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membrane gating charge movements (Q) demonstrated that channel open probability (i.e., G/Q ratio) was indistinguishable for full-length or truncated (at 1662) CaV1.1 expressed in *dysgenic* myotubes (Nature 360:169-171). Here we have investigated the effects of removing the distal C-terminus on depolarization-induced potentiation of CaV1.1. Specifically, tail currents were measured for repolarization to -30 mV following a 200 ms depolarization to either +40 or +90 mV. For both full-length and truncated CaV1.1, tail currents were both larger (~2.5-fold) and more slowly decaying (~2-fold) following the +90 mV depolarization. Thus, we find no evidence for a role of the CaV1.1 distal C-terminus in depolarization-induced potentiation.
We are currently examining the role of the CaV1.2 distal C-terminus by co-expression of full-length or truncated (1669) CaV1.2 in tsA-201 cells together with β_{2a} and $\alpha_2\delta 1$. In agreement with previous work (J Physiol. 576:87-102, and in contrast to CaV1.1, truncation of CaV1.2 resulted in ~4-fold increase in the G/Q ratio. We are currently investigating the ability of the truncated CaV1.2 to undergo depolarization-induced potentiation. Supported by NIH (NS24444) and MDA grants to KGB.

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Chimera of CaV1.2 and CaV3.1 alpha1 Subunits Suggests Role of the C-terminal Tail in Cytosolic Mg2+ Actions on CaV1.2 Gating
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Previous studies (Wang and Berlin, *Am. J. Physiol*. **291**:C83, 2006) have shown that gating properties of $Ca_V1.2$ channels (subunits α_1, β_{2A} and $\alpha_2\delta$) expressed in tsA201 cells are significantly altered by varying cytosolic Mg^{2+} across a range of physiologic concentrations. Alterations in gating include changes in peak current amplitude as well as kinetics of current inactivation. In contrast, when $Ca_V3.1$ (α_1 subunit only) is expressed, varying cytosolic Mg^{2+} across a similar concentration range has little or no effect on channel gating. To understand the molecular basis for the effects of cytosolic Mg^{2+} on these related Ca^{2+} channels, a chimera channel consisting of $Ca_V3.1$ (α_1 residues 1-1826) with the C-terminal region of $Ca_V1.2$ (α_1 residues 1515-2171) was constructed and expressed in tsA201 cells. Ca^{2+} currents were measured in cells whole-cell patch-clamped with electrodes containing salt solutions in which Mg^{2+} and Ca^{2+} concentrations were strongly buffered. The chimera Ca^{2+} channel had a similar membrane potential dependence for activation and steady-state inactivation as $Ca_V3.1$; however, the rate of current inactivation was slowed at least two-fold. Varying patch electrode Mg^{2+} concentration had little effect on the rate of current inactivation, similar to $Ca_V3.1$, but unlike $Ca_V1.2$. On the other hand, current amplitude was depressed in the chimera channel with increasing Mg^{2+}. These results show that the C-terminal tail of $Ca_V1.2$ affects kinetics of channel gating. At least in part, changes in channel availability with cytosolic Mg^{2+} can be attributed to the C-terminal tail of $Ca_V1.2$; however, this domain alone cannot be responsible for Mg^{2+}-dependent regulation of channel gating kinetics.

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$Ca_V1.4$ C-tail Segment (ICDI) Inhibits Ca_V Channel Inactivation by Competing with Calmodulin–Resolution by Holochannels and Calmodulin FRET Sensors
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An intriguing variation on calmodulin/Ca_V channel inactivation (CDI) is the action of a C-tail segment from $Ca_V1.4$ channels (ICDI) to eliminate CDI. Introducing ICDI into $Ca_V1.2$ or $Ca_V1.3$ channels nearly abolishes strong baseline CDI, and a like effect is observed when ICDI is present within $Ca_V1.4$ itself. In retina, the effect in $Ca_V1.4$ helps sustain Ca^{2+} influx despite maintained depolarization. Contrasting with clear-cut function, the underlying ICDI mechanism remains controversial. One group proposes that ICDI allosterically inhibits CDI (Wahl-Schott *et al* PNAS 2006), while another suggests direct competition between calmodulin and ICDI for the channel (Singh *et al* Nature Neurosci 2006). The discussion hinges on differing calmodulin versus channel peptide assays. Here, we perform functional interaction assays using holochannels within live cells. As baseline, we electrophysiologically characterized $Ca_V1.3$ channels fused to an ICDI-containing segment ($\alpha_{1D\text{-}ABI\text{-}F}$). These $\alpha_{1D\text{-}ABI\text{-}F}$ channels exhibited little CDI compared to wild-type $Ca_V1.3$. Critically, variations in the ambient calmodulin concentration would only affect competitive versus allosteric mechanisms. Indeed, when calmodulin was depleted by a 'calmodulin sponge,' residual CDI in $\alpha_{1D\text{-}ABI\text{-}F}$, was totally eliminated. More telling, when calmodulin was over-expressed with $\alpha_{1D\text{-}ABI\text{-}F}$, we observed a resurgence of CDI to wild-type $Ca_V1.3$ levels. To test for precise agreement with a competitive mechanism, we co-expressed $\alpha_{1D\text{-}ABI\text{-}F}$ channels with BSCaMIQ, a FRET biosensor of calmodulin (Black *et al* Biochemistry 2006). Accordingly, both CDI and calmodulin concentrations could be measured within single cells; and pooling data from cells exhibiting variable calmodulin levels permitted explicit resolution of an *in situ* calmodulin binding curve, in strict agreement with a competitive mechanism. In all, ICDI suppresses CDI by competing with calmodulin for the channel, raising the possibility that natural variations in calmodulin might customize CDI through this mechanism.

