the sustained phase is believed to be mediated via store depletion-activated Ca\(^{2+}\) entry. Using patch-clamp recording and Ca\(^{2+}\) imaging, we show here that Cav channels currents, while found in spermatogenic cells, are not detectable in epidermal sperm and are not essential for the ZP-induced [Ca\(^{2+}\)] change. Instead, CATSPER channels localized in the distal portion of sperm (the principal piece) are required for the ZP-induced [Ca\(^{2+}\)] change. Furthermore, the ZP-induced [Ca\(^{2+}\)] increase starts from the sperm tail and propagates toward the head.

**Voltage-gated Ca Channels II**

950-Pos Board B829

**A Simple Link Between Gating And Pore Occupancy Can Describe Complex Ion-Dependent Kinetics Of Ca\(^{2+}\) Channels**

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Several aspects of Ca\(^{2+}\) channel gating depend on permeant ions. These effects are difficult to describe in terms of regular “states-and-rates” models, which suggest that the channel does not change kinetic state(s) while it is open to pass current. We propose an approach to overcome this limitation. An open state is considered to have a minimum of two “sub-states”: one is occupied by permeant ion and the other is not. The sub-states are allowed to have different kinetic paths of exit from the open state.

A minimalistic model of this sort explains the U-shaped voltage-dependence of inactivation by incorporating our previous finding that the apparent affinity of the channel pore for permeant ions increases during inactivation (Babich et al., JGP, 2007). The model implies that the tighter binding of permeant ions to the pore prevents current through inactivated channels, as if the pore is the “inactivation gate.” This idea is in an apparent contradiction with the view that the mechanism of inactivation of Ca\(^{2+}\) currents, “C2D,” is independent from that of Ba\(^{2+}\) currents, “V2D” (e.g., Barret and Tsien, PNAS, 2008). Here we show that our model describes well effects of molecular interventions that appear to differentially alter “C2D” and/or “V2D.” Therefore, ion- and voltage-dependent components of inactivation may converge at the act of channel’s pore.

951-Pos Board B830

**Kinetic Modeling of CaV3.1**

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T-type calcium channel kinetics have been well characterized at the macroscopic level, but less well so at the single channel level. The most thorough single channel kinetic analyses have been performed using recordings from native tissues where the specific isoform(s) is present. As a result there is some discrepancy as to magnitude and voltage dependence of fundamental descriptors such as mean open time. It has been reported to be 0.5-2.5 ms and either weakly voltage dependent or voltage independent (summarized in Perez-Reyes 2005). Macroscopic current measurements, on the other hand, are consistent with a voltage dependent O→C transition, suggesting that mean open time is voltage dependent. Band-width differences and other issues associated with signal to noise ratio for these measurements in each of the four \(\alpha\)-subunit domain’s. In the four segments, also known as voltage sensors, every third position there is a positive charged residue (lysine or arginine). However, both high-voltage (HV) and low-voltage (LVA or T-type) activated Ca\(_{\alpha}\) channels, show S4 segments with very similar sequences.

We have utilized low noise recording methods to obtain higher bandwidth single channel data and paired it with maximum idealized point-likelihood analysis in QuB to estimate rate constants and their voltage dependence from single channel data for inclusion in developing models that correctly recapitulate macroscopic, single channel, and gating current data. Supported by F31-NS085833 (K.B.) and RO1-HL065680 (D.H.)

952-Pos Board B831

**Voltage-sensor Pharmacology Of Voltage-activated Calcium Channels (cav)**

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The four voltage sensors in Cav channels have distinct amino acid sequences, raising fundamental questions about their relative contributions to the function and regulation of the channel. Studies of Kv channels identified a S3b-S4 helix-turn-helix motif, termed paddle motif, which moves at the protein-lipid interface interface to drive activation of the voltage-sensors. This motif is an important pharmacological target for amphoteric neurotoxins and it has been suggested that is conserved in Cav and other voltage-gated ion channels. Here we show that the four S3b-S4 paddle motifs within the Cav channel could be transplanted into four-fold symmetric Kv channel to individually examine their contributions to the kinetics of voltage sensor activation and regulation by toxins.

