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P602

The hidden electrophysiological cardiotoxic effects of rofecoxib are prevented by ischaemic preconditioning

Husti Z.[1]; Varga RS.[1]; Brenner G.[2]; Bencsik P.[1]; Giricz Z.[2]; Gorbe A.[2]; Shulz R.[3]; Varro A.[1]; Ferdinandy P.[2]; Baczko I.[1]

[1]University of Szeged, Faculty of Medicine, Department of Pharmacology & Pharmacotherapy, Szeged, Hungary
[2]Semmelweis University, Department of Pharmacology and Pharmacotherapy, Budapest, Hungary
[3]Justus-Liebig University of Giessen, Institute of Physiology, Giessen, Germany

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Ischaemia-induced unexpected cardiac adverse events are major contributors to clinical trial discontinuation and drug attrition, and routine preclinical cardiotoxicity assessment fails to detect these effects. We found that rofecoxib possessed cardiac electrophysiological adverse effects only revealed during ischaemia/reperfusion, a phenomenon we termed "hidden cardiotoxicity". The conventional microelectrode technique was used to record action potentials from rat left ventricular papillary muscles. Under normoxic and ischaemic conditions, the following parameters were measured in the presence or absence of 1μM or 10 μM rofecoxib: conduction time, action potential amplitude, action potential duration at 75% and 90% repolarization (APD75, APD90).

The effects of rofecoxib during test ischaemia and reperfusion and the influence of preceding ischaemic preconditioning were studied. Rofecoxib (1 or 10 μM) did not influence electrophysiological parameters in normoxic conditions. Following 30 minute ischaemia, APD90 was significantly increased during reperfusion compared to APD90 in baseline: 67.9 ± 10.51 ms vs. 51.3 ± 7.13 ms ($p < 0.05$, $n = 6$), respectively. 1 μM and 10 μM rofecoxib further prolonged APD90: 83.1 ± 15.49 ms and 97.3 ± 5.57 ms ($p < 0.05$, $n = 6$), respectively. In groups, where ischemic preconditioning preceded test ischaemia, further increase in repolarization in the presence of rofecoxib was not observed upon reperfusion. This is the first demonstration that preclinical cardiac safety testing of a drug in ischemia/reperfusion may reveal its hidden cardiotoxicity, and that the cardiac electrophysiological adverse effects of rofecoxib can be prevented by ischaemic preconditioning.

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P603

A high-throughput system to reliably characterize normal human ventricular electrophysiology from living patients

Olivan-Viguera A.[1]; Fernandez-Bes J.[1]; Marigil MA.[2]; Vallejo-Gil JM.[3]; Ballerter-Cuenca C.[3]; Pueyo E.[1]

[1]University of Zaragoza, Zaragoza, Spain
[2]San Jorge Hospital, Department of Pathology, Huesca, Spain
[3]University Hospital Miguel Servet, Department of Cardiovascular Surgery, Zaragoza, Spain

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Background/Introduction: Cardiac tissue slices have been proposed as a research model that offers preserved three-dimensional tissue structure, cellular contacts and representative populations of cells in a less expensive and more controlled manner than in vivo studies or ex vivo whole heart analysis. In the case of left ventricular slices, to date these are generally obtained from tissue blocks of failing human hearts or of sacrificed animals.

Methods: In this study tru-cut left ventricular transmural biopsies representative of normal human myocardium were obtained from patients undergoing coronary artery surgery or valve repair. In addition, left ventricular transmural tissue blocks (surface area 5 × 5 mm) were obtained from explanted pig hearts and compared with tru-cut biopsies obtained from the same ventricular area. Both tru-cut biopsies and tissue blocks were sliced in a vibratome, providing a large number of viable 350 μm-thick slices, which were used for viability and histological evaluation as well as electrophysiological assessment. Transmembrane potential and intracellular calcium were optically measured and Action Potential Duration (APD) and calcium transient (CaT) characteristics were calculated at pacing frequencies ranging from 1 to 4 Hz. The response to beta-adrenergic stimulation was evaluated by application of isoproterenol at various concentrations.

Results: Viability assays indicated no damage effects associated with biopsy collection and slicing. Histological analysis of sections stained with haematoxylin-eosin and Masson's trichrome revealed well-preserved tissue structure. In pigs, mean optically mapped APD and CaT duration presented no significant differences between tissue blocks and biopsies for all tested pacing frequencies, both at baseline and in response to beta-adrenergic stimulation. In humans, APD and CaT duration could be successfully measured at all frequencies and a reduction in their mean values was quantified following beta-adrenergic stimulation.

Conclusions: Tru-cut left ventricular biopsies provide an affordable way to obtain normal human myocardial tissue from a wide range of patients, suitable for electrophysiological assessment and representative of the native myocardium.

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Pros and cons of human iPSC cardiomyocytes in drug testing and disease modelling: insights from computational modelling

Koivumaeki JT.; Paci M.; Hyttinen J.

Tampere University, Faculty of Medicine and Health Technology, Tampere, Finland

Funding Acknowledgements: Academy of Finland, Finnish Foundation for Cardiovascular Research, Paavo Nurmi Foundation, Tekes Finland, and Finnish Cultural Foundation

Background: Human induced pluripotent stem cells (hiPSCs) have emerged as a very promising approach for cardiac research.

Purpose: Exploitation of the full potential of hiPSC-derived cardiomyocytes (hiPSC-CMs) in drug testing or disease modelling boils down to a fundamental question: how well do hiPSC-CMs recapitulate the functional properties of native/mature human ventricular CMs (hV-CMs)? Here, we present our recent efforts to use computational methods and virtual hiPSC-CM models to answer that question.

Methods: We have developed mechanistic models that not only incorporate the standard mathematical formulations of transmembrane ionic currents and dynamics of intracellular ionic concentrations, but also account for characteristic (ultra-)structural (irregular shape, lack of regular t-tubular network, and unevenly distributed intracellular calcium stores) and functional (spontaneous electrical activity) features of hiPSC-CM.

Results: We have used the virtual cell models to elucidate 1) the mechanisms of automaticity, and 2) the characteristics of excitation-contraction coupling of hiPSC-CMs. Furthermore, we have employed the computational approach to investigate, how well the in vitro hiPSC-CM results translate to the human/patient context 1) in various familial heart diseases, and 2) when pharmacological agents are applied to block ion channels. Our simulation results suggest that the physiological properties of hiPSC-CMs differ from hV-CMs in a way that warrants caution, as hiPSC-CMs show less robustness and greater tendency for arrhythmic events than hV-CMs.

Conclusions: While recent efforts by multiple laboratories have succeeded in producing hiPSC-CMs with more mature-like electrical and contractile function, there are no common criteria for assessing these properties. Therefore, translation of findings from disease-related mutations or pharmacological interventions is not straightforward. Virtual hiPSC-CM models provide an important parallel strategy for overcoming the limitations of experimental in vitro models.

