potassium channel KcsA as the template, encompasses transmembrane helices 8-10 and provides an excellent description for the pore domain of RyR2 with the overall structure and arrangement of key structural elements of the model closely resembling those of KcsA (Biophysical Journal 2004, 87, 2335-2351). Although good progress has been made in understanding ion handling capabilities in RyR2 the exact mechanisms remain elusive and controversial. To test and define the analogy model we have constructed and expressed both the PFR of RyR2 alone and a KcsA_RyR2_PFR chimera whereby the 22 residue N-terminal helix and 41 residue C-terminal domain of KcsA have been added by primer extension to the RyR2 PFR region and cloned into a modified pET expression vector containing an N-terminal hexa-His tag. Just as voltage sensor modules are transferable among potassium channels, our chimera will give us information on the functionality and transferability of the RyR2 PFR. Preliminary results indicate that both the chimera and PFR constructs express in large amounts in rosetta bacterial cells and are targeted to the membrane with no detectable protein in the soluble fraction. Detergent trials have identified LDAO as the best candidate for solubilisation from the membrane although other commonly used detergents are also capable of solubilisation, albeit to a lesser degree. Both constructs were purified as tetramers following nickel-affinity and gel filtration chromatography. This suggests strongly that both the RyR2 PFR and chimera are functional proteins capable of tetramerisation.
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2646-Pos
Enhanced RyR1 Channel Activity by the Knock-In Mouse that Expresses Human Malignant Hyperthermia Mutation T4826I
Wei Feng[1], Tianzhong Yang[2], Isela T. Padilla[1], Genaro C. Barreintos[1], Paul D. Allen[2], Isaac N. Pessah[1].
[1]University of California, Davis, Davis, CA, USA, [2]Brigham and Women's Hospital, Boston, MA, USA.
Malignant hyperthermia (MH) is a life-threatening pharmacogenetic syndrome of skeletal muscle associated with mutations in the Ca^{2+} release channel - ryanodine receptor type 1 (RyR1). A genetically engineered knock-in mouse that expresses the human C-terminal MH mutation T4826I RyR1 was created. Both RyR1-T4826I/WT (HET) and RyR1-T4826I/T4826I (HOM) mice survive and thrive if unchallenged with triggering agents. Sarcoplasmic reticulum membranes from HET, HOM, and wild type (WT) mice were prepared to study the biochemical and biophysical properties of RyR1complexes using [^{3}H]ryanodine ([^{3}H]Ry) binding analysis, western blotting, and single channels incorporated in bilayer lipid membranes. The results from this study reveal: **(1)** Significantly elevated [^{3}H]Ry binding in the preparations with rank order HOM >>HET>WT), **(2)** significant diminution in the inhibitory potency of Ca^{2+} and Mg^{2+} for both HOM and HET, **(3)** initial rates of [^{3}H]Ry binding with rank order HOM>>HET>WT when measured at either 25 °C or 37°C, **(4)** Significantly greater open probability, longer mean open dwelling time and shorter mean closed times with HOM and HET channels. These data indicate that the T4826I RyR1 mutation confers significantly destabilizes the closed channel conformation of RyR1, and the altered channel properties may be mostly responsible for the abnormal intracellular Ca^{2+} homeostasis and the MH susceptibility. (Words: 202).

2647-Pos
AICAR Prevents Heat-Induced Death in Mice with Malignant Hyperthermia
Johanna T. Lanner, Cheng Long, JianJun Xu, Mariah R. Baker, Susan L. Hamilton.
Baylor College of Medicine, Houston, TX, USA.
The pharmacogenetic disorder malignant hyperthermia (MH) is serious and potentially life-threatening illness caused by mutations in the skeletal muscle Ca^{2+}-release channel (ryanodine receptor, RyR1) located in the membrane of the sarcoplasmic reticulum. An MH episode is triggered by general anesthetics, heat and exercise in warm conditions. Signs of an MH crisis are: elevated core temperature, arrhythmias, hyper-metabolism and rhabdomyolysis. The MH mice created in our laboratory with a $Y524S^{+/-}$ mutation in RyR1 ($RyR1^{Y524S+/-}$) die upon exposure to elevated environmental temperatures (37°C) (Chelu et al 2006). The muscle relaxant dantrolene is the only known treatment for an MH crisis, but its adverse side-effects preclude prophylactic usage. We find that the treatment of the $RyR1^{Y524S+/-}$ mice with 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), an activator of AMP-activated protein kinase (AMPK), prevents heat-induced sudden death. Less than 5% of untreated $RyR1^{Y524S+/-}$ mice survive a heat-challenge, but treatment with AICAR leads to 100% survival. Muscle AMPK phosphorylation ($AMPK\alpha^{Thr172}$) levels are not significantly altered after 10 min subcutaneous injection with AICAR compared to saline. This suggests that the rescue may not be due solely to AMPK activation, but may also involve off-target effect(s) of AICAR. Adenine nucleotides are known channel agonists and AICAR is a precursor of the AMP analog, ZMP. We tested the effects of AICAR on ^{3}H -ryanodine binding in the presence of non-hydrolysable ATP (AMP-PCP). We find that AICAR is a partial agonist of RyR1 and prevents full activation of RyR1 in the presence of cellular concentrations of ATP in both $RyR1^{Y524S+/-}$ and wild-type mice. AICAR prevents heat-induced death in mice with MH and, since it is thought to have few side-effects, it is a potential prophylactic treatment for heat induced death associated with some MH mutations.

