

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  $\beta_{2a}$  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 $\alpha_1$ Subunits Suggests Role of the C-terminal Tail in Cytosolic Mg<sup>2+</sup> Actions on CaV1.2 Gating

Min Wang<sup>1</sup>, Yoganand Yadari<sup>2</sup>, Joshua R. Berlin<sup>2</sup>.  
<sup>1</sup>Emisphere Technologies, Tarrytown, NY, USA, <sup>2</sup>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<sub>v</sub>1.2 channels (subunits  $\alpha_1$ ,  $\beta_{2A}$  and  $\alpha_{2\delta}$ ) expressed in tsA201 cells are significantly altered by varying cytosolic Mg<sup>2+</sup> 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<sub>v</sub>3.1 ( $\alpha_1$  subunit only) is expressed, varying cytosolic Mg<sup>2+</sup> across a similar concentration range has little or no effect on channel gating. To understand the molecular basis for the effects of cytosolic Mg<sup>2+</sup> on these related Ca<sup>2+</sup> channels, a chimera channel consisting of Ca<sub>v</sub>3.1 ( $\alpha_1$  residues 1-1826) with the C-terminal region of Ca<sub>v</sub>1.2 ( $\alpha_1$  residues 1515-2171) was constructed and expressed in tsA201 cells. Ca<sup>2+</sup> currents were measured in cells whole-cell patch-clamped with electrodes containing salt solutions in which Mg<sup>2+</sup> and Ca<sup>2+</sup> concentrations were strongly buffered. The chimera Ca<sup>2+</sup> channel had a similar membrane potential dependence for activation and steady-state inactivation as Ca<sub>v</sub>3.1; however, the rate of current inactivation was slowed at least two-fold. Varying patch electrode Mg<sup>2+</sup> concentration had little effect on the rate of current inactivation, similar to Ca<sub>v</sub>3.1, but unlike Ca<sub>v</sub>1.2. On the other hand, current amplitude was depressed in the chimera channel with increasing Mg<sup>2+</sup>. These results show that the C-terminal tail of Ca<sub>v</sub>1.2 affects kinetics of channel gating. At least in part, changes in channel availability with cytosolic Mg<sup>2+</sup> can be attributed to the C-terminal tail of Ca<sub>v</sub>1.2; however, this domain alone cannot be responsible for Mg<sup>2+</sup>-dependent regulation of channel gating kinetics.

#### 957-Pos Board B836

##### Ca<sub>v</sub>1.4 C-tail Segment (ICDI) Inhibits Ca<sub>v</sub> 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<sub>v</sub> channel inactivation (CDI) is the action of a C-tail segment from Ca<sub>v</sub>1.4 channels (ICDI) to eliminate CDI. Introducing ICDI into Ca<sub>v</sub>1.2 or Ca<sub>v</sub>1.3 channels nearly abolishes strong baseline CDI, and a like effect is observed when ICDI is present within Ca<sub>v</sub>1.4 itself. In retina, the effect in Ca<sub>v</sub>1.4 helps sustain Ca<sup>2+</sup> 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<sub>v</sub>1.3 channels fused to an ICDI-containing segment ( $\alpha_{1D-ABI-F}$ ). These  $\alpha_{1D-ABI-F}$  channels exhibited little CDI compared to wild-type Ca<sub>v</sub>1.3. 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  $\alpha_{1D-ABI-F}$  was totally eliminated. More telling, when calmodulin was over-expressed with  $\alpha_{1D-ABI-F}$ , we observed a resurgence of CDI to wild-type Ca<sub>v</sub>1.3 levels. To test for precise agreement with a competitive mechanism, we co-expressed  $\alpha_{1D-ABI-F}$  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 $\beta_1$ -subunits

Wanchana Jangsangthong<sup>1</sup>, Elza Kuzmenkina<sup>1</sup>, Ismail F.Y. Khan<sup>1</sup>, Roger Hullin<sup>2</sup>, Stefan Herzig<sup>1</sup>.

<sup>1</sup>University of Cologne, Cologne, Germany, <sup>2</sup>University Hospital Bern, Bern, Switzerland.

Auxiliary  $\beta$ -subunits of L-type Ca<sup>2+</sup> channel (L-VDCC) profoundly modulate properties of L-VDCC. Previously, we demonstrated that the N-terminus of  $\beta_2$ -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  $\beta_1$ -subunit. Three artificial  $\beta_1$ -subunit mutants with different N-terminus lengths,  $\beta_1A_{N18}$ ,  $\beta_1A_{N27}$  and  $\beta_1A_{N51}$ , were created. Their modulatory functions were investigated in recombinant L-VDCC and compared with the natural full-length isoform, termed  $\beta_1A_{N60}$ .

In whole-cell patch-clamp measurements, we confirmed functional expression of all  $\beta_1$ -subunit isoforms by a marked increase of current density and a leftward shift of activation, as compared to control transfections without any  $\beta$ -subunit. No obvious differences were found among  $\beta_1$ -subunit isoforms. In contrast, shortening 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, availability) revealed similar parameters among  $\beta_1$ -subunit isoforms, except for small deviations with  $\beta_1A_{N51}$ . Strikingly, the extent of the inactivation of ensemble average currents followed the length of the N-terminus ( $\beta_1A_{N60} > \beta_1A_{N51} > \beta_1A_{N27} > \beta_1A_{N18}$ ). For more detailed kinetic analysis, we performed Markov modeling using the scheme:

C-C-C-C-O

Ic-Ic-Ic-Ic-Io

with the rate constants for C-C and Ic-Ic: alpha, beta; C-O and Ic-Io: alpha, beta; 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, alpha, beta, beta) did not vary with the N-terminal length of the  $\beta_1$ -subunit.

Our results demonstrate that inactivation is under length-dependent control of the N-terminus of L-VDCC  $\beta_1$ -subunit. This could represent a general mechanism of  $\beta$ -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* (SmCav-beta), 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 Ca<sup>2+</sup> 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 Ca<sup>2+</sup>. 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 Ca<sup>2+</sup> into the excitable cells of platyhelminths.