

The role of na/ca exchanger in the ca-governed alternants in canine and human myocardium

Szlovak J.¹; Toth N.¹; Gazdag P.¹; Prorok J.¹; Tomoek J.²; Virag L.¹; Papp JGY¹; Varro A.¹; Nagy N.¹

¹University of Szeged, Department of Pharmacology and Pharmacotherapy, Szeged, Hungary

²University of Oxford, Life Sciences Interface DTC, Oxford, United Kingdom

Funding Acknowledgements: János Bolyai Res. Schol. of the Hungarian Academy of Sciences, NKFIH PD-125402, FK-129117, UNKP-18-4, GINOP-2.3.2-15-2016-00006, 00012, 040

OnBehalf: Department of Pharmacology and Pharmacotherapy

Introduction: The Ca²⁺-governed alternants refers a beat-to-beat oscillation of the action potential duration (APD) and the Ca²⁺ transient amplitude between a periodical long-short-long and large-small-large pattern respectively. Different Ca²⁺-dependent currents such as Na/Ca exchanger (NCX) and L-type Ca²⁺ currents assure the bidirectional coupling of the membrane potential and intracellular Ca²⁺ movements leading to the APD and transient amplitude alternants together. Therefore, the application of the selective NCX inhibition would be promising intervention against the development of alternants however the experiments were hampered because of appropriately selective inhibitor.

Purpose: In this study we aimed to characterize the role of NCX in the mechanism of alternants and we evaluated the therapeutic potential of selective NCX inhibition by using a novel inhibitor ORM-10962 against development of APD alternants in canine and human ventricular myocytes.

Methods: The action potentials were measured by conventional microelectrode technique, the ionic currents were monitored by the whole cell configuration of the patch-clamp technique while intracellular Ca²⁺ movements were recorded with fluorescent optical methods.

Results: The action potentials showed considerable APD alternants in frequencies faster than 250 ms, primarily in the APD₂₅ and APD₁₀ level which were markedly suppressed after application of 1 μ M ORM-10962 in canine papillary muscles. In parallel experiments we found similarly effective reduction of APD oscillation in human ventricle as well. Patch-clamp experiments revealed that 20 mM [Na⁺]_i intensified the alternation of the inward current and Ca²⁺ transient amplitude. However application of 1 μ M ORM-10962 show a clear tendency to decrease the transient alternation a clear conclusion requires further experiments.

Conclusion: Our results further support the previous assumptions regarding the role of NCX in the mechanisms of Ca²⁺-governed alternants. In line with this, selective NCX inhibition may reduce the magnitude of APD and transient oscillation providing a promising future pharmacological intervention in the arrhythmia therapy.

Subject: Intracellular calcium (dys)regulation and cardiac remodeling --

658

Characterization of changes in Ca^{2+} handling and contractile function in a rat model of exercise-induced cardiac hypertrophy

Gazdag P.¹; Hartai T.¹; Demeter-Haludka V.¹; Ordog B.¹; Szlovak J.¹; Nagy ZS.²; Nagy N.¹; Acsai K.²; Barta B.³; Olah A.³; Radovits T.³; Merkely B.³; Papp JGY²; Varro A.¹; Prorok J.¹

¹University of Szeged Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, Szeged, Hungary

²Hungarian Academy of Sciences, MTA-SZTE Research Group of Cardiovascular Pharmacology, Szeged, Hungary

³Semmelweis University, Heart and Vascular Center, Experimental Research Laboratory, Budapest, Hungary

Funding Acknowledgements: NKFIH (K-119992 and K-120277 GINOP-2.3.2-15-2016-00047) EFOP-3.6.2-16-2017-00006, Hungarian Academy of Sciences

Background: Regular intensive exercise induces adaptation of the cardiovascular system that involves increased cardiac mass (hypertrophy), commonly known as the „athlete's heart" and is associated with increased risk of sudden cardiac death. Abnormal calcium handling occurs in other forms of cardiac hypertrophy, such as hypertrophic cardiomyopathy. However, its role in the pathology of the exercise-induced cardiac hypertrophy is not known.