2648-Pos
Effects of a Y522S-RyR1 Mutation on Cerebellar Purkinje Cell Function
Jason A. Santiago, George C. Talbott, **Nancy M. Lorenzon**.
University of Denver, Denver, CO, USA.
The devastating consequences of calcium dysregulation are exemplified by the skeletal muscle diseases malignant hyperthermia (MH) and central core disease (CCD). These diseases result from mutations in the 'skeletal muscle-isoform' of the ryanodine receptor calcium release channel (RyR1). To investigate the etiology of MH and CCD, mouse models have recently been generated (Chelu et al. 2005). Skeletal muscle harboring the Y522S-RyR1 knock-in mutation exhibit Ca^{2+} leak from internal stores, basal cellular stress, and ultimately progressive mitochondrial and cellular damage (Durham et al. 2008). Although the effects of this MH-causing mutation have been characterized in skeletal muscle, the effects in the nervous system have not been documented. RyR1 is expressed highly in cerebellar Purkinje neurons. Y522S-RyR1 mice do not exhibit gross neurological defects suggesting that the defects may be subtle or that neuronal function is spared due to compensatory changes.
We have initiated studies investigating 3 main aspects of Y522S-RyR1 mouse Purkinje cells: intracellular calcium release, cellular organization/morphology, and cellular stress. In preliminary studies using imaging techniques with the calcium indicator dye Fura2-AM, RyR-mediated calcium release in Y522S-RyR1 Purkinje neurons exhibited a negative shift in the apparent EC_{50} for the agonist caffeine. In addition, the sensitivity of RyR to other activators (temperature, voltage, ryanodine) and intracellular calcium store content were investigated. Since calcium signaling is important during neuronal development and maturation, the morphology of Y522S-RyR1 Purkinje cells was examined using immunohistochemistry and confocal microscopy. Moreover, we have initiated studies to determine if altered RyR1 function results in basal cellular stress using Western blot and immunohistochemical analyses. Y522S-RyR1 mutant mice provide an excellent tool to address calcium dysregulation, its pathological consequences, and potential approaches for compensation of altered calcium signaling in the central nervous system.

2649-Pos
Distinct Properties of CPVT Mutations Located in the Central Domain of Human RyR2
Lynda Blayney[1], Michail Nomikos[2], Konrad Beck[1], Leon D'Cruz[1], Ewan McDonald[1], Julia Griffiths[1], **F. Anthony Lai**[1].
[1]Cardiff Univ, Cardiff, United Kingdom, [2]Demokritos Institute, Athens, Greece.
The cardiac ryanodine receptor (RyR2) Ca^{2+} release channel plays a central role in the rapid calcium release from the sarcoplasmic reticulum that is essential for muscle excitation-contraction coupling. Discrete mutations that have been discovered within the RyR2 associated with catecholaminergic polymorphic ventricular tachycardia (CPVT) are found to cluster in distinct regions of RyR2. These regions may represent important regulatory domains of the molecule that either directly or indirectly can affect channel gating. In response to a physiological trigger, RyR2 mutations are believed to cause diastolic Ca^{2+} leak which results in arrhythmia, making them an important potential therapeutic target.
We have expressed the RyR2 central domain CPVT-associated region and examined parameters which contribute to the structural and functional stability. A wild-type construct was compared with three constructs each containing a different CPVT mutation (P2328S, N2368I or A2387P). Circular dichroism spectroscopy revealed that none of the mutations significantly altered the percentage ellipticity of the protein, indicating that the overall conformation of the polypeptide backbone is only slightly affected by the mutations. Chemical denaturation using guanidine hydrochloride and monitoring tryptophan fluorescence suggested that the P2328S mutation was less stable than the wild-type or the other two mutations, indicated by a lower free energy change upon unfolding. A predicted ATP binding motif has previously been proposed in this domain and using fluorescence spectroscopy for the wild-type construct, ATP binding was observed with a K_d ~0.03mM.