953-Pos Board B832

**Independent Contributions Of Segments IS6 And IIS6 To Activation Gating Of CaV,1.2**

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Voltage dependence and kinetics of activation of Cav1.2 channels are affected by structural changes in pore lining S6 segments of the \(\alpha\)-subunit. Significant effects on channel activation are caused by either proline and threonine substitutions in the lower third of segment IS6 (‘bundle crossing region’). Here we report that S435P in IS6 results in a large shift of the activation (−26 mV) curve and slowed current kinetics. Threonine substitutions in positions Leu429 and Leu434 induced a similar kinetic phenotype with shifted activation curves.

Double mutations in segments IS6 and IS6E induced additive shifts of the activation curves, e.g.: L429T/L771T (+44±10), L434T/L771T (+50±3, +8), L429T/L779T (-22±5, 0.8) and L434T/L779T (-32.3±0.8). If the gating sensitive residues in the two neighboring segments IS6 and IS6E do not interact then the change in free energy of the two single mutations (ΔGmut IS6 see scheme, also Horovitz 1996). Double mutant cycle analysis revealed that the studied IS6 and IS6E mutations are energetically independent and thus have independent impacts on activation gating.

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954-Pos Board B833

**Role of S4 segments in Ca\(_{\alpha}\)1 and Ca\(_{\alpha}\)3 channels: gating and current density**

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Gating of voltage-dependent calcium channels (Ca\(_{\alpha}\)) is determined by S4 segments in each of the four \(\alpha\)-subunit domain’s. In the S4 segments, also known as voltage sensors, every third position there is a positive charged residue (lysine or arginine). However, both high-voltage (HAV) and low-voltage (LVA or T-type) activated Ca\(_{\alpha}\) channels, show S4 segments with very similar sequences. This methodology has already been investigated in some members of T-type Ca\(_{\alpha}\)3 family, namely Ca\(_{\alpha}\)3.1 (\(\alpha\)1G), and one report on Ca\(_{\alpha}\)3.2 (\(\alpha\)1H), but there is no data on the Ca\(_{\alpha}\)3.3 (\(\alpha\)1I). To investigate the role of S4 segments in the differences in gating between Ca\(_{\alpha}\)3.3 and Ca\(_{\alpha}\)1.2 we made a chimeric approach swapping the segment of II in Ca\(_{\alpha}\)3.3 with the corresponding S4 segment of Ca\(_{\alpha}\)1.2. We have used HEK-293 cells and the whole-cell patch clamp technique to characterize the functional expression of the constructs. Our preliminary results indicate that the substitution of the IS4 segment of Ca\(_{\alpha}\)3.3 for that of Ca\(_{\alpha}\)1.2 induce a 25 mV positive shift in the I-V peak with respect to the Ca\(_{\alpha}\)3.3 wild type (WT). Also, the Boltzmann parameters were significantly different between the WT and the chimeric channel I-V curves. There was no appreciable change in the kinetics of the currents. An unexpected result was a drastic decrease (<95%) in the current density of the chimeric channel. A possible explanation is that the IS4 (the whole or some residues of it) of Ca\(_{\alpha}\)1.2 is interacting with the rest of the channel protein in such way that makes more stable the closed state of the channel. Additional experiments are under way to further study this observation.

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955-Pos Board B834

**Depolarization-induced Potentiation Of Cav1.1 Does Not Require The Distal C-terminus**

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In adult skeletal muscle, the majority of the L-type Ca\(^{2+}\) channel Cav1.1 subunit is truncated post-translationally at residue 1664 (PNAS, 102:5274-79), raising the question of the functional role of the distal residues (1665-1873). It has been suggested (J Neurosci, 17:1243-55; J Biol Chem, 277:4079-87) that (i) the distal C-terminus is non-covalently associated with the remainder of the channel, (ii) reduces channel open probability, and (iii) loses this inhibitory effect as a result of phosphorylation during strong depolarization. In regard to point (ii), previous analysis of L-type ionic conductance (G) and
membrane gating charge movements (Q) demonstrated that channel open probability (i.e., G/Q ratio) was indistinguishable for full-length or truncated (at 1669) CaV1.2 in tsA-201 cells together with expression of full-length or truncated CaV1.1. 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 RGB.

