3713-Pos

Increased Ca Sparks and Waves Frequency and Unchanged $[Na]_i$ in Hearts from Ankyrin-B Heterozygous Mice

Emmanuel Camors[1], Peter J. Mohler[2], Donald M. Bers[1], Sanda Despa[1].
[1]University of California Davis, Davis, CA, USA, [2]University of Iowa, Iowa City, IA, USA.

The interplay between Na/Ca exchanger (NCX) and Na/K-ATPase (NKA) is essential in regulating cardiac $[Na]_i$, $[Ca]_i$ and contractility. The membrane targeting and stability of both NCX and NKA require direct interaction with cytoskeletal protein ankyrin B (AnkB). Humans with AnkB loss-of-function mutations and AnkB heterozygous mice (AnkB+/-) display a complex cardiac phenotype that includes ventricular arrhythmias and sudden death. Cardiac myocytes from AnkB+/- mice show reduced NCX and NKA expression at the T-tubules, larger cellular and SR Ca load and increased frequency of delayed afterdepolarizations (DADs). How the coordinated loss of NCX and NKA affects SR Ca release and $[Na]_i$ is poorly understood. We measured Ca sparks and waves in myocytes from AnkB+/- and wild-type (WT) mice with and without 1 μM isoproterenol. Under control conditions, Ca transients, SR Ca load and Ca sparks frequency (CaSpF), even normalized to SR Ca content, were higher in AnkB+/- mice at all frequencies. With isoproterenol, Ca transient amplitude and SR Ca content were similar in myocytes from WT and AnkB+/- mice, but CaSpF was still elevated in AnkB+/- mice. Ca waves occurred preponderantly in AnkB+/- mice. In some cases, Ca waves and sparks evolved into synchronous after-Ca transients that are indicative of DADs. This data suggest that SR Ca release is enhanced in AnkB+/- mice. We measured $[Na]_i$ to determine whether the increased CaSpF in AnkB+/- mice is due to higher $[Na]_i$, which may elevate diastolic Ca. $[Na]_i$ was slightly, but not significantly, lower in AnkB+/- vs. WT mice (11.4 ± 1.1 *vs.* 13.2 ± 1.7 mM in WT). Isoproterenol lowered $[Na]_i$ by a comparable amount in both AnkB+/- and WT mice (to 8.1 ± 0.9 mM and 11.1 ± 1.5 mM). Thus, altered $[Na]_i$ does not explain the increased Ca sparks frequency in AnkB+/- mice.

3714-Pos

Regulation of E-C Coupling Via TβRI and FKBP12

Cheng Long, JianJun Xu, Johanna Lanner, KeKe Dong, Susan L. Hamilton.
Baylor College of Medicine, Houston, TX, USA.

The immunophilin FKBP12 (12-kDa FK506 Binding Protein) binds to both the type I transforming growth factor (TGF-β) receptor (TβRI) and the skeletal muscle Ca^{2+} release channel (RyR1). Skeletal muscle contains significant amounts of TβRI which is an integral member of the TGF-β signaling pathway that consists of the extracellular domain and the intracellular domain. The cytoplasmic domain of TβRI directly interacts with several proteins including FKBP12. FKBP12 modulates both TβRI-mediated signaling and Ca^{2+} release from RyR1. The functional significance of the interplay between TGF-β signaling and intracellular Ca^{2+} homeostasis is not known. We assessed the effects of TGFβ on excitation-contraction (E-C) coupling and the level of intracellular Ca^{2+} stores in primary myotubes and on fatigue in adult muscle. We found that TGFβ increases the relative gain of E-C coupling in myotubes and enhances the development of fatigue in skeletal muscle. We compared the locations of TβRI and RyR1 in isolated single fibers from wild-type and FKBP12 deficient mice and found that TβRI and RyR1 co-localized at the triad in single fibers. Other data suggest that free FKBP12 is limiting in muscle such that RyR1 is not fully saturated with FKBP12. One possible side effect of activation of local TGF-β receptors would be a local increase in FKBP12 and subsequent effects on RyR1 activity. This work was supported by grants from NIH (AR041802) and MDA to S.L.H.

3715-Pos

Exposure to High Glucose Alters Action Potential-Induced Ca2+ Transients and Enhances NFAT5 Expression and Nuclear Translocation in Cultured Adult Mouse Skeletal Muscle Fibers

Erick O. Hernández-Ochoa, Minerva Contreras, Patrick Robison, Tiansheng Shen, Martin F. Schneider.
University of Maryland, Baltimore, MD, USA.

