able to increase wave frequency. On the other hand, FKBP12.6 was not (from 0.12 ± 0.04 Hz to 0.16 ± 0.04 Hz (SEM; n=4; $P>0.35$). Our results indicate that FKBP12 may have an important role as an activator of RyR2 in cardiac cells. Further work is required to determine the individual and combined roles of FKBP12 and FKBP12.6 in cardiac EC-coupling.
Supported by the British Heart Foundation.

1562-Pos
FKBP12 is a High Affinity, Reversible Activator of RyR2, and FKBP12.6 Antagonises Its Actions
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FKBP12.6 binds tightly to RyR2 and evidence suggests that it plays a vital physiological role in regulating channel activity. Moreover, changes in FKBP12.6/RyR2 interactions have been implicated in heart failure. Controversy exists, however, as to how FKBP12.6 affects the single-channel behaviour of RyR2. Furthermore, although higher levels of FKBP12 than FKBP12.6 are present in cardiac cells, the effects of FKBP12 on RyR2 are virtually unresearched. We have therefore compared the effects of FKBP12 and FKBP12.6 on the single-channel function of sheep RyR2 incorporated into bilayers under voltage-clamp conditions. We find that FKBP12 increases RyR2 open probability (Po) in a dose-dependant, reversible manner with an EC_{50} of 51 nM. In the presence of 10 μM cytosolic Ca^{2+}, physiological levels of FKBP12 (3 μM) increased Po from 0.187 ± 0.051 in controls to 0.657 ± 0.111 (SEM; n=14; $P<0.001$). In contrast, under identical experimental conditions, FKBP12.6 did not significantly increase or decrease RyR2 Po, however, it was able to antagonise the actions of FKBP12, shifting the EC_{50} value for FKBP12 to 4 μM. Our experiments demonstrate that FKBP12 has high affinity for RyR2 and that at physiological concentrations (1-3 μM) is an effective activator of the channel thereby suggesting that FKBP12 may have a more important role in cardiac excitation-contraction coupling than previously thought. We hypothesise that FKBP12.6 is a very low efficacy (but high affinity) partial agonist of RyR2 and that the balance between the effects of FKBP12 and FKBP12.6 is crucial for normal EC-coupling in cardiac cells.
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1563-Pos
Imperatoxin Induces a Biphasic Response in Ca^{2+} Sparks
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Imperatoxin induces a biphasic response in Ca^{2+} sparks
Imperatoxin A (IpTxa), isolated from the venom of the African scorpion *Pandinus imperator,* has been shown to specifically activate ryanodine receptors (RyR) and to be capable of translocating across cell membranes. IpTxa enhances [^{3}H]ryanodine binding to sarcoplasmic reticulum (SR) and stabilizes subconducting states in single channels. We previously demonstrated that IpTxa alters the amplitude of calcium transients in intact field-stimulated cells, causing a rapid increase in transient amplitude. This is followed by a gradual decrement in amplitude to a new steady state at lower amplitude than in control. The current study seeks to clarify how IpTxa acts on RyRs to perturb Ca^{2+} handling in cardiomyocytes. We employed visualization of IpTxa-modified Ca^{2+} sparks in saponin-permeabilized cells to facilitate direct titration of RyRs with known concentrations of IpTxa, ranging from 500pM to 50nM. In addition, we modified our sparks protocol to enable a comparison of the caffeine-releasable SR Ca^{2+} load before and after treatment with the toxin. Our results demonstrate that IpTxa induces a biphasic RyR response, typified by a transient increase in spark frequency, amplitude, FWHM, and FDHM, which is rapidly followed by a sharp decrease in the same parameters. Comparison of pre- and post-toxin caffeine-releasable SR Ca^{2+} consistently reveals that SR content has been reduced as a result of IpTxa perfusion to approximately 75% of control. These results are consistent with the biphasic response observed in Ca^{2+} transient experiments. We believe that IpTxa sensitizes RyR to luminal Ca^{2+}, leading to increased Ca^{2+} release and subsequent depletion of Ca^{2+} from the SR. Our findings have exciting implications for translational research into cardiac diseases such as catecholaminergic polymorphic ventricular tachycardia, in which acute RyR hyperactivity is hypothesized to trigger arrhythmias leading to sudden cardiac death.

