difference in current density between long and short channels for both Ba^{2+} and Ca^{2+} currents (I_{Ba} and I_{Ca}) while membrane expression of the pore-forming subunit was unchanged suggesting additional differences of unitary current properties. To further determine the mechanism underlying CTM modulation, we performed single-channel analysis of recombinant long ($Ca_V1.3_{42}$) or short ($Ca_V1.3_{42A}$) channels co-expressed with β_3 and $\alpha_2\delta$-1 in HEK-293 cells using either15mM Ba^{2+} or Ca^{2+} as charge carrier. We found a significant increase of channel availability given as fraction of active sweeps [%] (at −30mV for $Ca_V1.3_{42}$: 20.9 ± 3.1, $Ca_V1.3_{42A}$: 60.9 ± 9.7, $p<0.01$, Students t-test) which reflects the shift to hyperpolarized potentials of $Ca_V1.3_{42A}$ channels in whole-cell experiments. Furthermore, larger currents in $Ca_V1.3_{42A}$ channels were due to significantly increased open probability across all test potentials. Single-channel conductance was similar (16 and 15 pS for $Ca_V1.3_{42}$ and $Ca_V1.3_{42A}$). The inactivation of ensemble average I_{Ba} was similarly slow in both channels. In contrast, more pronounced single-channel I_{Ca} inactivation of $Ca_V1.3_{42A}$ compared to $Ca_V1.3_{42}$ was found (τ_{inact} [ms] at −10mV: $Ca_V1.3_{42}$: 35.5 ± 12.3, $Ca_V1.3_{42A}$: 8.2 ± 3.1).Taken together, single-channel properties reflect the differences in voltage- and Ca^{2+}-dependent activation and inactivation gating properties previously observed in whole-cell recordings for these two splice variants. We could explain the higher current density of $Ca_V1.3_{42A}$ channels by increased channel activity.

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2671-Pos

Both Voltage- and Ca^{2+}-Dependent Inactivation of $Ca_v1.2$ Ca^{2+} Channels are Suppresed in Myotubes, Likely Because of Triad Junction Proteins other than RyR1

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To investigate the relationship between calcium channel and EC coupling functions of CaV1.1 in skeletal muscle, we have compared its behavior with that of the cardiac L-type channel, CaV1.2. Using expression in tsA-201 cells, we showed that substituting CaV1.1 IQ motif residues into CaV1.2 IQ caused a loss of calmodulin binding and calcium-dependent inactivation (JBC 283:29301-11, 2008), raising the possibility that inactivation is maladaptive for CaV1.1 function in muscle. We thus compared inactivation of CaV1.2 in tsA-201 cells and *dysgenic* myotubes (null for CaV1.1). For CaV1.2 in tsA-201 cells (co-expressed with $\alpha2\delta1$ and $\beta2a$), $I_{50\ ms}/I_{peak}$ (R_{50}) at +20 mV was .69 in 10 Ca and .87 in 10 Ba, whereas R_{50} for CaV1.2 in dysgenic myotubes was .95 at +20 mV in 10 Ca, indicating that both voltage- and calcium-dependent inactivation were suppressed. In tsA-201 cells, co-expression of $\beta1a$ (the predominant skeletal muscle isoform) did not significantly alter R_{50} values from those with $\beta2a$. Furthermore, R_{50} values were similar for CaV1.2 expressed in *dysgenic* myotubes and myotubes null for both CaV1.1 and RyR1, suggesting that some component of muscle triad junctions other than RyR1 is responsible for the suppression of inactivation. We thus expressed CaV2.1 in *dysgenic* myotubes because this neuronal channel, unlike CaV1.2, is not targeted to triad junctions (PNAS 95:1903-1908, 1998). Consistent with the hypothesis CaV2.1 channels showed significant voltage- and calcium-dependent inactivation in *dysgenic* myotubes which were similar to those of the channel expressed in tsA-201 cells. We are currently seeking to identify the components involved in suppressing inactivation of CaV1.2 in myotubes. Supported by AHA0190016G to JDO and NIH/NIAMS (AR055104) and MDA4319 to KGB.

