

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 (~400 μ m 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/mm²) 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 ChR2 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 \pm 32.54 ms (n=17) in LMC cells, 1013.18 \pm 98.88 ms (n=11, p<0.05 vs. LMC) in LQT1 cells, and 1292.09 \pm 189.52 ms at 34 \pm 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 \pm 14.0 bpm, n=15; NP: 469.6 \pm 26.5 bpm, n=10; PP: 476.8 \pm 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 \pm 4% (n=7) and 27 \pm 7% (n=7) in P and NP mice, respectively (p=NS). Whereas, propranolol (250 μ M) reduced heart rate by 22 \pm 2% (n=7) and 30 \pm 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 \pm 18.11 bpm, n=11; NP 327.3 \pm 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.

2724-Pos

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; 1 μ M). 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.

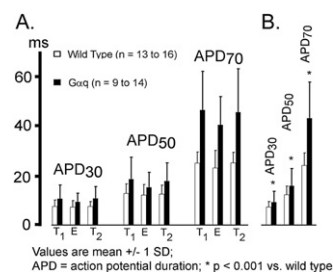
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Electrical Behavior At the Transverse-Axial Tubular Membrane of Hypertrophic Cardiomyocytes *in Situ*

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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 $G\alpha_q$ 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 T₁ and T₂) and at the end-to-end junctions (E) between



them. Action potential onset occurred synchronously within the TAT network of NTG and TG cardiomyocytes. Whereas the repolarization time course was similar between the three recording sites in NTG or TG hearts (Figure A), overall repolarization was prolonged in TG cardiomyocytes (Figure B). Thus, electrical coupling between the surface and TAT membrane was maintained despite pronounced TAT membrane restructuring in this model of cardiac hypertrophy.

2726-Pos

Unraveling of a Novel Cation Current in Cardiac Myocytes using Fenamates

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Interest in non-selective channels has increased recently following the discovery of transient receptor potential (TRP) proteins, which underlie many of these channels. We used the whole-cell patch-clamp technique on isolated ventricular myocytes to investigate the effect of fenamates on membrane ion currents. With voltage-dependent and other ion channels inhibited, all cells that were challenged with either N-(p-aminocinnamoyl)anthranilic acid (ACA, $\geq 3\mu\text{M}$), ONO-RS-082 ($\geq 100\mu\text{M}$) or flufenamic acid ($\geq 100\mu\text{M}$) responded with an increase in currents (induced current: -0.8 ± 0.06 pA/pF at -120 mV with $30\mu\text{M}$ ACA; $n=9$). ACA was the most potent ($K_{0.5} = 13\mu\text{M}$) of all drugs tested. The induced current reversed at $+43 \pm 2.2$ mV ($n=9$) and its inward but not outward component was suppressed in Na^+ -free extracellular conditions (Na^+ replaced by NMDG⁺). The current and its reversal potential (E_{rev}) were unaffected by lowering extracellular Cl^- concentration or by the removal of extracellular Ca^{2+} and Mg^{2+} . The current could not be induced by other non-fenamate anti-inflammatory drugs such as diclofenac, nor by non-fenamate phospholipase-A₂ inhibitors such as bromoeloin lactone and bromophenacyl bromide. Muscarinic or α -adrenergic receptor activation or application of diacylglycerol failed to induce or enhance the current. The lack of effect of removing extracellular divalent cations and the fact that the induced current could be obtained in the presence of high intracellular Mg^{2+} indicated that the channel implicated is not TRPM7. Given our experimental conditions, where Na^+ is the only ion with an equilibrium potential close to the above E_{rev} values, it is very likely that Na^+ carries the novel current induced by fenamates.

2727-Pos

Reductions in Ventricular Ca^{2+} Current Occur Independently of Cardiac Remodelling in Transgenic Mice with Cardiac Specific Overexpression of the Human Type 1 Angiotensin II Receptor

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Transgenic mice with cardiac specific overexpression of the human type 1 angiotensin II receptor (AT1R mice) develop hypertrophy and decreased cardiac contractility. However, it is unclear whether altered contractility is attributable to hypertrophy or AT1R overexpression and whether this differs between sexes. Since L-type Ca^{2+} current (I_{CaL}) is crucial for cardiac contraction, we characterized the effects of AT1R overexpression on ventricular I_{CaL} in the presence (older mice: 6-month) or absence (younger mice: 50-day) of cardiac hypertrophy in both male (M) and female (F) mice. Voltage-clamp recordings revealed the density of I_{CaL} did not differ between sexes for either age group for AT1R and wild-type (WT) mice. However, I_{CaL} density (in pA/pF) was significantly reduced in ventricular myocytes from 50-day male and female AT1R mice (at 0 mV, M: -4.2 ± 0.3 , $n=17$ and F: -3.2 ± 0.3 , $n=6$) compared to age-matched WT (M: -7.4 ± 0.4 , $n=20$ and F: -6.8 ± 0.9 , $n=5$) (all $p < 0.05$). Similarly, I_{CaL} was significantly reduced in 6-month male and female AT1R myocytes (at 0 mV, M: -3.6 ± 0.2 , $n=15$ and F: -3.0 ± 0.4 , $n=5$) in comparison to WT cells (M: -5.9 ± 0.2 , $n=17$ and F: 6.4 ± 0.2 , $n=10$) (all $p < 0.05$). Using real-time RT-PCR, we showed that ventricular $\text{Ca}_v1.2$ (L-type Ca^{2+} channel α -subunit) mRNA expression was decreased in 50-day and 6-month male and female AT1R mice compared to age- and sex-matched WT mice. Overall, the data indicates that the reduction in I_{CaL} and $\text{Ca}_v1.2$ in AT1R mice occurs independently of sex and cardiac remodelling. These alterations could contribute to the decreased cardiac contractility observed in AT1R mice.