Hyperosmolar non-ketotic syndrome (HNKS) is a life-threatening acute metabolic complication of uncontrolled type-2 diabetes characterized by severe hyperglycemia (600-900 mg/dL; 35-50 mM), dehydration, and muscle weakness; usually lasting for several days with a prognosis of 10-20% mortality. In the renal medulla, the transcription factor NFAT5 (or TonEBP) is a key regulator in protection from hypertonic stress. Here we evaluate the effects of sustained elevated extracellular glucose (50 mM of D or L-glucose for 48 hr) on Ca^{2+} signaling and endogenous NFAT5 activity in cultured adult murine FDB skeletal muscle fibers. Indo1-based ratiometric measurements showed no changes in resting Ca^{2+} in fibers challenged with high glucose. However, high glucose-exposed fibers displayed abnormal action potential-induced Ca^{2+} transients, manifest as a double Ca^{2+} spike upon a single 0.5 ms external field stimulus. Neither gadolinium (10 uM; a blocker of mechano-sensitive channels) nor apamin (1 uM; a Ca-dependent SK K^+ channel blocker) reversed the effect of high glucose on AP-induced Ca^{2+} transients. Di-8-anepps staining revealed that the normally highly regular transverse-tubule morphology was noticeably disrupted in fibers exposed to high glucose. Thus, elevated glucose did not modify resting Ca^{2+} homeostasis, but affected AP-induced Ca^{2+} transients, possibly via a modification of T-tubule cable properties. Western blot and immunostaining assays indicated that endogenous NFAT5 was present in control FDB fibers. High glucose increased NFAT5 protein expression and augmented its nuclear translocation. NFAT-driven luciferase assays demonstrated an increase in NFAT-mediated transcriptional activity in fibers keep in high glucose conditions. In conclusion, increased expression and augmented transcriptional activity and abnormal Ca^{2+} signaling in skeletal muscle may play a role in the pathophysiology of the HNKS. Supported by NIH-NIAMS Grants R01-AR055099, R01-AR056477 and T32-AR007592.

3716-Pos

Association of the Alpha1S I/II Loop Peptide with Beta1A Results in Translocation of the Complex to the Cell Surface and in Clustering

Alexander Polster, Symeon Papadopoulos.
Medical School Hannover, Hannover, Germany.

The cytoplasmic domains of the skeletal muscle dihydropyridine receptor (DHPR) are involved in processes like targeting to the junctions and coupling to the ryanodine receptor (RyR1). To investigate whether defined cytoplasmic DHPR regions possess affinity for the junctions, we expressed fluorescent protein labelled variants of the α_{1S} amino and carboxy terminus, its three loops connecting the homologous repeats, and the β_{1A} subunit, in dysgenic (α_{1S} null) myotubes. Individually expressed, no construct was able to recapitulate the fluorescence pattern typical of fluorescently labelled α_{1S} correctly targeted to the junctions. Rather, the proteins exhibited a diffuse intracellular distribution. The same was true for combinatory expression of the constructs, with one exception: When the α_{1S} loop connecting repeats I and II was co-expressed with β_{1A}, a translocation of the two interacting proteins from the cytoplasm to the surface of the myotube occurred. Moreover, the I/II loop - β_{1A} complex formed surface associated puncta reminiscent of fluorescently labelled junctional α_{1S}. In fact, we could observe a partial co-localization of the complex with fluorescently labelled RyR1 when the three proteins were co-expressed in dysgenic myotubes. According to these observations, it appears possible that the I/II loop - β_{1A} association could create a specific affinity for surface membrane regions and could thus contribute to the typical clustering of DHPRs. Additional experiments are being carried out to identify the regions within the I/II loop and within β_{1A} required for translocation of the complex.

3717-Pos

In Vitro Interactions Between the $Beta_{1a}$ Subunit of the Skeletal Muscle Dhpr and RyR1

Robyn Rebbeck[1,2], Yamuna Karunasekara[1,2], Esther M. Gallant[1,2], Llara Weaver[1,2], Nicole A. Beard[1,2], Marco G. Casarotto[1,2], **Angela F. Dulhunty**[1,2].
[1]JCSMR, Canberra City, Australia, [2]JCSMR, Canberra, Australia.

The $beta_{1a}$ subunit of the skeletal muscle DHPR plays two important roles in excitation-contraction (EC) coupling in skeletal muscle. $Beta_{1a}$ was originally found to target the $alpha_{1S}$ subunit of the DHPR to sarcolemmal tetrads which oppose skeletal ryanodine receptor (RyR1) channels in the sarcoplasmic reticulum. It was later found to also aid in transmission of EC coupling between the $alpha_{1S}$ subunit and RyR1 (1,2,3), through its C-terminal tail residues (1,2). This presumably depends on an interaction between the C-terminal tail of $beta_{1a}$ and RyR1. The full beta1a subunit binds to RyR1 in affinity chromatography experiments (4), but direct binding of the C-tail to RyR1 has not been reported, nor have direct functional interactions between the proteins. We show here that a peptide corresponding to the native sequence of residues in the C-terminus of $beta_{1a}$, but not a scrambled sequence, bind to RyR1. In addition the peptide and the full β1a subunit significantly increase both [^{3}H]ryanodine binding and single RyR1 channel activity at concentrations of 0.1 to 1nM, with maximum 3- to 5-fold activation at a concentration of ~10nM. The increase in RyR1 channel activity with both constructs is essentially irreversible within the lifetime of the bilayer, indicating tight binding. These results show that the C-terminal tail of the $beta_{1a}$ subunit is capable of directly binding to and activating RyR1 and hence suggest that $beta_{1a}$ may enhance EC coupling by virtue of its ability to activate RyR1.

1. Beurg M et al. Biophys J1999 *77*, 2953-2967.
2. Zhou W et al. Cell Calcium 2006 *39*, 227-236.
3. Schredelseker J et al. J Biol Chem 2009 *284,* 1242-51.
4. Cheng W et al. Proc Natl Acad Sci 2005 *102*, 19225-19230.