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 Ca_V channel currents, while found in spermatogenic cells, are not detectable in epididymal sperm and are not essential for the ZP-induced $[Ca^{2+}]_i$ changes. Instead, CATSPER channels localized in the distal portion of sperm (the principal piece) are required for the ZP-induced $[Ca^{2+}]_i$ changes. Furthermore, the ZP-induced $[Ca^{2+}]_i$ 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 ion 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, ''CDI'', is independent from that of Ba^{2+} currents, ''VDI'' (e.g., Barret and Tsien, PNAS, 2008). Here we show that our model describes well effects of molecular interventions that appear to differentially alter ''CDI'' and/or ''VDI''. Therefore, ion- and voltage-dependent components of inactivation may converge to act at the channel's pore.

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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) present is unknown. 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 0.5-2.5ms and either weakly voltage dependent or voltage independent (summarized in Perez-Reyes 2003). Macroscopic current measurements, on the other hand, are consistent with a voltage dependent O->C transition, suggesting that mean open time is voltage dependent. Bandwidth differences and other issues associated with signal to noise ratio for these conductance channels have no doubt contributed to measured durations. The development of gating models for these channels has been hampered by the paucity of high quality single channel data. Furthermore, previously published gating models of $Ca_V3.1$ correctly approximate the voltage dependence and time course of the macroscopic currents of $Ca_V3.1$ but fail to appropriately recapitulate the gating currents (Serrano et al 1999, Burgess et al 2002). 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-NS058334 (K.B.) and RO1-HL065680 (D.H.)

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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 amphipathic 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 $Ca_V1.2$

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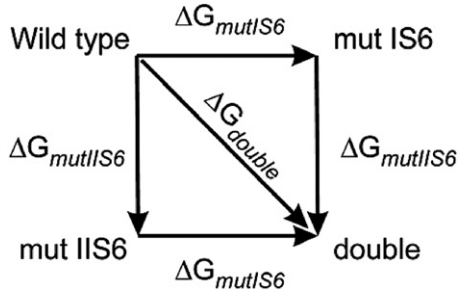
Voltage dependence and kinetics of activation of $Ca_V1.2$ channels are affected by structural changes in pore lining S6 segments of the α_1-subunit. Significant effects on channel activation are induced by either proline or threonine substitutions in the lower third of segment IIS6 ('bundle crossing region'). Here we report that S435P in IS6 results in a large shift of the activation (−26mV) 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 IIS6 induced additive shifts of the activation curves, e.g.: L429T/I781T (-44.0 ± 1.0), L434T/I781T (-50.3 ± 0.8), 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 IIS6 do not interact then the change in free energy (ΔG_{double}) of the double mutant is equal to the sum of the changes in free energy of the two single mutations ($\Delta G_{mut\ IS6}$ and $\Delta G_{mut\ IIS6}$ see scheme, see also Horovitz 1996). Double mutant cycle analysis revealed that the studied IS6 and IIS6 mutations are energetically independent and thus have independent impacts on activation gating.



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Role of S4 segments in Ca_v1 and Ca_v3 channels: gating and current density

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Gating of voltage-dependent calcium channels (Ca_v) is determined by S4 segments in each of the four α1-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 (HVA) and low-voltage (LVA or T-type) activated Ca_v channels, show S4 segments with very similar sequences. This issue had already been investigated in some members of T-type Ca_v3 family, mainly in $Ca_v3.1$ (α1G), and one report on $Ca_v3.2$ (α1H), but there is no data on the $Ca_v3.3$ (α1I). To investigate the role of S4 segments in the differences in gating between $Ca_v3.3$ and $Ca_v1.2$ we made a chimerical approach swapping the S4 segment of domain II of $Ca_v3.3$ with the corresponding S4 segment of $Ca_v1.2$. 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 IIS4 segment of $Ca_v3.3$ for that of $Ca_v1.2$ induce a 25 mV positive shift in the *I-V* peak with respect to the $Ca_v3.3$ 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 IIS4 (the whole or some residues of it) of $Ca_v1.2$ 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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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