2193-Plat Effects of Tryptophan Substitutions for Highly-Conserved Phenylalanines in the Skeletal Muscle L-Type Calcium Channel Roger A. Bannister1, Ong Moua1, Christopher A. Ahern2, Kurt G. Beam1, 1Univ. Collo. Sch. of Medicine, Aurora, CO, USA, 2Univ. of British Columbia, Vancouver, BC, Canada.

The motif FxxExxxKR is highly conserved in S2 segments of voltage-gated cation channels, including the four repeats of the z1b subunit of the skeletal muscle L-type CaV3.3 channel, and appears to represent an important structure for voltage-sensing. Mutating the conserved S2 phenylalanine (F290) within the Shaker K+ channel monomer to tryptophan causes a ~40 mV hyperpolarizing shift in activation (Tao et al., 2010; Lacroix & Bezanilla, 2011) due to an introduced cation-π interaction with S4 charges (Pless et al., 2011). We made homologous F to W substitutions in repeats I (F97W), II (F97W), III (F843W) and IV (F1161W) of YFP-α1S, and tested the mutants’ ability to conduct L-type voltage-sensing and to serve as the voltage sensor for excitation-contraction (EC) coupling after expression in dysgenic myotubes. Except for R1161W, each of these constructs encoded channels that were targeted to SR-membrane junctions and supported EC coupling. In contrast to the large hyperpolarizing shift in activation reported for Shaker F290W, no activation shift was apparent for F97W relative to YFP-α1S (p<0.05) and substantial depolarizing shifts were evident for F475W and F843W (ΔV1/2 = 39.0±2.9 mV and 44.1±2.5 mV, respectively; vs. 32.4±0.8 mV for YFP-α1S; p<0.001, ANOVA). These depolarizing shifts in activation may have been a consequence of impaired gating charge transfer because the Q-F relationships for F475W and F843W were bi-sigmoidal, containing a component of charge that moved at more depolarized potentials than in YFP-α1S. Our results indicate that, despite having sequence homology, the structure and function of the voltage-sensing modules in four-repeat CaVs and NaVs (Pless et al., this meeting) channels diverge significantly from those of homotetrameric Kv and bacterial Na+ channels. Supported by NIH AR055104 (K.G.B) and AG038778 (R.A.B.), and MDA4319 (K.G.B.)

2194-Plat The Distal Carboxy Tail (DCT) of CaV1.4 Modulates More than Ca2++/CaM-Dependent Inactivation (CDI) Paul J. Adams, Ivy E. Dick, Manu B. Johny, Hojat Bazzazti, Phil S. Yang, David T. Yue. The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

CaV1.4 L-type Ca2+ channels populate retinal ribbon synapses, and mediate tonic neurotransmitter release. Mutations in the CACNA1F gene encoding CaV1.4 are implicated in congenital stationary night blindness (CSNB2). The K1591X CSNB2 mutation yields a premature stop that deletes the DCT. Interestingly, the DCT contains a module that competes with Ca2+-free calmodulin (apoCaM) for binding at the IQ-domain of CaV1.4, thereby tuning channel affinity for apoCaM (Nature 463:968). Since only channels ‘charged’ with apoCaM exhibit Ca2+-/CaM-mediated inactivation (CDI), wildtype CaV1.4 channels exhibit little or no CDI, while deleting the DCT yields a resurgence of CDI. The latter effect in K1591X channels has suggested that pathogenesis involves abnormally diminished Ca2+ influx with resulting impairment of photoreceptor signaling. Here, however, we observe a dramatic and unrecognized effect of DCT deletion. Single-channel recordings indicate that wild-type CaV1.4 feature diminutive open probability P0. (A. Top, exemplar single-channel trace during voltage ramp. Bottom, P0-F relation averaged over multiple patches). By contrast, K1591X channels exhibit strikingly enhanced P0. (B). Hence, this form of CSNB2 likely involves an unexpected Ca2+-overload phenomenon, raising the possibility of therapeutics involving CaV1.4 channel blockade.

2195-Plat Analysis of Competition between CaBP1 and Calmodulin on the Voltage-Gated Calcium Channel CaV1.2 Felix Findeisen, Daniel L. Minor. UCSF, San Francisco, CA, USA.