Objectives: Therefore, we undertook to characterize Ca^{2+} handling and contractile function in the rat model of exercise-induced cardiac hypertrophy.

Methods: Rats were randomly divided into control (CTL, $n = 18$) and exercised groups (EX, $n = 18$) and were subjected to previously published training protocol (Radovits et al, 2013). Left ventricular developed pressure (LVDP) and ECG were recorded in Langendorff-perfused hearts. Arrhythmias induced by hypokalemic (2,7mM K^{+}) solution. The caffeine-induced NCX currents, L-type calcium currents and repolarizing currents I_{to} and I_{K1} were measured by whole cell patch-clamp. The Ca^{2+} transients (CaT) and cell shortening were measured by optical method in enzymatically dissociated cardiomyocytes. The qPCR and western blotting were used to detect mRNA and protein levels.

Results: LVDP and cell shortening were markedly increased in EX, compared to CTL group. The half decay time of CaT was decreased in EX vs the CTL group, while the amplitude remained unchanged. The amplitude of I_{Ca} did not change either. No significant changes found in the properties of repolarizing potassium currents I_{to} or I_{K1} . Caffeine-induced NCX currents were significantly increased in the EX group, indicating elevated SR Ca^{2+} content. In line with this, the mRNAs of the Ryanodine receptor and calsequestrin increased, similarly to the relative protein density of the monomeric form of phospholamban. Hypertrophy increased the number of extrasystole and hypocalemic provocation caused more pronounced arrhythmia incidence in EX hearts compared to the CTL group.

Conclusions: Significant cardiac hypertrophy emerged in the animals submitted to intense exercise protocol. In this study, we show that exercise-induced cardiac hypertrophy is associated with increased SR Ca^{2+} load and increased contractile function. These results suggest that in the athlete's heart model, in parallel to the hypertrophy of the cardiac muscle, the Ca^{2+} homeostasis adapts as well to meet the increased demand for cardiac output during training.

Subject: Intracellular calcium (dys)regulation and cardiac remodeling --

659

Differentiating the exercise-induced effects of acute beta-adrenergic stimulation and stretch on calcium and force dynamics using a novel electromechanical cardiomyocyte model

Lyon A.¹; Dupuis L.J.¹; Arts T.¹; Delhaas T.¹; Prinzen F.W.²; Heijman J.³; Lumens J.¹

¹Cardiovascular Research Institute Maastricht (CARIM), Department of Biomedical Engineering, Maastricht, Netherlands

²Cardiovascular Research Institute Maastricht (CARIM), Department of Physiology, Maastricht, Netherlands

³Cardiovascular Research Institute Maastricht (CARIM), Department of Cardiology, Maastricht, Netherlands

Funding Acknowledgements: Netherlands Organisation for Scientific Research (NWO-ZonMw, VIDI grant 016.176.340)

Background: Cardiac arrhythmias have traditionally been considered electrical phenomena, but recent evidence suggests that electromechanical interactions play a key role in arrhythmogenesis. Physical exercise modulates both cardiac electrophysiology and mechanics and can be an important proarrhythmic trigger. However, the complex dynamic interplay between electrophysiology and mechanics complicates the understanding needed for arrhythmia risk prediction and treatment. Computational models provide a unique environment to untangle these multiscale interactions.

