to sham operated animals of the same genotype (SH). Contraction of enzymatically isolated left ventricular myocytes was measured during electrical field stimulation; membrane currents and $[Ca^{2+}]_i$ were measured under whole-cell patch clamp, with Fluo-3 as Ca^{2+} indicator, all at 35°C. Data are shown as mean ± SEM. **Results.** In WT, TAC induced an increase in cell width (from 28 ± 0.7μm in SH to 30 ± 0.9μm in TAC, P<0.05), but not in cell length. This increase was prevented in TG mice (28 ± 0.7μμ ιν ΣΗ *νσ*. 28 ± 0.8μμ ιν TAX). At 1 Hz, unloaded cell shortening amplitude was not altered with TAC in both genotypes. However, the prolongation with TAC of time to peak (to 63 ± 3ms *vs*. 49 ± 2ms, P<0.05) and half-time relaxation (to 98 ± 8ms vs. 77 ± 3ms, P<0.05) in WT was less in TG (to 58 ± 4ms *vs*. 55 ± 4ms for time to peak and 91 ± 6ms *vs*. 84 ± 6ms for half-time relaxation). $[Ca^{2+}]_i$ transient amplitude, L-type Ca^{2+} current density and SR Ca^{2+} content were unchanged with TAC in both genotypes. RyR spontaneous activity (sparks) increased with frequency in SH animals. This frequency effect was reduced by TAC in WT but not in TG animals (P<0.05). **Conclusion**. FKBP12.6 overexpression reduces cellular hypertrophy and blunts functional remodeling in the setting of pressure-overload.

569-Pos Board B448

FKBP12.6 Binding Characteristics of Ryanodine Receptor Mutations Associated with Arrhythmogenic Cardiac Disease

Spyros Zissimopoulos, N. Lowri Thomas, F. Anthony Lai.
Wales Heart Research Institute, Cardiff, United Kingdom.

The cardiac muscle ryanodine receptor-calcium release channel (RyR2) and its interaction with an accessory protein, FK506-binding protein (FKBP12.6), have been implicated in the molecular pathogenesis of acquired and inherited cardiac disease (e.g. catecholaminergic polymorphic ventricular tachycardia; CPVT). We have assessed the FKBP12.6 binding characteristics of recombinant wild-type and CPVT mutant RyR2 channels under basal and oxidising conditions. We find that the R176Q and S2246L mutations exhibit an increased FKBP12.6 binding by ~40% and ~20% respectively, whereas R4497C was similar to wild-type. The redox sensitivity of the FKBP12.6 interaction with the mutant RyR2 channels was very similar to wild-type. The oxidising reagents H2O2 and diamide resulted in reduced FKBP12.6 binding by ~10% and by ~50%, respectively, compared to the untreated state for all four RyR2 proteins. These results suggest that the FKBP12.6 regulation of RyR2 is unlikely to be defective in inherited arrhythmogenic cardiac disease.

570-Pos Board B449

Impact of RyR2 Mutation Responsible for Cathecolaminergic Polymorphic Ventricular Tachycardia (CPTV) on the Short Term Interval-Force Relationship of Atrial and Ventricular Myocardium

Cecilia Ferrantini[1], Raffaele Coppini[1], Beatrice Scellini[1], Alexandra Belus[1], Chiara Tesi[1], Barbara Colombi[2], Elisabetta Cerbai[1], Carlo Napolitano[2], Silvia Priori[2], Corrado Poggesi[1].
[1]University of Florence, Florence, Italy, [2]University of Pavia, Pavia, Italy.

Mutant RyR2 responsible for CPVT can trigger on stress and exercise catecholaminergic induced-ventricular arrhythmias and sudden death. Defective RyR2, ubiquitously expressed in the heart, is responsible for sarcoplasmic reticulum dysfunction that may lead to atrial and ventricular contractile changes also detectable under basal conditions. Left atrial and right ventricular trabeculae were dissected from control (WT) and heterozygous mice (HE) carrying one of the most common CPVT-related RyR2 mutations (R4496C). Preparations underwent various stimulation protocols, including pause-delayed and premature stimuli while isometric tension was recorded. For intracellular Ca^{2+} and action potential recordings ventricular and atrial cardiomyocytes were isolated. In HE myocytes maximum post-rest increase in the amplitude of intracellular Ca^{2+}transients was reduced compared to WT. Maximum post-rest potentiation of isometric tension was lower in HE than in WT trabeculae (e.g.228 ± 37% vs.386 ± 71%, p<0.05, in atrial trabeculae) and was reached at shorter rest-intervals in HE preparations. Steady-state frequency and isoproterenol-induced positive inotropic responses were less pronounced in HE vs. WT trabeculae. These results seem consistent with the ''leaky phenotype'' of the mutant RyR2. Electrical refractoriness was the same in HE and WT myocytes but in HE trabeculae mechanical refractoriness was shorter and restitution of isometric tension was significantly faster than in WT. The rate constant of mechanical restitution at 1 Hz basal frequency was 10.07 ± 1.40 s^{-1} in HE atrial trabeculae vs. 5.28 ± 1.25 s^{-1} in WT and 3.68 ± 0.37 s^{-1} in HE ventricular trabeculae vs. 1.49 ± 0.99 s^{-1} in WT (p<0.05). Isoproterenol accelerated mechanical restitution of all types of trabeculae but did not abolish the difference between HE and WT preparations. A faster recovery from refractoriness of the mutant RyR2 may be responsible for these findings. Supported by Telethon-Italy GGP06007.