Voltage-gated calcium channel (CaV) activity is regulated by calcium sensors including calmodulin (CaM) and calcium-binding protein 1 (CaBP1). CaBP1 inhibits CaM-mediated calcium-dependent inactivation (CDI). We investigated the origins of functionally important differences between CaM and CaBP1 by creating a number of CaM/CaBP1 chimeras. The exchange of C-terminal lobes between CaM and CaBP1 is accompanied by few functional changes, suggesting CaBP1 and CaM C-lobe can substitute for each other. In contrast, we identified the linker and the N-terminal lobe of CaBP1 as elements that set it functionally apart from CaM. CaBP1 and CaM are thought to modulate CaV function by competing for binding to the CaV C-terminal IQ-domain, but this assumption has never been tested directly. By determining CaV1.2 CDI under conditions with different ratios of CaM and CaBP1, we demonstrated direct competition between CaBP1 and CaM for its CaV1.2 binding site. In order to extend our analysis of CaBP1/CaM competition we used an isothermal titration calorimetry to determine the affinity of both CaM and CaBP1 in both calcium-bound and apo-states for the IQ-domain, suggesting that competition occurs mainly in the apo-state. Analysis of an IQ domain mutant shows unexpected differences between CaM and CaBP1 in IQ domain binding, suggesting a possible mechanism for their different functional effects on CaV1.2 inactivation. Overall, the data reveal those parts of CaBP1 that set it functionally apart from CaM and provide a framework for understanding how CaBP1 and CaM differentially regulate CDI on CaV1.2.

2196-Plat Nanometer Resolution of Ca+-Generated Ca2++ Gradients Michael R. Tadross1, Richard W. Tsien2, David T. Yue2. 1Stanford University, Stanford, CA, USA, 2Johns Hopkins University, Baltimore, MD, USA.

Diffusion theory predicts that each pA of Ca++ flux through a voltage-gated Ca2+ channel (CaV) should generate ~100 μM free [Ca2+] in the channel nanodomain, with steep spatial gradient and little sensitivity to intracellular Ca2+ buffers. This is widely quoted, and bears on synaptic transmission, activity-dependent plasticity, and excitation-contraction coupling. Yet, experimental verification has been difficult because diffusible Ca2+ indicators report space-averaged [Ca2+], and Ca2+-tethered fluorescent indicators cannot distinguish between active and silent channels. Here, we used Ca2+-/calmodulin-dependent inactivation (CDI) of CaV1.3 channels themselves as a nearfield indicator of nanodomain [Ca2+], noting that CaBP1 provides an ionic readout based only on active channels. We calibrated the [Ca2+] sensitivity of CDI by monitoring the decay of Li+ currents following spatially-uniform Ca2+ uncaging steps. We then ran Ca2+ through the same channels to generate local [Ca2+] gradients, and titrated the external Ca2+ concentration until CDI diminished ~50%. The corresponding unitary flux was resolved through a combination of single-channel and whole-cell recordings, enabling determination of the relationship between unitary Ca2+ flux and nanodomain [Ca2+]. Contrary to theory, nanodomain [Ca2+] signals are larger and more buffer-sensitive than expected: ~800 μM/pA in 10 mM intracellular BAPTA, and ~200 μM/pA in 60 mM BAPTA. These findings indicate that diffusion of free Ca2+ ions is ~10-fold slower in the nanodomain versus other regions of the cell, and that calmodulin (the Ca2+-sensing subunit for CDI) is ~7 nm from the channel pore. Our results suggest that the nanodomain is a tortuous environment, where a Ca2+ ion would collide with an obstacle in 9 out of every 10 of its Brownian movements. Under physiological buffering, we predict that Ca2+, by virtue of their crowded nanodomains, convert small Ca2+ fluxes into enormous Ca2+ concentrations, ~1 mM/pA over distances of 10-20 nm.

2197-Plat Inhibitory Cav2.2 Peptides Effects in Superior Cervical Ganglion Neurones Giovanna Bucci1, Christian Vogl1, Cesare Usai1, Sumiko Mochida3, Gary J. Stephens1. 1The University of Reading, Reading, United Kingdom, 2National Research Council, Genova, Italy, 3Tokyo Medical University, Tokyo, Japan. We have been developing inhibitory CaV2.2 peptides based on reported interaction sites between voltage-dependent Ca2+ channels (VDCCs) and G protein βγ subunits. We have shown that an alpha interaction domain CaV2.2[377-393] ‘AID peptide’ and a CaV2.2[45-55] amino terminal ‘NT peptide’ act to reduce synaptic transmission via a blockade of VDCCs in superior cervical ganglion neurones (SCGNs) (Bucci et al., 2011, J Physiol 589:3085-101). Here, we compare the effects of the AID peptide and an ‘AID W-A’ peptide containing a (W391A) mutation to a tryptophan residue previously implicated in VDCC function (both peptides used at 1 μM).

