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Voltage-Dependent Stochastic Gating Models of TRIC-B Channels

Antoni Matyjaszkiewicz[1], Elisa Venturi[1], Daiju Yamazaki[2], Miyuki Nishi[2], Krasimira Tsaneva-Atanasova[1], Hiroshi Takeshima[2], Rebecca Sitsapesan[1].
[1]University of Bristol, Bristol, United Kingdom, [2]Kyoto University, Kyoto, Japan.

TRIC-A and TRIC-B are two, related, trimeric intracellular cation channels present in sarcoplasmic/endoplasmic reticulum (SR) and are thought to provide counter-current for SR Ca^{2+}-release. TRIC-B knockout mice die immediately after birth demonstrating the importance of this isoform [Yazawa et al., 2007, *Nature*, **448**, 78-82]. To study the distinct single-channel gating behaviour of TRIC-B, we incorporated skeletal muscle light SR from TRIC-A knockout mice into artificial membranes under voltage-clamp conditions in symmetrical 210 mM K-PIPES, pH 7.2. We developed Markov models of TRIC-B gating, with up to 4 distinct sub-conductance states (S_1-S_4), using both QuB [Qin F., 2004, *Biophys J.,* **86**(3), 1488-501] and our own software. Our models incorporate different connectivity schemes to account for the intrinsic variability in gating that was observed between different channels. Despite the variability, some obvious trends emerged. TRIC-B activity was higher at positive than at negative holding potentials. At positive potentials, the majority of channels exhibited long bursts of openings where predominant gating transitions were between the full open state and S_1, the largest sub-conductance state. Some channels, however, gated preferentially in sub-states S_3 and S_4, only visiting the full open state briefly. At negative potentials, channel activity consisted primarily of brief transitions between sub-conductance states. Closed lifetime distributions at positive potentials comprised of fast components ($\tau \approx 1$ ms), corresponding to brief transitions from the full open state, as well as slower components corresponding to inter-burst intervals. At negative potentials, inter-burst intervals were orders of magnitude longer demonstrating that the frequency of channel opening is heavily dependent on voltage. It will be important to develop comprehensive models of TRIC-B channel gating in order to fully understand the role of this important ion-channel in intracellular Ca^{2+}-release.

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Different Modes of Interaction between Junctin and Ryanodine Receptors

Shamaruh Mirza, Linwei Li, Nicole Beard, Angela Dulhunty.
Australian National University, Canberra, Australia.

The sarcoplasmic reticulum (SR) Ca^{2+} store in skeletal and cardiac muscle is important in regulating Ca^{2+} release and the strength of contraction, through key proteins including the ryanodine receptor (RyR), calsequestrin (CSQ) and intrinsic membrane co-proteins, junctin and triadin, which link CSQ to RyRs. Junctin contributes to SR Ca^{2+} release, store size, CSQ2 depolymerisation and activates RyR1 and RyR2 in lipid bilayers when added to the luminal solution (1). Two regions of junctin's luminal domain interact with luminal loops on RyR2 (2), indicating that junctin modulates RyR2 from within the SR lumen. Similar experiments have not been done with RyR1 and cytoplasmic interactions between junctin and RyRs have not been fully explored in RyR1 or RyR2.

To explore these interactions, we isolated full length junctin (FLjun) from skeletal muscle, expressed the C-terminal domain of junctin (Cjun) in E. coli and synthesised the Njun peptide corresponding to cytoplasmic N-terminal domain. Deletion constructs of cytoplasmic and luminal domains of RyR1 were expressed in HEK293 cells. Affinity chromatography and co-immunoprecipition confirmed that the FLjun binds to RyR1 and RyR2 and revealed that Njun binds to cytoplasmic regions of RyR1, while Cjun binds to two luminal loops on RyR1, one between TM5 and TM6 (aa4583-4626), and the second in a construct containing the pore helix and adjacent luminal residues (aa4861-4910). Results from lipid bilayers (Li, Mirza, Beard, Dulhunty abstract) indicate that junctin regulates RyR1 and RyR2 via cytoplasmic and luminal interactions and, unexpectedly, the cytoplasmic interaction is functionally dominant. Njun binding to the cytoplasmic domain of RyR1 must underly this action. We predict that Njun will also bind to the cytoplasmic domain of RyR2.

1.Wei L et al. (2009) Int J Biochem Cell Bio 41, 2214.
2.Altschafl et al. (2011) J Physiol 589, 6063.

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A Cytoplasmic Interaction between Junctin and RyRs with Major Consequences for RyR1 and RyR2 Activity In Vitro

Linwei Li, Shamaruh Mirza, Nicole A. Beard, **Angela F. Dulhunty**.
JCSMR, Canberra City, Australia.

