$ loss influences electrical propagation in monolayers of neonatal rat ventricular myocytes (NRVMs) using a method of local perfusion of a portion of the monolayer with the mitochondrial oxidative phosphorylation uncoupler, FCCP (a protonophore; 1μM). Propagation of the electrical wave through the monolayer was recorded by optical mapping with a 464-element photodiode array using the voltage-sensitive fluorescent dye, di-4-ANEPPS. Using a custom-built perfusion device, a 5mm circular zone in the center of the coverslip (full diameter 2cm) was exposed to FCCP with the remainder perfused with normal Tyrode's buffer, and the monolayer was stimulated from one edge at 1Hz. Upon encountering the metabolic sink, the wave of electrical depolarization was slowed and the amplitude of the action potentials in the FCCP-perfused area decreased significantly. The results indicate that heterogeneous $\Delta\Psi_m$ collapse can significantly alter the electrical substrate in a manner that could promote reentry.

2725-Pos

Electrical Behavior At the Transverse-Axial Tubular Membrane of Hypertrophic Cardiomyocytes *in Situ*

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Cardiac hypertrophy is associated with restructuring of the transverse-axial tubular (TAT) system. It is unknown whether such changes are accompanied by altered electrical behavior at the transverse-axial tubules. Here, we used confocal microscopy and the voltage-sensitive dye ANNINE-6plus to visualize the TAT network and to resolve action potentials at the surface and TAT membrane of *in situ* left ventricular cardiomyocytes within normal and hypertrophic mouse hearts. Transgenic (TG) hearts overexpressing Gαq and non-transgenic (NTG) hearts were perfused in Langendorff-mode during image acquisition. Cardiomyocytes in hypertrophic TG hearts exhibited patchy loss and gain, respectively, of transverse and axial elements. Action potentials were recorded at the TAT membrane within two adjacent cardiomyocytes (denoted T_1 and T_2) and at the end-to-end junctions (E) between