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P605

Advanced morpho-functional analysis on ventricular and atrial tissue reveals sarcomere energetic impairment in hcm patients

Lazzeri E.[1]; Vitale G.[2]; Giardini F.[1]; Costantini I.[1]; Mazzamuto G.[1]; Ferrantini C.[2]; Tesi C.[2]; Pavone FS.[1]; Poggesi C.[2]; Sacconi L.[3]

[1]LENS - European Laboratory for Non-Linear Spectroscopy, Sesto Fiorentino, Italy
[2]University of Florence, Florence, Italy
[3]INO - National Institute of Optics, florence, Italy

Mutations in MYBPC3, the gene coding for cardiac myosin-binding protein-C (cMyBP-C), are the most common cause of Hypertrophic CardioMyopathy (HCM). The E258K-MyBP-C is a highly penetrant missense mutation with poorly understood molecular mechanisms. Mechanics of contraction as well the energy cost of tension generation were investigated using left ventricular (LV) and atrial tissue from E258K HCM patients and from donor hearts. Maximal ATPase and isometric active tension were simultaneously measured in permeabilized LV and atrial strips. Direct measurements in skinned LV and atrial strips showed that force production was lower while tension cost was higher in E258K preparations compared to controls. To check whether cardiomyocyte disarray, typical of HCM hearts, may have contributed to artificially decrease the force or increase the tension cost measured in the HCM preparations, the strips used for mechanical investigations were immunostained, clarified and imaged at mesoscale level. An immunostaining protocol using alpha-actinin antibody is optimized to visualize sarcomere structure. Then, an advanced tissue clearing method in combination with two-photon microscopy was employed to reconstruct the 3D image of the strips at sub-micrometer spatial resolution. A 3D cytoarchitecture analysis tool based on 3D Fourier Transform was developed and applied to determine cardiomyocyte orientation across and along the strips. Both global and local statistics of spatial disarray were derived and correlated to mechanical and energetic data. The results did not highlight structural differences between donor and HCM patients, but normalizing the force production on alignment and the tension cost on dispersion, the results show an increase in significance. In conclusion, mechanics of contraction as well the energy cost of tension generation were influenced by the structure but the E258K mutation primarily impairs sarcomere energetics.

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P606

Istaroxime improves diabetic diastolic dysfunction through SERCA stimulation

Torre E.[1]; Lodrini AM.[1]; Barassi P.[2]; Ferrandi M.[2]; Boz E.[3]; Bussadori C.[3]; Ferrari P.[2]; Bianchi G.[2]; Rocchetti M.[1]

[1]University of Milan-Bicocca, Milan, Italy
[2]CVie Therapeutics Limited , Taipei, Taiwan ROC
[3]Clinica Veterinaria Gran Sasso, Milan, Italy

Calcium handling is generally impaired in heart failure (HF). Mechanisms that can restore cardiac relaxation (lusitropic effect) improving cellular Ca2+ cycling, represent a promising therapeutic approach for HF. Istaroxime is a Na-K ATPase (NKA) inhibitor with the property of accelerating Ca2+ re-uptake into sarcoplasmic reticulum (SR) through the SR Ca2+ pump (SERCA) stimulation by displacing the interaction between SERCA and its inhibitor, phospholamban (PLB).

The project aims to characterize Istaroxime effects in a model of mild diabetes (type 1) with diastolic dysfunction and preserved global systolic function. Istaroxime was tested at a concentration mostly unaffecting NKA to isolate its effects dependent on SERCA only. Streptozotocin (STZ)-treated rats were evaluated at 9 weeks after STZ injection in comparison to control (CTR) ones. STZ-induced changes were evaluated in vivo (echocardiography), in isolated left ventricular (LV) myocytes and in SERCA2a-enriched microsomes. SERCA and PLB protein levels were measured by western blot and SERCA activity as 32P hydrolysis. Action potential rate-dependency and intracellular Ca2+ handling were evaluated in patch clamped or field-stimulated (2Hz) cells.

STZ-induced cardiomyopathy was characterized by cardiac hypotrophy, heart rate and cardiac output reduction. Echo parameters showed impaired diastolic relaxation which was associated to reduced SERCA protein level and activity at the cellular level. Moreover, the monomeric PLB/SERCA ratio was increased, implying that SERCA was not only reduced but also much more inhibited in STZ-treated animals. In STZ group, action potentials (AP) were significantly prolonged at each cycle length and the beat-to beat variability increased; Ca2+ transients were characterized by slower decay, delayed onset and increased diastolic Ca2+. Istaroxime at a concentration of 100 nM significantly stimulated SERCA activity and SR Ca re-uptake after caffeine depletion in STZ group only. Moreover, Istaroxime reduced STZ-induced diastolic Ca2+ enhancement, without altering Ca2+ transient amplitude and SR Ca2+ content. STZ-induced AP prolongation was not affected by Istaroxime.

Overall, SERCA stimulation can be considered a promising therapeutic approach for diastolic dysfunction treatment.

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P607

Mechanism of sinus node dysfunction in carriers of the E161K mutation in the SCN5A gene

Wilders R.

Academic Medical Center, University of Amsterdam, Department of Medical Biology, Amsterdam, Netherlands

Background: Some but not all carriers of the E161K mutation in the SCN5A gene, which encodes the Nav1.5 pore-forming α-subunit of the ion channel underlying the cardiac fast sodium current (INa), show sinus bradycardia and occasional exit block. Voltage clamp experiments on wild-type and mutant INa channels in mammalian expression systems have revealed a mutation-induced 2.5 to 4-fold reduction in INa peak current density as well as a +19-mV shift and reduced steepness of the steady-state activation curve. The highly common H558R polymorphism in Nav1.5 limits the shift in steady-state activation to +13 mV, but also introduces a −10-mV shift in steady-state inactivation.

Purpose: We assessed the cellular mechanism by which the E161K mutation causes sinus node dysfunction in heterozygous mutation carriers as well as the potential role of the H558R polymorphism.

Methods: We incorporated the mutation-induced changes in INa into the Fabbri-Severi model of a single human sinoatrial node cell and the Maleckar et al human atrial cell model, and carried out computer simulations under conditions of normal autonomic tone and vagal tone.

Results: In H558 background, the E161K mutation increased the intrinsic cycle length of the sinoatrial node cell by 54 ms. Under vagal tone, through the simulated presence of 10–25 nM acetylcholine, this increase was raised to 104–347 ms, reducing the beating rate at 25 nM acetylcholine from 41 to 33 beats/min. The increase in cycle length was the result of a significant slowing of diastolic depolarization. The mutation-induced reduction in INa window current had reduced the contribution of the mutant component of INa to the net membrane current during diastolic depolarization to effectively zero. Highly similar results were obtained in R558 background. Atrial excitability was reduced, in either background, as reflected by an increase in threshold stimulus current and a concomitant decrease in capacitive current of the atrial cell.

Conclusions: We conclude that the experimentally identified mutation-induced changes in INa can explain the clinically observed sinus node dysfunction. Furthermore, we conclude that the common H558R polymorphism does not significantly alter the effects of the E161K mutation and can thus not explain the reduced penetrance of the E161K mutation.