956-Pos Board B835
Chimeras 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 CaV1.2 channels (subunits α1, β2, and δ3) expressed in tsA201 cells were signiﬁcantly altered varying cytosolic Mg2+ 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 Caα3.1 (α1 subunit only) is expressed, varying cytosolic Mg2+ across a similar concentration range has little or no effect on channel gating. To understand the molecular basis for the effects of cytosolic Mg2+ on these related Ca2+ channels, a chimera channel consisting of Caα3.1 (α1 residues 1-1826) with the C-terminal region of Caα1.2 (β2 residues 1515-2171) was constructed and expressed in tsA201 cells. Ca2+ currents were measured in cells whole-cell patch-clamped with electrodes containing salt solutions in which Mg2+ and Ca2+ concentrations were strongly buffered. The chimera Caα3.1 channel had a similar membrane potential dependence for activation and steady-state inactivation as Caα3.1; however, the rate of current inactivation was slowed at least two-fold. Varying patch electrode Mg2+ concentration had little effect on the rate of current inactivation, similar to Caα3.1, but unlike Caα1.2. On the other hand, current amplitude was depressed in the chimera channel with increasing Mg2+. These results show that the C-terminal tail of Caα1.2 affects kinetics of channel gating. At least in part, changes in channel availability with cytosolic Mg2+ can be attributed to the C-terminal tail of Caα1.2; however, this domain cannot alone be responsible for Mg2+ dependence regulation of channel gating kinetics.

957-Pos Board B836
Cα1.4 C-tail Segment (ICDI) Inhibits Cα1 Channel Inactivation by Competing with Calmodulin—Resolution by Holochannels and Calmodulin FRET Sensors
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An intriguing variation on calmodulin/Cav channel inactivation (CDI) is the action of a C-tail segment from Caα1.4 channels (ICDI) to eliminate CDI. Introducing ICDI into Caα1.2 or Caα3.1 channels nearly abolishes strong baseline CDI, and a like effect is observed when ICDI is present within Caα1.4 itself. In retina, the effect in Caα1.4 helps sustain Ca2+ influx despite maintained depolarization. Contrasting with clear-cut function, the underlying ICDI mechanism remains controversial. One group proposes that CDI 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 as says. Here, we perform functional interaction assays using holochannels within live cells. As a baseline, we electrophysiologically characterized Caα1.3 channels fused to an ICDI-containing segment (α1DABL). These α1DABL Channels exhibited little native CDI compared to wild-type Caα1.3. 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 α1DABL was totally eliminated. More telling, when calmodulin was over-expressed with α1DABL, we observed a resurgence of CDI to wild-type Caα1.3 levels. To test for precise agreement with a competitive mechanism, we co-expressed α1DABL channels with BSA-MIQ, 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 Bl-subunits
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Auxiliary β-subunits of L-type Ca2+ channel (L-VDCC) profoundly modulate properties of L-VDCC. Previously, we demonstrated that the N-terminus of B2-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 B1a-subunit. Three artificial B1a-subunit mutants with different N-terminus lengths, B1aN18, B1aN27 and B1aN51, were created. Their modulatory functions were investigated in recombinant L-VDCC and compared with the natural full-length isoform, termed B1aN60. In whole-cell patch-clamp measurements, we confirmed functional expression of all B1a-subunit isoforms by a marked increase of current density and a leftward shift of activation, as compared to control transfected without any B-subunit. No obvious differences were found among B1a-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 B1a-subunit isoforms, except for small deviations with B1aN51. Strikingly, the extent of the inactivation of ensemble average currents followed the length of the N-terminus (B1aN60>B1aN51>B1aN27>B1aN18). For more detailed kinetic analysis, we performed Markov modeling using the scheme: 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: alpha, beta; C-I and O-I: gamma, delta. Channel open probability, availability, and single-channel open-time and close-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, beta, beta), did not vary with the N-terminal length of the B1a-subunit. Our results demonstrate that inactivation is under length-dependent control of the N-terminus of L-VDCC B1-subunit. This could represent a general mechanism of B-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 accesso-