Junctin is an intrinsic sarcoplasmic reticulum (SR) membrane protein in cardiac and skeletal muscle. The bulk of the protein is within the lumen of the SR where it binds to ryanodine receptor (RyR) calcium release channels and regulates Ca^{2+} release from the SR. We have previously reported that junctin added to the luminal solution increases RyR1 activity in lipid bilayers [1].

The contributions of the luminal and cytoplasmic domains of junctin to RyR activation have not previously been explored, although it is assumed that activation depends on interactions between their luminal domains. To explore this further, we expressed junctin's C-terminal domain (Cjun), synthesised a cytoplasmic N-terminal domain peptide (Njun) and isolated full length junctin (FLjun) from skeletal muscle. Consistent with previous findings [1,2], FLjun in luminal solutions activated purified RyR1 and RyR2. We predicted that luminal Cjun would similarly activate RyRs if the proteins interact only via their luminal domains. Unexpectedly, (a) luminal Cjun strongly inhibited RyR1 and RyR2 and (b) cytoplasmic Njun activated channels to a greater degree than luminal FLjun and also bound to cytoplasmic fragments of RyR1 [3]. Neither luminal Njun, nor scrambled Njun in cytoplasmic solution influenced channel activity. To explore cytoplasmic Njun and luminal Cjun effects further, we added both domains sequentially to channels. Excess activation by Njun was reduced to FLjun levels by adding Cjun, while significant inhibition by Cjun added first was reduced by Njun, it remained significantly different from the activation by FLjun. Therefore cytoplasmic interactions between junctin and RyRs determines the overall effect of junctin on channel activity.

1. Wei et al. (2009) *Int J Biochem Cell Biol***41;**2214.
2. Gyorke et al. (2004) *Biophys J* **86**:2121.
3. Mirza et al. (2013) Biophysics abstract.

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Human Aging and Expression of Proteins Interacting with the Ryanodine Receptor in Skeletal Muscle

Hermia Willemse[1], Paul N. Smith[2], Philip G. Board[1], Marco G. Casarotto[1], Angela F. Dulhunty[1].
[1]John Curtin School of Medical Research, The Australian National University, Canberra City, Australia, [2]Trauma and Orthopaedic Research Unit, Canberra Hospital, Canberra, Australia.

Sarcopenia is characterised by reduced muscle mass and reduced force that is only partially attributable to muscle atrophy. Fiber type distribution changes with aging (fast twitch decreases, slow twitch increases). Excitation-contraction coupling (ECC) may be impaired by an uncoupling of the dihydropyridine receptor (DHPR) and the ryanodine receptor (RyR1), possibly due to decreased expression of the DHPR α_{1s} subunit (Delbono et al., J Membr Biol. 1995;148:211-22). The DHPR β_{1a} subunit, which may contribute to ECC (Rebbeck et al., Biophys J. 2011;100:922-30), increases (Taylor et al., Aging cell 2009;8:584-94). The 12KDa FK506 binding protein (FKBP12) stabilizes RyR1 and its dissociation may contribute to decreased ECC (Andersson et al., Cell Metab.2011; 14:196-207). Previous studies have used animal models; here we investigate the expression of these proteins in aging human muscle.

Human muscle samples were obtained from 42 male and female donors (age 40-90) undergoing knee (vastus medialis) and hip (gluteus minimus or gluteus medius) replacements. Results of fiber type distribution in muscle homogenates from female subjects shows fast twitch fibers decreasing and slow twitch increasing with age. Preliminary data shows that levels of FKBP12 relative to RyR1, determined in microsomal vesicles using western blot and densitometry decrease with age. Expression levels of the DHPR α_{1s} and β_{1a} subunits relative to that of actin determined in muscle homogenates using western blot and densitometry are being analysed. We find that β_{1a} subunit is mostly in the homogenate and not associated with membrane fractions. The preliminary data indicates that the levels of several of the proteins that associate with RyR1 and modulate its activity change with age and could affect the release of Ca^{2+} during ECC and muscle function in human sarcopenia.

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Carboxyl-Terminal Domain of DHPR β1A is Essential for DHPR Tetrad Formation

Claudio F. Perez[1], Monique Thomas[1], Clara Franzini-Armstrong[2].
[1]Brigham and Women's Hospital, Boston, MA, USA, [2]University of Pennsylvania, Philadelphia, PA, USA.

Lack of expression of DHPR-β1a subunit in skeletal muscle cells severely compromises the DHPRs-RyR1 stereospecific association that results in the assembly of ordered DHPR tetrads arrays. This, in turn results in the disruption of the EC-coupling DHPR-RyR signal. Previously, using expression of β1a subunits with progressive truncations of the carboxyl-terminal (C-term) domain in β1-null myotubes we found that aa residues 488-512 of β1a were critical to the restoration of the EC-coupling signal. Here we explore the effect of C-term truncations on DHPR tetrad formation and whether the ability of different truncated β1a to restore EC-coupling correlate with their ability to restore DHPR