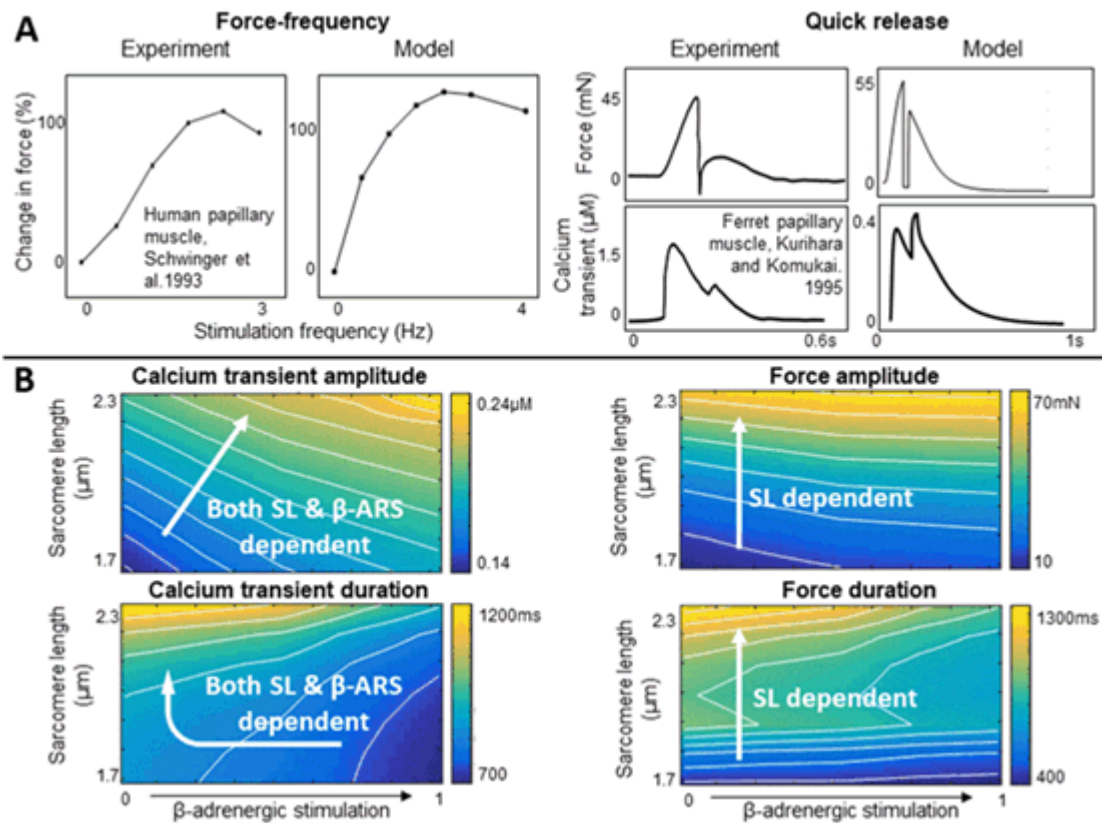
Purpose: To differentiate the exercise-induced effects of acute β -adrenergic stimulation (β -ARS) from that of cardiomyocyte stretch (increased sarcomere length, SL) on calcium transient (CaT) dynamics and force generation using a newly developed computational model of cardiac electromechanics linking human ventricular electrophysiology and sarcomere mechanics.

Methods: We implemented a bidirectional coupling between two extensively validated models: the O'Hara-Rudy model of human ventricular electrophysiology and the MechChem model of sarcomere mechanics, in which calcium induces force generation and contractile properties determine calcium buffering. The coupled model was validated using a range of experimental data from large mammals or human samples (isotonic contractions, force frequency, and quick release) (Panel A). CaT and force were simulated with varying degrees of acute β -ARS (0 to 3-fold increase in L-type calcium current, 0 to 50% increase in Na-K pump current, 0 to 50% increase in SERCA2a calcium affinity and 0 to 7-fold increase in slow delayed rectifier potassium current) and various SL (1.7 to 2.3 μ m).

Results: The model reproduced experimental data including force-frequency and quick release experiments, validating the bidirectional interaction between electrics and mechanics (Panel A). Increasing β -ARS and SL both increased CaT and force amplitude (positive inotropy), but the influence of β -ARS on force amplitude was smaller than that of SL (Panel B top). Increasing β -ARS and SL had opposite effects on CaT and force duration (reflecting lusitropy), with increased β -ARS decreasing CaT and force duration whereas increased SL resulted in increased CaT and force duration (Panel B bottom). These results suggest that electrical effects (acute β -ARS) and mechanical changes (increased SL), as may occur with cardiovascular disease or in endurance athletes, may have both synergistic and opposing effects on CaT and force properties.