571-Pos Board B450

An L433P Arrhythmia-linked Mutation In RyR2 Uncouples Agonist-evoked Ca^{2+} Release From Homeostatic Ca^{2+} Cycling

Aaron I. Clack, Debra L. Fry, N. Lowri Thomas, F. Anthony Lai, Christopher H. George.
Wales Heart Research Institute, Cardiff University, Cardiff, United Kingdom.

A common RyR2 polymorphism (G1885E) modulates the caffeine-sensitivity of an arrhythmogenic RyR2 mutation (L433P). However, despite the markedly different caffeine sensitivities that characterised homotetrameric channels formed from mutant subunits (L433P) or subunits in which the mutation was complemented *in cis* with the polymorphism (L433P/G1885E), cells expressing these channels exhibited equivalent reductions in the propensity for spontaneous intracellular Ca^{2+} oscillations under non-stimulated conditions. This finding suggested that a common mechanistic basis of perturbed basal Ca^{2+} handling may exist. We explored whether other factors, in addition to altered agonist sensitivity, contributed to the altered homeostatic Ca^{2+} signalling in these cells. We analysed the relationships between the amplitude and kinetic profiles of caffeine-evoked Ca^{2+} responses and multiple indices of basal Ca^{2+} homeostasis including mean Ca^{2+} levels, propensity for spontaneous oscillation and Ca^{2+} signal noise. Under non-stimulated conditions, intracellular Ca^{2+} signal noise was comparable in cells expressing WT RyR2, L433P, G1885E or L433P/G1885E. The amplitude and temporal characteristics of caffeine-evoked Ca^{2+} release was linked to basal Ca^{2+} cycling in cells expressing WT and G1885E channels. However, in cells expressing L433P the relationships between basal Ca^{2+} cycling and the amplitude and kinetic profiles of sub-maximal caffeine-triggered Ca^{2+} release was significantly altered. Under these conditions, the sequestration mechanisms that underlie the decay kinetics of the Ca^{2+} transients were uncoupled from basal Ca^{2+} handling events. *In cis* complementation with the G1885E polymorphism only partially reversed this phenomenon. Our data suggest that arrhythmogenic RyR2 mutations may perturb the regulatory link between homeostatic Ca^{2+} cycling events in non-stimulated cells and the cellular Ca^{2+} response following agonist stimulation. This functional uncoupling, that was a feature of channels exhibiting very different caffeine sensitivities, may be an important mechanistic defect that contributes to dysfunctional Ca^{2+} release in arrhythmogenesis.

572-Pos Board B451

A G1885E RyR2 Polymorphism Modulates The Caffeine Sensitivity Of An Arrhythmia-linked Mutation

Aaron I. Clack, N. Lowri Thomas, Christopher H. George, F. Anthony Lai.
Wales Heart Research Institute, Cardiff University, Cardiff, United Kingdom.

More than 70 arrhythmia-linked mutations have been identified in the cardiac ryanodine receptor (RyR2). The observation that different mutations underlie similar clinical phenotypes supports the concept of a common mechanism of mutant channel dysfunction. However, RyR2 mutations are not exclusively 'gain-of-function' and some mutations may underpin different arrhythmogenic mechanisms. Additional mechanistic complexity may be conferred by the putative role of sequence polymorphisms in modulating the clinical severity of arrhythmias arising from ion channelopathies. A common RyR2 polymorphism (G1885E), that occurs at a frequency of approximately 6% in normal populations, is found on the same allele (in cis) as RyR2 mutations in symptomatic families (Tiso et al., (2001) Hum. Mol. Genet. 10 189–194). We investigated the modulatory effects of the G1885E polymorphism on human wild-type (WT) and L433P mutant channel function. eGFP-tagged WT RyR2, or RyR2 containing the L433P mutation, G1885E polymorphism or L433P and G1885E in cis (L433P/G1885E) were expressed in HEK293 cells. Consistent with previous findings (Thomas et al., (2005)), L433P channels exhibited decreased sensitivity to caffeine-evoked Ca^{2+} release although maximal responses were comparable to WT. Despite a trend toward increased caffeine sensitivity, G1885E channels were similar to WT. Unexpectedly, complementation of L433P in cis with G1885E reversed the right-shifted dose response characteristic of L433P channels and produced channels that exhibited significantly increased caffeine sensitivity when compared to WT RyR2. Despite their markedly different sensitivities to caffeine, cells expressing L433P or L433P/G1885E exhibited a reduced propensity of spontaneous Ca^{2+} oscillations under non-stimulated conditions. The mechanistic basis of these phenomena has been explored. We have extended the study to examine the functional consequences of L433P and G1885E complementation in trans. This data provides the first evidence that RyR2 polymorphisms may be a critical determinant of mutant channel dysfunction.

573-Pos Board B452

Decoding The Molecular Basis Of Anti-Apoptotic Cardiac Ca^{2+} Signalling Via Human RyR2 Splice Variants

Wai Yin Yeung, Matthew Davies, F. Anthony Lai, Alan J. Williams, Christopher H. George.
Wales Heart Research Institute, Cardiff University, Cardiff, United Kingdom.