1564-Pos
Role of Hydrophobic Interactions in the Block of the Ryanodine Receptor by *Shaker* B Inactivation Peptides
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Shaker B K^+ channel NH_2-inactivation peptides (*Sh*BP) block both sheep and mouse cardiac ryanodine receptor Ca^{2+} release channels (RyR2). We provide new evidence for the presence of hydrophobic residues in the conduction pathway of RyR2 and their role in the block by wild-type (WT) and mutant *Sh*BP. RyR2 proteins were expressed in HEK cells, purified and their single channel activity recorded in lipid bilayers. Four peptides were synthesised and tested: i) WT *Sh*BP MAAVAGLYGLGEDRQHRKKQ, ii) a ''less hydrophobic'' peptide (LHBP) MAQVQGLYGLGEDRQHRKKQ, and 2 ''more hydrophobic'' peptides (MHBP), iii) MHBPI MAVVAGLYGLGEDRQHRKKQ and iv) MHBPII MAAVVGLYGLGEDRQHRKKQ. All peptides blocked the ryanodine-modified RyR2 channel from the cytosolic face in a concentration- and voltage-dependent manner. At a holding potential of +50 mV in symmetrical 210 mM KCl, we found an affinity constant K_D of 39.54 ± 3.90 μM for WT *Sh*BP, 65.90 ± 12.99 μM for LHBP, 27.79 ± 4.29 μM for MHBPI and 44.56 ± 9.38 for MHBPII. The association rates K_{on} of the peptides varied with concentration (2.92 ± 0.08 $s^{-1}.\mu M^{-1}$ for WT *Sh*BP, 3.85 ± 0.32 $s^{-1}.\mu M^{-1}$ for LHBP, 2.96 ± 0.22 $s^{-1}.\mu M^{-1}$ for MHBPI and 2.31 ± 0.25 $s^{-1}.\mu M^{-1}$ for MHBPII). Dissociation rates K_{off} were independent of concentration (115.30 ± 7.90 s^{-1} for WT *Sh*BP, 253.52 ± 24.49 s^{-1} for LHBP, 82.14 ± 5.48 s^{-1} for MHBPI and 102.97 ± 8.30 s^{-1} for MHBPII). Furthermore the block induced by the peptides could be reduced by an increase of the salt concentration at the luminal side of the channel. These findings indicate that hydrophobic interactions between RyR2 and inactivation peptides are necessary for the block, and that the binding sites of the peptides are within the pore. This research was supported by the BHF.

1565-Pos
Crystallographic Insights into the Cardiac Ryanodine Receptor N-terminal Domain and its Disease Mutants
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Ryanodine receptors (RyRs) are large channels governing the release of Ca^{2+} from the sarcoplasmic or endoplasmic reticulum. They are required for the contraction of both skeletal (RyR1) and cardiac muscles (RyR2). Mutations in RyR genes have been associated with severe genetic disorders, but high-resolution data describing the disease variants in detail has been lacking. We have solved the crystal structures of the N-terminal domains of both RyR2 (2.55Å) and RyR1 (3.0Å), along with structures of various RyR2 disease mutants. The N-terminal domain in both RyR1 and RyR2 consists of a core beta trefoil domain flanked by an alpha helix. Two cysteine pairs display a highly increased flexibility, making them ideal candidates to receive redox modifications. Crystal structures of several RyR2 disease mutants (1.7Å - 2.2Å) show that most of the mutations cause distinct local changes to the surface of the protein, highlighting at least two putative binding interfaces required for normal RyR function. One RyR2 disease mutant causes significant changes in the thermal stability of the N-terminal domain, accompanied by large conformational changes in the structure.

1566-Pos
Structural Mapping of the Ryanodine Receptor Type 1 Using A Fret-Based Method
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The type 1 ryanodine receptor (RyR1) mediates excitation contraction coupling in skeletal muscle by releasing stored intracellular calcium in response to cellular depolarization. This 2.2 MDa homotetrameric protein is associated with numerous regulatory proteins that modulate its activity in vivo. Understanding the structure and conformational dynamics of this immense macromolecular complex is an enormous challenge in skeletal muscle biology. In this report, structural determinations of RyR1 were performed using Förster resonance energy transfer (FRET) measurements. In this system, the FRET donor was green fluorescent protein (GFP) fused to RyR1, which could then transfer energy to Cy3NTA, a site-specific FRET acceptor targeted to poly-histidine segments inserted into RyR1. Energy transfer was monitored as a decrease in GFP fluorescence occurring when Cy3NTA was bound to a His tag in close proximity to the GFP donor fused either to position 1 or position 618 of RyR1. Cy3NTA was targeted to each of three ''divergent regions'' (DR) poorly conserved among the three RyR isoforms (DR1; position 4430, DR2; position 1323, DR3; position 1861). While minimal FRET was detected between N-terminally fused GFP and Cy3NTA targeted to these divergent regions, significant energy transfer was detected from GFP at position 618 to Cy3NTA targeted to DR2 or DR3. These experiments indicate that these donor and acceptor sites are in close proximity to each other and also demonstrate the utility of this FRET-based technique for further structural mapping of RyR1. (Supported by NIH grant R21ARO56406).