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Structure-function Relationship of N-terminal Deletion Mutants of Cardiac L-type Calcium Channel ß1-subunits
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Auxiliary ß-subunits of L-type Ca2+ channel (L-VDCC) profoundly modulate properties of L-VDCC. Previously, we demonstrated that the N-terminus of ß2-subunit serves as a length-dependent structural determinant of channel inactivation (Herzig et al., FASEB J. 2007). Here, we tested the role of the N-terminus of ß1a-subunit. Three artificial ß1a-subunit mutants with different N-terminus lengths, ß1aN18, ß1aN27 and ß1aN51, were created. Their modulatory functions were investigated in recombinant L-VDCC and compared with the natural full-length isoform, termed ß1aN60.
In whole-cell patch-clamp measurements, we confirmed functional expression of all ß1a-subunit isoforms by a marked increase of current density and a leftward shift of activation, as compared to control transfections without any ß-subunit. No obvious differences were found among ß1a-subunit isoforms. In contrast, shortenening of the N-terminus progressively decreased the rate and extent of time-dependent inactivation at all test voltages.
Descriptive analysis of the single-channel data (e.g., peak ensemble average current, open probability, availablility) revealed similar parameters among ß1a-subunit isoforms, except for small deviations with ß1aN51. Strikingly, the extent of the inactivation of ensemble average currents followed the length of the N-terminus (ß1aN60>ß1aN51>ß1aN27>ß1aN18). For more detailed kinetic analysis, we performed Markov modeling using the scheme:
C-C-C-C-C-O
Ic-Ic-Ic-Ic-Ic-Io
with the rate constants for C-C and Ic-Ic: alpha, beta; C-O and Ic-Io: alphá, betá; C-I and O-I: gamma, delta. Channel open probability, availability, and first-latency, open-time and closed-time histograms were well fitted simultaneously. We found significant linear correlation between the inactivation rates gamma and delta and the N-terminus length. The other parameters alpha, alphá, beta, betá) did not vary with the N-terminal length of the ß1a-subunit.
Our results demonstrate that inactivation is under length-dependent control of the N-terminus of L-VDCC ß1-subunit. This could represent a general mechanism of ß-subunit modulation.

959-Pos Board B838
Modulation Of Calcium Currents By Acidic Domains Of Calcium Channel Subunits: A Novel Feedback Mechanism?
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Voltage-gated calcium (Cav) channels are essential to the function of excitable cells. Cav channels are multimeric proteins that consist of a pore forming subunit (alpha1) and several accessory subunits. We are characterizing an accessory beta subunit from the human pathogen Schistosoma mansoni (SmCavbeta), using a human alpha1E channel (Cav2.3) as the modulatory substrate and the whole-cell patch-clamp technique. SmCavbeta modulates Cav2.3 currents in a conventional manner, but it induces them to run-down to ~75% of their initial amplitudes within two minutes of establishing the whole-cell configuration. SmCavbeta has a unique poly-acidic motif of 15 aspartate and glutamate residues in its N-terminus. A mutant version of SmCavbeta lacking the first forty-six amino acids, which comprise the entire poly-acidic motif, did not induce run-down of the calcium current. Smaller deletions of this region provide a higher-resolution profile of the structures required for run-down. A deletion of the N-terminus that eliminates the amino acids preceding the acidic motif reduces the Ca2+ current to the same extent as the wild type subunit, by ~29% within 4 minutes of patch disruption. A deletion that eliminates the first six residues of the poly-acidic motif also reduces the calcium current significantly, but to a lesser extent than the wild-type subunit (by ~17% within the same time-frame). A deletion mutant subunit without the first nine acidic residues of the poly-acidic motif did not induce run-down. Based on the structural homology between the poly-acidic motif of SmCavbeta and that of calcium binding phosphoproteins, we speculate that SmCavbeta binds Ca2+. Since similar N-terminal poly-acidic motifs are found in all platyhelminths examined, it is tempting to hypothesize that these structures represent a feedback mechanism that modulates influx of extracellular Ca2+ into the excitable cells of platyhelminths.