Conclusions: We propose a coupled human electromechanical model integrating ventricular electrophysiology and sarcomere mechanics. Our results identify differential effects of acute β -ARS and changes in SL on CaT dynamics and force generation, modulating inotropy and lusitropy. Integrated in a whole-heart model, this work will have the potential to untangle the effects of electrical and mechanical substrates during exercise and investigate arrhythmogenicity.

Abstract 659 Figure.



Subject: Intracellular calcium (dys)regulation and cardiac remodeling --

660

Beta3-adrenergic receptor subtype and Na^+/K^+ -atpase relationship in catecholamine-induced myocardial remodeling

Badone B.¹; Kayki Mutlu G.²; Ronchi C.¹; Rocchetti M.¹; Zaza A.¹

¹University of Milano-Bicocca, Milano, Italy

²Ankara University, Ankara, Turkey

Background: The role of β_3 -adrenoceptors (β_3 -ARs) in catecholamine-induced myocardial remodeling is complex, because stimulation of these receptors includes potentially beneficial and harmful effects at the same time. A decrease in Na^+/K^+ -ATPase (NKA) activity, by a potentially reversible post-translational modification, contributes to cell damage and contractile dysfunction in the remodeled myocardium. β_3 -AR stimulation has been reported to acutely increase NKA activity via an oxidative modification in healthy hearts; nonetheless, whether it may preserve NKA function in the context of overall chronic adrenergic activation is unknown. Aim: Here we investigate the effects of a change in the contribution of β_3 -ARs to catecholamine-induced remodeling of NKA activity in ventricular cardiomyocytes (CMs). Methods: 1) Cellular model: isolated rat CMs were incubated with noradrenaline (NA) 10 μM at 37°C for 3 h to simulate damage induced by prolonged catecholamine exposure. BRL 37344, a specific β_3 agonist, was added to the incubation medium at 100 nM concentration (NA + BRL). NA and NA + BRL treatment groups were compared to CMs incubated in normal Tyrode's solution (CTRL). All incubation solutions were supplemented with ascorbic acid (100 μM) to prevent catecholamine oxidation. 2) Electrophysiological recordings: membrane currents were measured in the whole-cell configuration at 35°C. NKA current (I_p) was recorded, at a holding potential of -30 mV, as the current change induced by switching from a K^+ -free solution (blocking NKA), to the same solution containing 4 mM K^+ . K^+ -sensitive currents other than I_p were blocked by Ba^{2+} , 4-AP and TEA. Complete blockade of the K^+ -sensitive current by 20 μM ouabain was verified. Results: In CTRL CMs I_p showed an initial peak, followed by a sag, as previously described. NA increased membrane capacitance (C_m , 158.6 ± 14.5 vs 121.6 ± 7.5 pF NA n = 7, CTRL n = 10; $p < 0.05$) and markedly reduced I_p amplitude (I_p , 33.4 ± 2.6 vs 91.1 ± 1.8 pA NA n = 7, CTRL n = 10; $p < 0.05$) without changing its kinetics. In NA + BRL CMs C_m returned to control values (115.6 ± 8.2 pF; $p < 0.05$ vs NA) and I_p amplitude was increased (148.1 ± 6.2 ; $p < 0.05$ vs NA); furthermore, an I_p component with very slow activation was superimposed on the fast one in the majority of CMs. Conclusion: An increase in the β_3 -ARs component of adrenergic stimulation prevents downregulation of NKA function by NA. We speculate that the change in the kinetics of I_p onset may reflect relocation of NKA to a compartment more slowly equilibrated with the extracellular solution; experiments are ongoing to test this hypothesis.