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P608

Blockade of the adenosine 2A receptor mitigates the cardiomyopathy induced by loss of plakophilin-2 expression

Van Opbergen C J M[1]; Malkani K.[2]; Irrera N.[3]; Zhang M.[2]; Van Veen TAB[1]; Cronstein B.[4]; Delmar M.[2]; Cerrone M.[2]

[1]University Medical Center Utrecht, Department of Medical Physiology, Utrecht, Netherlands
[2]New York University Langone Medical Center, Leon H. Charney Division of Cardiology, New York, United States of America
[3]University of Messina, Department of Clinical and Experimental Medicine, Messina, Italy
[4]New York University Langone Medical Center, Division of Translational Medicine, New York, United States of America

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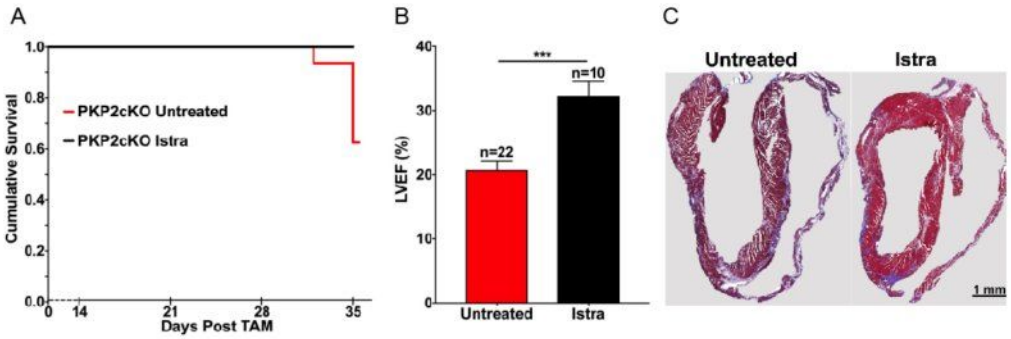
OnBehalf: Department of Medical Physiology

Background: Mutations in plakophilin-2 (PKP2) are the most common cause of familial Arrhythmogenic Right Ventricular Cardiomyopathy, a disease characterized by ventricular arrhythmias, sudden death and progressive fibrofatty cardiomyopathy. The relation between loss of PKP2 expression and structural cardiomyopathy remains under study, though paracrine activation of pro-fibrotic intracellular signaling cascades is a likely event. Previous studies have indicated that ATP release into the intracellular space, and activation of adenosine receptors, can regulate fibrosis in various tissues. However, the role of this mechanism in the heart, and in the specific case of a PKP2-initiated cardiomyopathy, remains unexplored. The aim of this study was to investigate the role of ATP/adenosine in the progression of a PKP2-associated cardiomyopathy.

Methods and Results: HL1 cells were used to study PKP2- and Connexin43 (Cx43)-dependent ATP release. HL1 cells silenced for PKP2 showed increased ATP release compared to control. Knockout of Cx43 in the same cells blunted the effect. A cardiac-specific, tamoxifen-activated PKP2 knock-out murine model (PKP2-cKO) was used to define the effect of adenosine receptor blockade on the progression of a PKP2-dependent cardiomyopathy. Transcriptomic data of PKP2-cKO mice revealed overexpression of genes involved in adenosine-receptor cascades. Treatment with Istradefylline (an adenosine 2A receptorblocker) tempered the progression of fibrosis and mechanical failure observed in PKP2-cKO mice (see Fig. B,C). In contrast, PSB115, a blocker of the 2B adenosine receptor, showed opposite effects.

Conclusion: Paracrine adenosine 2A receptor activation contributes to the progression of fibrosis and impaired cardiac function in animals deficient in PKP2. Given the limitations of the animal model, translation to the case of patients with PKP2 deficiency needs to be done with caution.

Abstract P608 Figure. Istradefylline treatment PKP2-cKO mice



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P609

Distinct effect on Ca2+ cycling depending on the level of cardiac specific FKBP12.6 overexpression

Gandon-Renard M.[1]; Lefebvre F.[1]; Courilleau D.[2]; Gomez S.[1]; Gerbaud P.[1]; Gomez AM.[1]; Mercadier JJ.[1]

[1]University of Paris-Sud 11, Laboratory of Signaling and Cardiovascular Pathophysiology, INSERM UMR-S 1180, Chatenay-Malabry, France
[2]University of Paris-Sud 11, UMS-IPSIT, Chatenay-Malabry, France

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Background: FKBP12.6 belongs to the FK506 binding proteins (FKBP) immunophilin family, and, in cardiomyocytes, is a regulator of ryanodine receptors, RyR2, with a strong affinity for these Ca2+ channels which have an essential role in cardiac excitation-contraction coupling. In the heart, another isoform, FKBP12, is known to regulate RyR2 with a weaker affinity than FKBP12.6. Furthermore, in cardiomyocytes, FKBP12.6 is less abundant than FKBP12. In heart failure (HF), FKBP expression decreases, which may participate in arrhythmia development. Moreover, arrhythmias induced by β-adrenergic stimulation are prevented in mice with cardiac FKBP12.6 overexpression, but the anti-arrhythmic mechanism remains unknown.

Methods: To address this issue, we developed 2 transgenic mouse lines with cardiac-specific moderate- (TG1) and high- (TG2) overexpression levels of FKBP12.6 to characterize cardiac function, [Ca2+]i cycling and β-adrenergic signaling.

Results: Both TG mouse lines developed cardiac hypertrophy, mild for TG1 and marked for TG2. Basal cardiac function was increased only in TG1 mice, whereas TG2 mice were associated with intraventricular asynchrony. In stimulated isolated cardiomyocytes, [Ca2+]i transient amplitude, measured by confocal microscopy, was higher in TG1 than in wild type (WT) mice, without a significant difference in their SR Ca2+ content. The β-adrenergic stimulation obtained by adding 50 nM of isoproterenol, had lower effect in TG1 than in WT mice, associated with a prevention of pro-arrhythmogenic Ca2+ release events, like Ca2+ waves. In contrast, TG2 mice showed [Ca2+]i handling characteristics similar to HF, with slower [Ca2+]i transient relaxation. Interestingly, and contrary to HF, pro-arrhythmogenic Ca2+ release events were also reduced in TG2.

Conclusion: These results indicate distinct effects depending on the level of FKBP12.6 overexpression on cardiac function and on Ca2+-cycling and its response to β-adrenergic stimulation.

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P610

A clinically applicable method for the identification of slow electrical conduction using rescaled electrograms

Winter J.

University of Birmingham, Birmingham, United Kingdom

Funding Acknowledgements: British Heart Foundation

Background: Electrophysiological mapping is used clinically to guide catheter ablation procedures for the treatment of monomorphic ventricular tachycardia. One approach, known as substrate mapping, attempts to determine regions of fibrotic scar through the use of indirect electrical indices, the most common of which is bipolar electrogram amplitude. However, amplitude is a complex function of multiple factors, including conduction velocity, wall thickness, electrode orientation, heart rate, etc. Thus, better indices may improve outcomes in substrate mapping procedures.

