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.

956-Pos Board B835

Chimera of CaV1.2 and CaV3.1 alpha1 Subunits Suggests Role of the C-terminal Tail in Cytosolic Mg2+ Actions on CaV1.2 Gating

Min Wang[1], Yoganand Yadari[2], **Joshua R. Berlin[2]**.
[1]Emisphere Technologies, Tarrytown, NY, USA, [2]UMDNJ-New Jersey Medical School, Newark, NJ, USA.

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.

957-Pos Board B836

$Ca_V1.4$ C-tail Segment (ICDI) Inhibits Ca_V Channel Inactivation by Competing with Calmodulin–Resolution by Holochannels and Calmodulin FRET Sensors

Xiaodong Liu, Philemon S. Yang, Vincent Wu, Wanjun Yang, David T. Yue.
Johns Hopkins University, Baltimore, MD, USA.

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.

958-Pos Board B837

Structure-function Relationship of N-terminal Deletion Mutants of Cardiac L-type Calcium Channel ß1-subunits

Wanchana Jangsangthong[1], Elza Kuzmenkina[1], Ismail F.Y. Khan[1], Roger Hullin[2], Stefan Herzig[1].
[1]University of Cologne, Cologne, Germany, [2]University Hospital Bern, Bern, Switzerland.

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?

Vicenta Salvador-Recatala, Robert M. Greenberg.
University of Pennsylvania, Philadelphia, PA, USA.

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.