2672-Pos

Electromechanical Coupling During the Activation of $Ca_v1.2$

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The $Ca_V\alpha_1$ subunit of L-type calcium channel $Ca_V1.2$ is formed of a voltage sensing domain (segments S1 to S4) and a pore-forming domain (segments S5-S6). Although the electromechanical coupling between these regions during opening and closing of the channel, in which movement of the S4 voltage sensor is mechanically transferred to the gate via movement of the S4-S5 linker, has been well established in K^+ channels (Long et al. 2005), the molecular mechanism linking the movement of the voltage sensor to the opening of the pore remains elusive in voltage dependent Ca^{2+} channels. Glycine mutations at position I781 in the distal IIS6 helix in $Ca_V1.2$ (Timin et al., 2009) and the equivalent I701 residue in $Ca_V2.3$ (Raybaud et al., 2007) have been shown to significantly decrease the free energy of voltage-dependent activation in both channels, suggesting that IIS6 plays a key role in coupling the pore opening and the voltage-sensing domain. To examine the electromechanical model of channel opening in $Ca_V1.2$, cross-linking studies were undertaken with pairs of cysteine mutants introduced in the S4-S5 linker(s) and I781C in IIS6. The biophysical properties of the double mutants expressed in *Xenopus* oocytes, are currently tested in the presence of the bridging reagent $tbHO_2$ at 0.5 mM. We hypothesize that if cysteine residues in the S4-S5 linker come in atomic proximity ($\leq$ 3 Å) with I781 during channel activation, addition of $tbHO_2$ will promote the formation of a disulfide bridge. This effect should be reversible by addition of DTT. Supported by grants from the Canadian Institutes of Health Research and the Heart and Stroke Foundation of Canada to LP.

2673-Pos

Activation Gating Determinants in Segments IS6 - IIIS6 of $Ca_v1.2$

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We have previously shown that mutations in pore lining segment IIS6 of Cav1.2 induce leftward shifts of the activation curve reflecting a destabilization of the closed and a simultaneous stabilization of the open channel state (Beyl et al. 2009). Systematic substitutions of a cluster of hydrophobic residues (LAIA motive) by residues of different size and polarity (except proline) revealed a strong correlation between changes in hydrophobicity (ΔH) and the shifts of the activation curve ($\Delta V_{0.5}$). A similar analysis in segment IS6 revealed no correlation between ΔH and $\Delta V_{0.5}$. Here we show that amino acid substitutions in the corresponding sequence stretch of segment IIIS6 (F1191 - I1196) shift the activation curves ($\Delta V_{0.5}$ F1191T=-7.1 ± 1.3mV, V1192T= 8.2 ± 1.2mV , G1193T=-31.2 ± 1.3mV, F1194T=2.1 ± 1.2mV, V1195T= -10.3 ± 1.1mV, I1196T=-17.9 ± 1.1mV). A reduction in hydrophobicity in positions V1195 (ΔH(V1195T)=-4.9) and I1196 (ΔH(I1196T)=-5.2) apparently destabilized the closed channel state. Other mutations (V1192T, G1193T and F1194T) did not fit this paradigm. Potential interactions between residues in segments IS6, IIS6 and IIIS6 were analysed by means of double mutant cycle analysis. Our data reveal a positional specific interaction between gating determinants in segments IS6 and IIS6 as well as IIS6 and IIIS6. Rate constants of the voltage sensing machinery and pore stability were estimated using a 4 state gating model (Beyl et al. 2009).

2674-Pos

Effects of Alanine Substitutions for Highly-Conserved Phenylalanines in the Skeletal Muscle L-Type Calcium Channel

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The motif FxxExxxK/R is highly conserved in S2 segments of voltage-gated cation channels, including the four repeats of the α_{1s} subunit of the skeletal muscle L-type Ca^{2+} channel, and appears to represent an important structure for voltage-sensing. Moreover, mutating the conserved S2 phenylalanine within the drk1 K^+ channel monomer to alanine causes a ~50 mV depolarizing shift in activation (Li-Smerin et al., JGP 115:33-49). Here, we made homologous F to A substitutions in Repeat I (F97A), Repeat III (F843A) and Repeat IV (F1161A) of α_{1S} N-terminally tagged with yellow fluorescent protein (YFP-α_{1S}), and tested the ability of the mutants to conduct L-type Ca^{2+} current and serve as the voltage sensor for excitation-contraction (EC) coupling after expression in *dysgenic* (α_{1S} null) myotubes. Confocal imaging of the YFP tag indicated that each of these constructs was targeted to plasma membrane junctions with the sarcoplasmic reticulum (SR). Measurement of intramembrane charge movements showed no significant difference in mutant expression relative to YFP-α_{1S} ($p > 0.05$, *ANOVA*). The F843A and F1161A mutants both supported L-type currents and myoplasmic Ca^{2+} transients with similar amplitude and voltage-dependence to those of YFP-α_{1S}. In contrast, the F97A mutant displayed substantial depolarizing shifts in activation of L-type current (24 mV) and SR Ca^{2+} release (13 mV). Together, these results indicate that: *i*) voltage-induced conformational changes in repeat I are directly important for activation of both L-type current and EC coupling, and *ii*) either repeats III and IV are less directly important for these functions or the F-A mutations have little influence on voltage-dependent conformational changes of these repeats. We are currently assessing the effects of the F475A mutation in Repeat II. Supported by NIH AR055104 and MDA4319 to K.G.B. and MDA4155 to R.A.B.