Aim

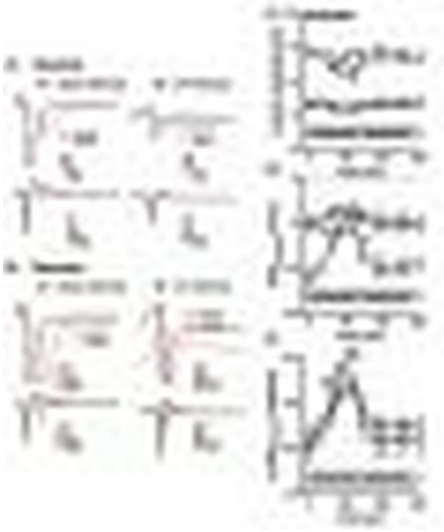
To test the hypothesis that the amplitude-independent frequency components of locally recorded electrograms could be used as an index of cardiac conduction and as an alternative to electrogram amplitude.

Methods: Experiments were conducted in isolated perfused mouse hearts instrumented for the recording of 5 unipolar electrograms from the endocardial surface of the left ventricle. Hearts were paced from the endocardium or epicardium. Ischaemia was induced by lowering the flow-rate of the peristaltic pump (25% of initial flow-rate). Drugs were added directly to the perfusate. For amplitude-independent analysis, electrograms were rescaled between 0 and 1. The minimum dVdt of the rescaled (amplitude-normalised) electrogram was used to quantify the amplitude-independent components of unipolar electrogram recordings (n_dVdtmin).

Results: Figure 1 presents data on the impact of a short period of low-flow global ischaemia on electrogram indices during endocardial and epicardial pacing. For endocardial pacing, ischaemia was associated with a gradual reduction in electrogram amplitude (Figure 1C) and a corresponding fall in dVdtmin (Figure 1D). With epicardial pacing, electrogram amplitude was substantively smaller than for endocardial pacing (Figure 1A&C). Data for rescaled electrograms are shown in Figure 1B&E. Notably, n_dVdtmin was similar between pacing protocols and exhibited a comparable response to ischaemia and tissue reperfusion. Thus, in comparison to electrogram amplitude, n_dVdtmin is less dependent on the electrical activation sequence of the ventricle. Both sodium channel blockade by flecainide (1-4microM), and gap junction inhibition by carbonoxolone (10-50microM), caused slowing of n_dVdtmin, indicating that n_dVdtmin is a measure of local tissue conduction velocity. Comparable findings were observed for calculated bipolar electrogram recordings.

Conclusions: The derivative of rescaled (amplitude-normalised) electrograms is a sensitive index of ventricular conduction that exhibits less dependence of electrical activation sequence of the ventricle (i.e. pacing site) when compared with traditional amplitude-based indices.

Abstract P610 Figure 1



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Transgenic LQT5, LQT2 and LQT2-5 rabbit models with decreased repolarization reserve for more reliable prediction of drug induced ventricular arrhythmias

Hornyik T.[1]; Castiglione A.[1]; Franke G.[1]; Perez-Feliz S.[1]; Major P.[2]; Hiripi L.[2]; Koren G.[3]; Bosze ZS.[2]; Varro A.[4]; Brunner M.[1]; Bode C.[1]; Baczko I.[4]; Odening K.[1]

[1]Heart Center University of Freiburg, Department of Cardiology and Angiology I, Freiburg, Germany
[2]NARIC Agricultural Biotechnology Institute, Gödöllo, Hungary
[3]Brown University, Cardiovascular Research Center, Providence, United States of America
[4]University of Szeged, Department of Pharmacology and Pharmacotherapy, Szeged, Hungary

Background: For more reliable prediction of pro-arrhythmic side-effects of novel drug candidates, different transgenic LQTS rabbit models with impaired repolarization reserve were generated by overexpressing loss-of-function mutations of human HERG (HERG-G628S, loss of IKr; LQT2), KCNE (KCNE1-G52R, decreased IKs; LQT5), or both transgenes (LQT2-5) in the heart.

Purpose: The effects of K+-channel blockers on cardiac repolarization and arrhythmia (AR) development were studied in wild type (WT), transgenic LQT5, LQT2, and LQT2-5 rabbits.

Methods: In vivo (QTc, Tpeak-Tend [Tp-e], STVQT) and ex vivo (action potential duration (APD75), triangulation and reverse use-dependence) pro-arrhythmic biomarkers were monitored by ECG and monophasic action potential (MAP) measurements in Langendorff-perfused hearts. Arrhythmia development was provoked ex vivo by low K+ (2.0mM) and IK1-blocker BaCl2 (10μM) (5 minutes).

Results: At baseline, QTc, Tp-e (ms ± SEM) and STVQT were similar in LQT5 (144.3 ± 1.2, 30.2 ± 0.7, 2.0 ± 0.1) as in WT (137.0 ± 2.4, 29.8 ± 0.8, 1.9 ± 0.1) but were significantly prolonged in LQT2 and LQT2-5 (LQT2: 161.9 ± 5.8, 40.2 ± 1.3, 2.9 ± 0.1 and LQT2-5: 164.7 ± 3.2, 36.0 ± 1.1, 2.5 ± 0.2; all $p < 0.05$ vs. WT or LQT5). Slight IKr-blockade by dofetilide (0.02mg/kg, iv.) increased STVQT (Δ:+0.7 ± 0.2), prolonged Tp-e (Δ:+2.1 ± 0.5) and QTc (Δ:+7.7 ± 1.9) only in LQT5 (all $p < 0.05$ vs. baseline). IK1-blocker BaCl2 (0.1mg/kg, iv.) prolonged QT and Tp-e more pronouncedly in LQTS animals as in WT (ΔQTc, LQT2: +20.4 ± 3.0, LQT2-5:+24.0 ± 7.4 vs. WT:+10.2 ± 0.9; ΔTp-e, LQT5:+4.9 ± 1.3, LQT2:+6.8 ± 1.7, LQT2-5:+6.0 ± 1.0 vs. WT:+1.5 ± 0.3; all $p < 0.05$) and increased STVQT only in transgenic rabbits (all $p < 0.05$ vs. baseline) but not in WT. Following IKs 'pre'-activation by iv. isoproterenol, QT prolongation by IKs-blocker HMR-1556 (0.1 mg/kg iv.) was more pronounced in WT or LQT2 as in LQT5 or LQT2-5 (ΔQTc, WT:+11.8 ± 1.7 vs. LQT5:+4.1 ± 1.1 or LQT2:+16.5 ± 1.8 vs. LQT2-5:+0.2 ± 3.1; all $p < 0.05$), indicating impaired IKs function in LQT5 and LQT2-5. Ex vivo prolongation of APD75, triangulation of APD and reverse use-dependence were more pronounced upon IK1 (10μM BaCl2) or combined IK1/IKs (10μM BaCl2 + 100nM HMR-1556) -blockade in LQT2 and LQT2-5 than in WT. Ultimately, 5 min. perfusion by low K+(2.0mM) and IK1-blocker BaCl2(10μM) resulted in higher incidence of arrhythmia (VT, LQT5:50%(2/4) LQT2:100%(3/3), LQT2-5:100%(3/3) vs. WT:0%(0/7); VF, LQT2:67%(2/3), LQT2-5:67%(2/3) vs. WT:0%(0/7), all $p < 0.05$, $\chi 2$-test) and longer duration (total AR in %±SEM, LQT5: 57.3 ± 18.4, LQT2: 83.2 ± 8.4, LQT2-5: 82.6 ± 9.0 vs. WT: 16.2 ± 5.9; all $p < 0.05$) in LQTS animals compared to WT.

