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Mechanical Effects of Late Na-Current Blockers in Human Hypertrophic Cardiomyopathy Myocardium

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Diastolic dysfunction and left ventricular outflow trait (LVOT) obstruction are major determinants of symptoms and disability in hypertrophic cardiomyopathy (HCM) patients. Disopyramide (Dis), Ranolazine (Ran) and the novel compound GS-967 are late Na-current blockers with progressively increasing selectivity for late vs. peak Na current. Dis is employed in HCM patients as an agent to relieve obstruction, but the cellular basis of its negative inotropic effect remains unknown. We previously showed that Ran ameliorates the diastolic properties of trabeculae from HCM patients, with no effects on baseline systolic force (Coppini et al, Circulation 2013). Here, we aim to study the effects of late Na-current blockers on diastolic function and contractility of HCM myocardium. Patch-clamp studies and intracellular-Ca^{2+} recordings were performed in isolated myocytes from myectomy samples of obstructive HCM patients; intact trabeculae were used for mechanical measurements. Dis (5μM) reduced twitch tension in a dose dependent manner (EC50: 5.29 ± 1.55μM) and accelerated contraction kinetics in HCM trabeculae. Ran (10μM), despite no significant effect on the amplitude of baseline contraction, significantly reduced isometric twitch tension when applied on top of isoproterenol 10-6M (Iso+Ran). Contraction kinetics in Iso+Ran were still significantly faster than baseline. The late Na-current blocker GS-967 (1μM) did not reduce baseline twitch force but accelerated contraction kinetics, highlighting qualitatively similar effects compared to Ran, albeit at 1/10 concentration. Intracellular Ca^{2+} measurements and patch clamp studies performed in HCM cardiomyocytes suggest that most of these mechanical effects are mediated by inhibition of the up-regulated INaL via normalization of NCX function and intracellular [Ca^{2+}]. From the clinical perspective: (i)all the three drugs may reduce diastolic dysfunction by speeding up contraction kinetics; (ii)Ran and GS-967 may reduce septal contractility only at peak exercise, representing a safer option to treat obstruction compared to Dis.

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Myocardial Dysfunction in Hypertrophic Cardiomyopathy: Primary Effects of Sarcomeric Mutations Versus Secondary EC-Coupling Remodelling

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In cardiac muscle from HCM patients primary changes in myofilament function, related to the presence of disease-causing mutations in sarcomeric proteins, are always associated with secondary abnormalities due to adverse remodeling of cardiomyocyte EC-coupling(Coppini et al,Circulation 2013). The latter are likely major contributors of the mechanical dysfunction and arrhythmogeneity of HCM human hearts. Here we characterize the changes in sarcomere function and EC-coupling that occur in two HCM mouse models carrying different mutations in cTnT (R92Q and E163R). Echocardiography showed LV hypertrophy, enhanced contractility, diastolic dysfunction and enlarged left atria in both HCM models; the phenotype was more pronounced in the R92Q mice. In E163R ventricular myofibrils, in spite of a significant increase in the rate of the initial isometric slow phase of relaxation, overall relaxation from maximal activation was impaired and prolonged vs WT and R92Q myofibrils that exhibited similar relaxation kinetics. Resting tension was higher in the E163Q compared to WT and R92Q myofibrils. Isometric ATPase both at rest and at maximal Ca^{2+}-activation and the energy cost of tension generation were increased in E163R vs WT and R92Q skinned trabeculae. Myofilament Ca^{2+}-sensitivity was increased in both mutant lines compared to WT; the change was larger in the R92Q preparations. R92Q intact cardiomyocytes and trabeculae compared to WT and E163R preparations showed blunted response to inotropic interventions, reduced amplitude and slower decay of Ca^{2+}-transients with reduced SERCA function. Twitch kinetics were prolonged in both HCM mouse models, despite Ca^{2+}-transient kinetics was faster and SERCA function unchanged in the E163R mice. Intact preparations of both HCM mouse models showed increased probability of arrhythmogenic behavior that increased in response to isoproterenol. The results suggest that similar HCM phenotypes can be generated through different pathogenic pathways. Grant Telethon-GGP13162.

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R21C Mutation in Cardiac Troponin I Imposes Differences in the Degree of Order and Kinetics of Myosin Cross-Bridges of Left and Right Ventricles

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The effect of the TnI R21C mutation in the human cardiac troponin I, the mutation that is linked to hypertrophic cardiomyopathy, on muscles of the left (LV) and right (RV) ventricles was examined in the knock-in mice. Experiments were on a mesoscopic scale where the contribution of a single molecule makes difference to the overall signal. We examined 3-4 actin molecules in ex-vivo myofibrils prepared from LV and RV muscles. Experiments revealed that the mutation imposed significant differences in the distribution of actin angles: during contraction, actin from LV were much more tightly distributed compared to actin from RV. They also revealed that the mutation imposed significant difference on the kinetics cycle of cross-bridges (XBs) of the LV and RV: XBs from RV displayed a 3-fold decrease in the rate of power stroke and a 2-fold decrease in the rate of dissociation from thin filaments as compared to LV. We speculate that mutation operates differently in both ventricles because of the differences in external load between LV and RV. Kinetic results suggest that an increase in isometric tension observed in mutated RV results from an inability of XBs to dissociate promptly from thin filaments.

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Spatial Distribution of Actin and Mechanical Cycle of Myosin are Different in Right and Left Ventricles of Healthy Mouse Hearts

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The contraction of the right ventricle (RV) expels blood into the pulmonary circulation, and the contraction of the left ventricle (LV) pumps blood into the systemic circulation through the aorta. The respective afterloads imposed on the LV and RV by aortic and pulmonary artery pressures create very different mechanical requirements for the two ventricles. Indeed, differences have been observed in the contractile performance between left and right ventricular myocytes in muscle growth, in dilated cardiomyopathy, in congestive heart failure, and in energy usage and speed of contraction at light loads in healthy hearts. In spite of these functional differences, it is commonly believed that the right and left ventricular muscles are identical because there were no differences in stress development, twitch duration, work performance and power among the RV and LV in dogs. This report shows that on a mesoscopic scale (when only a few molecules are studied (here 3-6 molecules of actin in ex-vivo ventricular myofibrils), the two ventricles in rigor differ in the degree of orientational disorder of actin within thin filaments, and during contraction they differ in the kinetics of the cross-bridge cycle.

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Upregulation of α1A-Subtype Adrenergic Signaling is Beneficial in Failing Right Ventricle (RV)

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Compared to non-failing RV, we reported that in failing RV the inotropic response to stimulation of α1-adrenergic receptors (α1-ARs) was increased. Recently we found that a single α1-AR subtype (α1A) was responsible. However, the significance of increased α1A responses remains unclear.
Goal: Determine if increased α1A-mediated inotropy is beneficial in RV failure and if the α1A-subtype is a potential therapeutic target.
Methods: We used a RV-specific failure model in mice. Tracheal instillation of the fibrogenic antibiotic bleomycin induced pulmonary fibrosis, pulmonary hypertension, and RV failure within 2-3 wk. We assessed survival and investigated the cellular mechanisms contributing to increased α1A inotropy in failing RV. Furthermore, in the context of bleomycin induced RV failure, we determined if there was a beneficial effect of chronic (2 wk.) treatment of mice with the α1A agonist A61603 using osmotic mini-pump infusion.
Results: For non-failing RV, acute α1A stimulation with A61603 induced a negative inotropic effect. In contrast, for failing RV, acute α1A stimulation