2728-Pos

Sphingosine-1-Phosphate Regulates Volume-Sensitive Chloride Current in Ventricular Myocytes by Means of ROS

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We previously demonstrated that bacterial sphingomyelinase (SMase) activates a volume-sensitive current, $I_{\text{Cl,swell}}$, by a pathway that involves mitochondrial ROS production. SMase activity generates endogenous ceramides from sphingomyelin in the outer plasma membrane leaflet and, in turn, ceramides are metabolized to several sphingolipids, including sphingosine-1-phosphate (S1P). We tested whether ceramide metabolites are responsible for eliciting $I_{\text{Cl,swell}}$. Under isosmotic conditions that isolate anion currents, SMase-induced $I_{\text{Cl,swell}}$ was abrogated by blockade of ceramidase (converts ceramide to sphingosine) with D-erythro-MAPP ($10\mu\text{M}$). SMase-induced $I_{\text{Cl,swell}}$ also was suppressed by inhibition of sphingosine kinase with DL-threo-dihydrosphingosine ($10\mu\text{M}$). These data suggested that the ceramide metabolite S1P is likely to stimulate $I_{\text{Cl,swell}}$. As expected, exogenous S1P (500 nM) elicited an outwardly rectifying Cl^- current that was fully inhibited by the $I_{\text{Cl,swell}}$ -specific blocker DCPIB ($10\mu\text{M}$). As seen with SMase-induced $I_{\text{Cl,swell}}$, S1P-induced $I_{\text{Cl,swell}}$ was fully inhibited by the mitochondrial Complex I blocker rotenone ($10\mu\text{M}$), which suppresses extramitochondrial ROS release by Complex III. In contrast to results with SMase, S1P-induced current was partially inhibited by blockade of NADPH oxidase (NOX) with apocynin ($500\mu\text{M}$). These data indicate that S1P is a necessary component of SMase-induced $I_{\text{Cl,swell}}$ activation and that the action of exogenous S1P involves ROS from both mitochondria and NOX. Importantly, exogenous C₂-ceramide ($2\mu\text{M}$), a synthetic short-chain ceramide, also elicits $I_{\text{Cl,swell}}$ even though C₂-ceramide is not metabolized to S1P in native cells. Thus, it seems likely that ceramides can elicit $I_{\text{Cl,swell}}$ via S1P and also by a distinct pathway and that both pathways converge at mitochondrial ROS.

2729-Pos

Modeling the Dynamic Currents Recorded under Action Potential-Clamp in Cardiac Myocytes

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The rhythm and shape of the cardiac action potential (AP) adapt on a moment-to-moment basis to our physical activity, emotional state, even our breathing. Underlying this exquisite adaptability is a constellation of ion channels and transporters that respond to extracellular and intracellular signals and the membrane voltage itself. At any moment, the dynamical behavior of the AP is governed by the sum of all ionic currents. Subtle changes in the kinetics or magnitudes of some currents can upset the precise choreography and generate, for example, early after-depolarization (EAD), which are often precursors to ectopic arrhythmias. Current cardiac AP models can reproduce the steady state AP properties but is less successful in accurately describing the transient/dynamic behavior of the AP such as those during adaptation and restitution, which are indicators of arrhythmias.

We hypothesized that this inaccuracy could be due to the experimental methods used to obtain the data for creating models. Current models are largely based on the experimental data obtained from traditional voltage clamp experiments using square pulse protocol and non physiological milieu. To overcome this limitation, we used the *self AP-clamp* technique to record the dynamic ionic currents under the cell's own AP in physiological milieu, which provides an accurate measure of the ionic currents experienced by the cell *in situ*. Here we report the modeling results describing the dynamic behavior of the ionic currents measured during the AP in guinea pig ventricular myocytes.

2730-Pos

The Role of Kinesin I and a Small Gtpase in the Forward Trafficking of Kv1.5 Channels

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Kv channels play important roles in the repolarization phase of the action potential in cardiac cells. The regulation of functional Kv1.5 surface expression has been reported to be modulated by retrograde trafficking through dynein motor but little is known about regulation by forward trafficking. Here, we use electrophysiological and immunocytochemical methods to investigate the mechanisms and regulation of anterograde trafficking of newly synthesized Kv1.5 channel proteins in cultured cells and in adult cardiomyocytes. Over-expression of a kinesin I isoform (Kif5b) increased outward K^+ current by two fold in cultured cells stably expressing Kv1.5. This enhancement of Kv1.5 current by Kif5b was blocked by a six hour treatment with Brefeldin A. Over-expression of Kif5b increased Kv1.5 current additionally with inhibition of endocytosis by p50 over-expression and dynamin inhibitory peptide. Deletion of a specific SH3-binding domain in Kv1.5 that is essential for internalization of the channel similarly enhanced Kif5b-induced Kv1.5 current. Expression of a dominant negative Kif5b mutant prior to induction of Kv1.5 in a tetracycline-inducible system almost completely blocked Kv1.5 current. These results