Conclusion: LQT5 and LQT2 or LQT2-5 rabbits with mild/severe reduction of repolarization reserve are more sensitive to K+-channel blockers as WT animals demonstrating not only more pronounced increase in various pro-arrhythmic biomarkers but also higher incidence, longer duration and more malignant type of arrhythmia development.

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P612

Caval vein myocardium demonstrates automaticity and properties of a leading pacemaker

Ivanova AD.; Kuzmin VS.; Filatova TS.

M.V. Lomonosov Moscow State University, Biological department, Moscow, Russian Federation

Funding Acknowledgements: RFBR grant 18-315-00253

Introduction. In adult mammals, including humans and rodents, the heart rhythm is maintained by the primary pacemaker – sinoatrial node (SAN), that is located at the junction of the superior caval vein (CV) and the right atrium. CV contain myocardial tissue that it is present here at all stages of ontogenesis. CV and SAN myocardium shares the same precursors, that have pacemaker properties. It was shown that during early stages of development the level of transcriptional factors that determine electrophysiological phenotype changes in CV myocardium. However, it is not clear how CV myocardium electrophysiological properties change at the same time.

Purpose. The aim of this project is to investigate the CV pacemaker properties during the early postnatal ontogenesis.

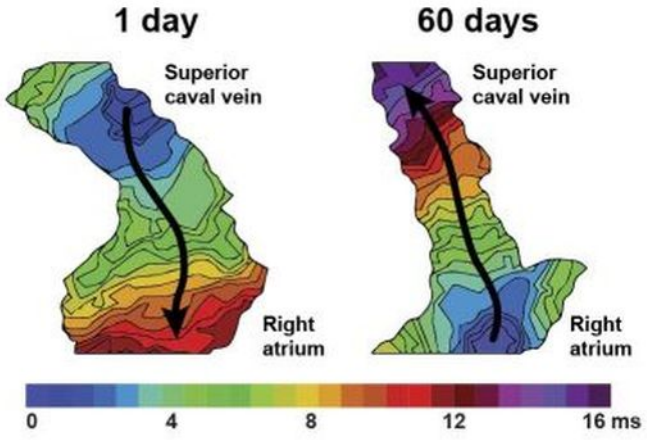
Methods. All the experiments were performed in accordance with the "Principles of laboratory animal care" (NIH Publication no. 85-23 revised 1985). The experiments were carried out on isolated Tyrode perfused preparations of CV with the usage of standard microelectrode technique or CV-atrial preparations with the optical mapping technique. Preparations were dissected from male Wistar rats of different ages (1, 3, 7, 14, 21 and 60 day of postnatal development).

Results. Without electrical pacing in 50% of CV preparations of 1-7 days old rats demonstrated a spontaneous activity that appeared in a permanent manner. In 67% of spontaneously active preparations action potentials (AP) had a phase of slow diastolic depolarization resembling pacemaker-like AP. During aging, the AP shape turned to atrial-like, with rapid upstroke phase, and the ability of isolated CV preparations to perform automaticity gradually decreased. There was no spontaneous AP under basal conditions in 60 days old rats' CV.

In optical mapping experiments in CV-atrial preparations almost in all cases we observed an anterograde propagation of excitation and the focus of automaticity initiation was located in the right atrium region. However, in 30% of newborn rats on the 1 day of postnatal development we observed retrograde conduction with the excitation focus in CV myocardium and not in the right atrium region (Fig.1).

Conclusion. In this study we demonstrated for the first time that the formation of a leading pacemaker continues during early postnatal life. We also showed that at the early steps of postnatal development CV myocardium can play a role of a normal pacemaker. Probably, the observed processes can be a reflection of changes of the level of transcription factors that determine the electrophysiological phenotype in CV myocardium.

Abstract P612 Figure. The propagation of excitation in CV.



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A platform for assessing pro- and anti-arrhythmic effects of drugs based on isogenic human iPSC-derived cardiomyocytes

Campostrini G.[1]; Sala L.[1]; Ward-Van Oostwaard D.[1]; Van Meer BJ.[1]; Tertoolen LGJ[1]; Bartulos-Encinas O.[2]; Braam SR.[2]; Ijzerman AP.[3]; Mummery CL.[1]; Bellin M.[1]

[1]Leiden University Medical Center, Leiden, Netherlands
[2]NCardia BV, Leiden, Netherlands
[3]Leiden Academic Center for Drug Researh, Leiden, Netherlands

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Background: The incidence of cardiac arrhythmias is constantly increasing in Western world, not only because of population aging but also because of unexpected side effects of drugs, such as chemotherapeutics. Cardiotoxicity is the main cause of drugs withdrawal from the market. Some drugs bind to the cardiac hERG ion channel and cause a prolongation of the heart QT interval, inducing arrhythmias that can eventually lead to sudden cardiac death. Notably, individuals with congenital long QT syndrome (LQTS) are more prone to develop drug-induced arrhythmia. Molecules acting as hERG allosteric modulators are able to shorten the QT interval in vitro and we have previously shown that the LUF7346 allosteric modulator can counteract arrhythmia in LQTS human iPSC-derived cardiomyocytes (hiPSC-CMs).

Purpose: Here we want to establish a drug-screening platform based on healthy and diseased (LQTS) hiPSC-CMs to i) identify molecules with hERG allosteric modulator activity and ii) assess the pro- and anti-arrhythmic effect of drugs. This platform will be useful to prevent life-threatening arrhythmias both in the general and in LQTS patient populations.

Methods: Compound libraries are screened to identify molecules with structure similarity to LUF7346. Allosteric modulator activity is first assessed by in vitro radio ligand binding assays. Secondly, the most active drugs are tested, both alone and in association with pro-arrhythmic drugs, on hiPSC-CMs plated on 96-well Multi-Electrode Arrays (MEAs). Finally, selected compounds are tested on a new integrated system that we have developed, based on fluorescent dyes to record simultaneously voltage, calcium and contractile properties of hiPSC-CMs. The effects are compared between isogenic wild type and diseased hiPSC-CMs.

Results: We used two isogenic hiPSC lines: one carrying in homozygosis a KCNQ1 mutation, previously associated with Jervell Lange-Nielsen syndrome (JLNS), which is a severe form of LQTS and its isogenic wild-type line, generated with CRISPR/Cas9 technology. Both lines were differentiated into cardiomyocytes. The electrophysiological properties of these hiPSC-CMs were evaluated both by MEA recordings of spontaneous beating activity and by patch clamp recordings of action potentials stimulated at 1Hz. JLNS hiPSC-CMs action potential duration (APD) was prolonged compared to the isogenic wild type line. We then optimized the seeding and recording conditions in 96-well MEA plates and tested the effect of LUF7346 (as a reference) and of other 3 identified molecules by measuring the QT interval duration. Finally, for the first time we simultaneously tested the effect of the hERG allosteric modulators on APD, intracellular calcium transient, and contraction, using the integrated system.