In SCGN synapses, EPSPS amplitude was significantly reduced by presynaptic injection of AID (20.9±3.9 %, n=6, P<0.05) and AID W-A (34±3.9 %, n=6, P<0.05) peptides; with increased inhibitory effects for the AID W-A peptide (P<0.05 vs AID). In isolated SCGNs, Ca2+ current-density at 0 mV (control: 40±6.5 pA/pF, n=9) was significantly reduced by AID (25±4.6 pA/pF, n=9, P<0.05) and AID W-A (14±2.7 pA/pF, n=9, P<0.001) peptides; representing increased AID W-A peptide inhibitory effects (P<0.05 vs AID). Consistent
with these effects, intracellular Ca\textsuperscript{2+} accumulation in response to depolarizing voltage steps to 0 mV (control: 60.8 ± 3.0 nM, n=7) was significantly reduced by AID (6.4 ± 1.6 nM, n=7, P<0.001) and AID W-A (18.6 ± 3.4 nM, n=7, P<0.001) peptides; again representing increased inhibitory AID W-A peptide effects (P<0.05 vs AID). Under these conditions, both AID and AID W-A peptides also attenuated G-protein modulation of Ca\textsuperscript{2+} current (as induced by 1 μM somatostatin).

These data are consistent with inhibitory CaV2.2 peptides acting to reduce synaptaptic activity due to a direct effect on presynaptic VDCCs. The increased inhibitory effect of the AID W-A peptide may represent a starting point to develop inhibitory agents in aberrant CaV2.2 signalling pathways, such as in nociception.

2198-Plat
Ca\textsuperscript{2+} Signaling Amplification by Oligomerization of L-Type Cav1.2 Channels
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Ca\textsuperscript{2+} influx via L-type Cav1.2 channels is essential for multiple physiological processes including gene expression, excitability, and contraction. Amplification of the Ca\textsuperscript{2+} signals produced by the opening of these channels is a hallmark of many intracellular signaling cascades, including excitation-contraction (EC) coupling in heart. Using optogenetic approaches, we discovered that Cav1.2 channels form clusters of varied sizes in ventricular myocytes and that physical interaction between these channels via their C-tails renders them capable of coordinating their gating thereby amplifying Ca\textsuperscript{2+} influx. Light-sensitized fusion of wild-type (WT) Cav1.2 channels with channels carrying a gain-of-function mutation that causes arrhythmias and autism in humans with Timothy syndrome (Cav1.2-TS) increased Ca\textsuperscript{2+} currents, diastolic and systolic Ca\textsuperscript{2+} levels, contractility, and the frequency of arrhythmogenic Ca\textsuperscript{2+} fluctuations in ventricular myocytes. Our data indicate that these changes Ca\textsuperscript{2+} signaling resulted from Cav1.2-TS increasing the activity of adjoining WT CaV1.2 channels via protein-to-protein interactions. Our data support the novel concept that oligomerization of Cav1.2 channels can control the amplification of Ca\textsuperscript{2+} influx in excitable cells.

Platform: Interfacial Protein-Lipid Interactions II

2199-Plat
Synaptotagmin C2 Domain Membrane Targeting: Kinetic and Mechanistic Diversity Among Isolforms From Different Cell Types
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Synaptotagmin (Syt) triggers Ca\textsuperscript{2+}-dependent membrane fusion during secretion via its tandem C2 domains, termed C2A and C2B. The seventeen known human isoforms are active in different secretory cell types, including neurons (SytI and others) and pancreatic β cells (SytVI and others). Here, quantitative fluorescence measurements reveal notable differences in the membrane docking affinities, kinetics, and molecular driving forces for C2A and C2B domains from SytI and SytVI, using vesicles comprised of physiological target lipid mixtures. In agreement with previous studies, the C2\textsuperscript{2+} sensitivity of membrane binding is greater for both domains from SytI than for their counterparts in SytVI, but for C2A, this increased sensitivity is due to a stronger SytVIIC2A membrane interaction, which involves substantial contribution from the hydrophobic effect. Association and dissociation rate constants for both SytVI domains are found to be significantly slower than their counterparts in SytI. For SytVIIC2A, the dissociation rate constant is ~50-fold slower than SytIIC2A and is reminiscent of the cPLA2C2 domain of their counterparts in SytI. We demonstrate that for C2A, this increased sensitivity is due to the membrane binding is greater for both domains from SytI than for their counterparts in SytVI. We demonstrate that for C2A, increased sensitivity is due to the increase electrostatic. The inclusion of phosphatidylinositol-4,5-bisphosphate (PIP\textsubscript{2}) in membrane mixtures leads to increased affinity and slower dissociation for both C2A and C2B domains, but has minimal effects on C2A domains. Overall, highly homologous domains from these two proteins exhibit distinct mechanisms of membrane binding that may reflect their functions in different cell types.