Conclusion: This platform can identify active molecule able to shorten APD and our approach will provide evidence for the value of using hiPSC-CMs in preclinical drug testing for predicting cardiotoxicity.

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Nanokicking promotes cell adhesion complex formation in human induced pluripotent stem cell-derived cardiomyocytes

Costa A.[1]; Childs P.[2]; Salmeron-Sanchez M.[2]; Dalby M.[3]; Smith GL.[1]

[1]University of Glasgow, Institute of Cardiovascular and Medical Science, Glasgow, United Kingdom
[2]University of Glasgow, Biomedical Engineering, Glasgow, United Kingdom
[3]University of Glasgow, Institute of Molecular Cell & Systems Biology, Glasgow, United Kingdom

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Introduction: Traditionally, cardiotoxicity studies have primarily been performed in animal models during the early stages of drug development. Recently, monolayers of human induced-pluripotent stem cell-derived cardiomyocytes (hIPSC-CMs) have been used as an alternative to adult primary cells, but the lack of reproducibility of hIPSC-CM monolayer culture limits applicability. Cell adhesion to scaffold is an important variable in monolayer cultures and is determined by mechanical cues in the local environment.

Purpose: To modulate cell adhesion to scaffold via altered expression of focal adhesion proteins using novel mechanostimulation in commercial hIPSC-CMs.

Methods: Commercial hIPSC-CMs were cultured in bovine fibronectin-coated T25 cell culture flasks and mechanostimulation was applied via the base of the culture flask by vibration at 1kHz (~40nm displacement) using a Nanokick system for 24h, 48h or 72h. The cells were detached and plated at 30,000 cells/well in glass-bottom 96 well plates. On day 5 after mechanostimulation ceased, the culture was loaded with 5μM of voltage-sensitive dye, di-4-ANEPPS. Electrophysiological and contractile behaviors were recorded in a CellOPTIQ platform. The cells were treated with 3μM thapsigargin for 30min, before voltage and contractility were recorded. The cultures were fixed and the hIPSC-CMs were stained for vinculin and actin, and cell adhesions were measured using image analysis.

Results: Mechanostimulation using the Nanokick system for 24h had no functional effects nor did it affect cell adhesion protein expression. Response to thapsigargin, a SERCA blocker, was the same in controls and after 24h of mechnostimulation (NK group), but intrinsic cycle length (CL) and action potential duration (APD90) were both prolonged. After mechanostimulation for 48h there was a prolongation of CL (control 1936 ± 50ms vs NK 3038 ± 449ms) and APD90 (control 475 ± 14ms vs NK 638 ± 48ms). CL and APD90 in the NK group were unaffected by 3μM thapsigargin, while the same intervention increased both CL and APD90 in the control group. Cell adhesion complexes were unaffected in the 48h NK group. After 72h of mechanostimulation, the replated cells displayed a prolonged CL in NK group compared to control (3656 ± 211ms and 2183 ± 90ms, respectively). APD90 in NK was longer at 905 ± 29ms compared to 567 ± 30 in controls. Vinculin adhesions were 52% longer in NK 72h.

Conclusion: Conditioning the hIPSC-CMs with mechanostimulation for up to 3 days leads to prolongation of intrinsic cycle length and action potential duration in commercial hIPSC-CMs. Larger focal cell adhesions complexes were evident when cells are subjected to mechanostimulation for 72h but are not detectable at 24hrs. The cell adhesion complexes are responsible for adhesion to substrate, and this data suggests that mechanostimulation using the "Nanokick" system can promote cell/scaffold adhesion but with parallel effects on spontaneous electrical activity.

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Aspects of oestrogen action on cardiomyocyte calcium homeostasis

Thong EGHE[1]; Firth JM.[1]; Francis AJ.[1]; Islam N.[1]; Yang H-Y[2]; Macleod KT.[1]

[1]Imperial College London, National Heart and Lung Institute, London, United Kingdom
[2]National Defense Medical Center, Division of Cardiovascular Surgery, Department of Surgery, Tri-Service General Hospital, Taipei, Taiwan ROC

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Background: Gender differences in cardiovascular disease risk are well-documented, implying a cardioprotective effect of ovarian hormones. We aim to investigate the influence of long-term ovarian hormone deficiency on three cardiomyocyte calcium efflux mechanisms: Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase (SERCA), Sodium/Calcium Exchanger (NCX), and the Slow transporters.

Methods: Female guinea-pigs underwent ovariectomy (OVx) or sham operations. Left ventricular cells were enzymatically isolated 60 days post-surgery, loaded with a fluorescent calcium indicator, and calcium transient (CaT) parameters recorded.

Results: CaT amplitude was 9% greater in sham than OVx at steady-state stimulation. CaT decay rate attributable to SERCA activity was 17% slower in OVx cells compared with sham, with SERCA contributing less to calcium efflux. The rates associated with NCX and the Slow transporters were 12% and 22% faster respectively in OVx compared with sham cells, contributing more to calcium efflux.

Conclusions: Guinea-pig cardiomyocytes exhibited significant changes in CaT parameters and calcium efflux mechanisms 60-days post-OVx. These results differ to those obtained in previous studies at 150-days post-OVx, suggesting that changes at 150 days may be in response to the earlier alterations observed in this study. This highlights the complexity of oestrogen action on calcium efflux mechanisms and suggests an adaptive remodelling process.

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Mutation R591C associated with long QT syndrome type 1: clinical, genetic, and functional analysis

Svecova O.[1]; Bebarova M.[1]; Baiazitova L.[2]; Policarova M.[1]; Hosek J.[3]; Andrsova I.[4]; Valaskova I.[5]; Synkova I.[5]; Gaillyova R.[5]; Vit P.[6]; Novotny T.[4]

[1]Masaryk University, Faculty of Medicine, Department of Physiology, Brno, Czech Republic
[2]Brno University of Technology, Faculty of Electrical Engineering and Communication, Department of Biomedical Engineering , Brno, Czech Republic
[3]University of Veterinary and Pharmaceutical Sciences Brno , Faculty of Pharmacy, Department of Molecular Biology and Pharmaceutical Biotechnology, Brno, Czech Republic
[4]University Hospital Brno and Faculty of Medicine, Masaryk University, Department of Internal Medicine and Cardiology, Brno, Czech Republic
[5]University Hospital Brno and Faculty of Medicine, Masaryk University, Department of Medical Genetics, Brno, Czech Republic
[6]University Hospital Brno and Faculty of Medicine, Masaryk University, Department of Paediatrics, Brno, Czech Republic

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Introduction: Inherited arrhythmogenic syndromes are characterized by atypical ECG findings and high occurrence of arrhythmias. These syndromes are associated with mutations in various genes, most often encoding structure of cardiac ion channels. In this study, we focused on complex analysis of a mutation in the KCNQ1 gene associated with long QT syndrome type 1 (LQT1). This gene encodes α-subunit (Kv7.1) of the slowly activating delayed rectifier potassium (IKs) channel.

Purpose: The aim of this study was to analyse genetic, clinical, and functional characteristics of a C-terminal mutation G1772C in the KCNQ1 gene (R591C-Kv7.1) that was identified in a LQT1 patient and has not been studied so far.

Methods: Clinical analysis (ECG at rest, during and after ergometry) and genetic analysis (mutation and pedigree analysis) were performed. Chinese hamster ovary cells transiently expressing wild type (WT) and/or R591C human IKs channels (KCNQ1/KCNE1/Yotiao, 1:2:4) were used for the functional analysis. Measurements of electrophysiological characteristics were performed by the whole cell patch clamp technique at 37°C. Expression of the channels in the cell membrane was studied using the confocal microscopy.

Results: The heterozygous R591C mutation was identified in a woman with history of resuscitated syncope. The resting QTc interval of 435 ms was prolonged to 540 ms during ergometry. According to pilot data, R591C mutation leads to a complete loss of function of the channels, due to their absent cell membrane expression as confirmed by the confocal microscopy. A significant reduction of the tail current amplitude was observed in co-expressed WT and R591C channels. Significant changes of the steady-state activation curve and the channel activation kinetics were not observed.

Conclusions: The R591C mutation associated with LQT1 in a patient results in non-functional IKs channels due to their absence in the cell membrane. Co-expression of R591C and WT subunits shows haploinsufficient character of the mutation.

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P617

Oxytocin blocks IKs and IK1 - thus exerting harmful cardiac repolarization prolonging effects particularly in context of drug-induced LQTS

Kreifels P.; Bodi I.; Franke G.; Perez-Feliz S.; Castiglione A.; Ziupa D.; Brunner M.; Bode C.; Odening KE.

University of Freiburg, University Heart Center Freiburg, Department of Cardiology and Angiology I, Freiburg, Germany

Introduction: Oxytocin, is used therapeutically in patients with autism or borderline disorders. Many of these patients receive anti-depressant and/or anti-psychotic drugs, which cause acquired long QT 2 (LQT2) by blocking IKr. We have previously identified a QT-prolonging effect of oxytocin in transgenic LQT2 rabbits due to an oxytocin-induced reduction of IKs.

Purpose: We aimed at elucidating whether a combination of the two therapeutic strategies may be harmful due to severe QT prolongation and pro-arrhythmia.

Methods: Adult female wildtype rabbits were subjected to in vivo12-lead ECGs 1) at baseline, 2) during perfusion with risperidone (risp, 0.3mg/kg bolus, followed by 3mg/h iv; alone and with oxytocin, oxy 6U/h iv, n = 21) or 3) with fluoxetine (fluo, 1 mg/kg bolus, followed by 9 mg/h iv; alone and with oxy, n = 23) and 4) oxytocin alone (oxy, 1,5 U bolus, followed by 6U/h iv, n = 13). Ex vivomonophasic action potentials were measured in Langendorff-perfused rabbit hearts and patch clamping experiments were performed in isolated cardiomyocytes exposed to oxy (200ng/l, n = 8), risp (1µM, n = 8) or fluo (3µM, n = 10) alone and with oxy (200ng/l).

Results: Oxytocin as well as fluo and fluo + oxy prolonged QTc compared to baseline (ms, bsl, 251.1 ± 4.8 vs. oxy, 262.5 ± 5.6; $p < 0.001$, 244.1 ± 2.4 vs. fluo, 259.0 ± 3.1 vs. fluo + oxy 267.5 ± 3.5; $p < 0.0001$). The fluo + oxy combination showed an additional QTc-prolonging effect ($p < 0.001$). Similar effects were found for risp and risp + oxy (ms, bsl, 248.3 ± 2.1 vs. risp, 288.7 ± 3.9 vs. risp + oxy 296.4 ± 4.3; $p < 0.0001$).

Oxy prolonged mean action potential duration (APD75, ms, bsl, 127.1 ± 7.6 vs. oxy, 136.3 ± 10.1, n = 8, $p < 0.05$). Fluo + oxy prolonged APD75compared to baseline (bsl, 120.6 ± 4.4ms vs. fluo + oxy, 140.2 ± 2.8ms; $p < 0.001$) and the combination fluo + oxy further increased APD (ms, fluo, 129.9 ± 5.0 vs. fluo + oxy, 140.2 ± 2.8, $p < 0.05$) with similar results found for risp and risp + oxy (ms, bsl, 122.5 ± 3.9 vs. risp, 149.2 ± 6.3 vs. risp + oxy, 152.9 ± 5.9; $p < 0.001$). Similar APD prolonging effects were observed in isolated cardiomyocytes ($p < 0.05$). This is due to differential effects of oxy, risp and fluo on repolarizing ion currents: Oxy reduced IKs, while both fluo and risp reduced IKr, resulting in additive effects on IKtotal-tail (+40mV, % reduction of pA/pF, oxy -35% (n = 12), ´fluo -67% (n = 17), fluo + oxy -72% (n = 10); risp -55% (n = 10), risp + oxy -75% (n = 7)). In addition, oxy reduced IK1(-120mV pA/pF, bsl, -35.4 ± 3.7 vs. oxy, -29.9 ± 2.6, n = 11, $p < 0.05$) thus further reducing the repolarization reserve in cardiomyocytes while while risp and fluo alone had no effects on IK1.

Conclusion: Oxytocine, risperidone and fluoxetine prolong QTc and APD. Combined treatment with fluo + oxy further prolongs QTc and APD due to differential effects of oxy and risp / fluo on IKsand IK1(block by oxy) and IKr(block by risp and fluo) leading to pronounced impairment of repolarization reserve. Oxytocin should therefore be used with caution in the context of acquired LQTS.

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INa characterization from compound variants in SCN5A from a large founder population with excess sudden cardiac death

Altrocchi C.[1]; Spatjens RL.[1]; Sutanto H.[1]; Ter Bekke RM.[1]; Seyen S.[1]; Heijman J.[1]; Moreno C.[2]; Volders PG.[1]

[1]Cardiovascular Research Institute Maastricht (CARIM), Cardiology, Maastricht, Netherlands
[2]Johns Hopkins University of Baltimore, Baltimore, United States of America

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Recent clinical investigations in a Dutch-German founder population with excess sudden cardiac death, revealed striking phenotypic heterogeneity: long QT-syndrome, cardiac conduction disease, (drug-induced) Brugada syndrome, isorhythmic atrioventricular dissociation and overlap. Ventricular tachyarrhythmia often occurred during mental or physical stress. DNA sequencing identified the pathogenic SCN5A deletion c.4850_4852delTCT, encoding for Nav1.5-DelF1617, and a common polymorphism c.1673A > G, Nav1.5-H558R.

In the present study, we characterized the sodium current by performing whole-cell patch-clamp on Chinese hamster ovary cells (CHO), transiently transfected with wild-type (WT) Nav1.5, Nav1.5-DelF1617 or Nav1.5-DelF1617-H558R. Furthermore, given the location of the F1617, we investigated the interaction of the (mutant) channels with the β1 subunit, which was co-transfected with the aforementioned variants on Nav1.5. The data we generated were used to run in-silico action potential (AP) simulations, in order to evaluate the impact of the differential biophysical properties of the mutant channels on the AP.

The characterization of the Nav1.5-DelF1617 showed overall a marked loss-of-function phenotype, with a significant reduction in the current density, only partially recovered by the presence of the H558R. The voltage dependence of the mutant channels was not altered, but the time constant of inactivation was slower in Nav1.5-DelF1617(-H558R) and recovery from inactivation faster. No TTX-sensitive persistent current was detected. AP simulation confirmed a reduction of the phase 0 upstroke velocity for Nav1.5-DelF1617 and Nav1.5-DelF1617-H558R, due to a decreased peak INa. AP duration was slightly prolonged, especially at low pacing rate (1Hz). When the β1 subunit was co-expressed, the current density of Nav1.5-DelF1617 was not different from WT, but, interestingly Nav1.5-DelF1617-H558R showed a significant reduction. The voltage dependence of activation was shifted towards more positive potentials, causing, together with an incomplete inactivation, a 3-fold increase in the window current.

Taken together, these data suggest a differential interaction of the mutant channels with the β1 subunit, stressing the importance of the modulatory effect of other proteins (i.e. interacting protein forming the sodium channel macromolecular complex) on the cellular electrophysiological phenotype. Further investigation addressing the role of the β1 subunit and other modifier genes that might be of interest in this family is ongoing.

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P619

Molecular and functional mapping of cardiac electrophysiological differentiation

Liu J.[1]; Akerboom B.[1]; Tsonaka R.[2]; Mei H.[3]; Schalij M.[1]; Pijnappels D.[1]; De Vries A.[1]

[1]Leiden University Medical Center, Department of Cardiology, Leiden, Netherlands
[2]Leiden University Medical Center, Department of Biomedical Data Sciences, Medical Statistics Section, Leiden, Netherlands
[3]Leiden University Medical Center, Department of Medical Data Sciences, Sequencing Analysis Support Core, Leiden, Netherlands

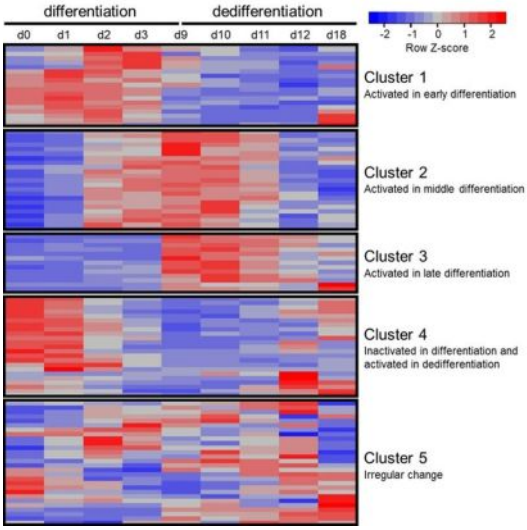
Background: Insight in the gene expression patterns underlying the development of cardiac excitability is limited. Moreover, studies on the developmental changes in the electrophysiological properties of cardiomyocytes have been restricted to single cells. We recently conditionally immortalized atrial myocytes designated iAM-1 so that their proliferation and differentiation could be controlled by the composition of culture medium.

Methods: Whole transcriptome analysis of iAM-1 cells was performed at 9 time points during one cycle of cardiac differentiation and dedifferentiation by RNA sequencing. In parallel, the electrophysiological properties of these cells were studied by optical voltage mapping together with pharmacological interventions for additional functional insight.

Results: Genes encoding proteins involved in ion transport were identified and the dynamical changes in their expression levels during iAM-1 cell differentiation and dedifferentiation were assessed. Hierarchical clustering identified 5 clusters that were highly correlated with different time points of differentiation and dedifferentiation. Genes in cluster 1 were highly expressed only during the early phase of differentiation and inactivated subsequently, suggesting a function in the initiation of differentiation process. Cluster 2 contained genes that were upregulated in the middle of differentiation suggested they are related to both regulation of cardiac differentiation and function. Cluster 3 comprised genes that were only highly expressed in differentiated cardiomyocytes and thus may play an important role in cardiac electrophysiological function. Gene cluster 4 contained genes whose expression was repressed during cardiomyogenic differentiation but reactivated during dedifferentiation, suggesting a potential function in cell proliferation. Genes in cluster 5 showed changes in expression profile that did not correlated with differentiation or dedifferentiation. Taken together, cardiac differentiation caused increases in the expression of several key ion channel genes involved in cardiac excitation. Subsequent induction of proliferation led to a drop in the expression of these genes. The changes in gene expression caused the cells to become excitable at day 3 of differentiation, to show a subsequent gradual decrease in action potential duration and to reach a maximum speed of action potential propagation of ± 20 cm/s at differentiation day 9. The functional significance of key ion channel genes was confirmed by optical mapping in the presence of specific drugs.

Conclusions: This study reveals the temporal changes of ion transport-related transcripts and their functional consequences during the development of cardiac excitability. These data provide novel insight into the molecular determinants of cardiac excitation and may thereby guide future research into the genetics of arrhythmogenic disease and the development of novel therapies for heart rhythm disorders.

Abstract P619 Figure. Heat map of ion transport related genes



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Optical membrane potential acquisition in human atrial engineered heart muscle constructs and human atrial biopsies

Seibertz F.[1]; Solano RA.[1]; Ort K.[2]; Cyganek L.[3]; Kutschka I.[2]; Zimmermann WH.[1]; Voigt N.[1]

[1]Institute of Pharmacology and Toxicology, UMG, Göttingen, Germany
[2]Department of Thoracic and Cardiovascular Surgery, UMG, Göttingen, Germany
[3]Stem Cell Unit Göttingen, UMG, Göttingen, Germany

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Background: Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) constitute extremely useful electrophysiological assays for in vitro action potential characterisation and patient specific drug screening. Current electrophysiological intracellular microelectrode techniques however are hampered by low throughput and a high degree of complexity, prompting the need for alternative methods.

Methods: We describe the application of a highly sensitive recently derived potentiometric probe, Voltage-Fluor2.1Cl, in order to investigate action potential alterations in human atrial myocytes and tissue. Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) were generated from healthy donors. Atrial differentiation was enhanced by application of retinoic acid (1 μM). Human right atrial biopsies were obtained from patients without structural heart disease undergoing open heart surgery.

Results: Robust optical action potentials (APs) were acquired from field stimulated (0.5 Hz) and electrically uncoupled (blebbistatin 5 μM) wild type iPSC-CMs as isolated cells in sparsely seeded monolayers. AP duration at 50% and 90% of repolarization was 23.4% and 27% shorter respectively in atrial vs. ventricular preparations (Panel A). In addition, we also investigated FluoVolt's efficacy in reporting APs in human atrial engineered heart muscle (EHM) and freshly isolated human atrial trabeculae (Panel B).

Changes in AP morphology in response to pharmacological intervention were appropriate, with significant AP shortening of up to 20% occurring upon application of carbachol, a muscarinic receptor agonist, in atrial iPSC-CM and human atrial trabeculae (Panel C).

Conclusion: We pertinently demonstrate the use of this indicator as a functionally equivalent method to increase throughput and precisely quantify repolarization mechanics of APs in native atrial preparations and atrial models. This platform can be extended further for electrophysiological validation of cardiac drug safety and atrial screening studies using patient specific iPSC-CMs.

Abstract P620 Figure. Optical APs in IPSC-CM, EHM, Trabeculae