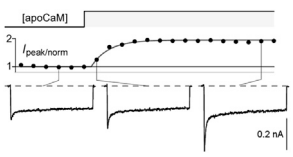
Ca^{2+}-free CaM (apoCaM) first bind to channels (preassociate), even before Ca^{2+} elevation. Subsequent Ca^{2+} binding to this 'resident' CaM then induces CDI; channels lacking preassociated apoCaM cannot undergo CDI. Here, using a novel generator of step increases in apoCaM, we find that apoCaM binding has another powerful effect. As shown below, apoCaM elevation not only heightens CDI as expected, but strongly boosts peak Ca^{2+} current (confirmed to increase P_O in single-channel assays). This latter effect could result from the binding of a second CaM to channels, atop the CaM required for CDI. However, plotting normalized peak current as a function of CDI strength invariably resolves a *single* relationship, over multiple experiments and conditions. This invariance furnishes compelling evidence that the binding of one and the same apoCaM imparts increases in both CDI and opening. This newly recognized apoCaM effect to increase P_O opens new dimensions through which Ca^{2+} homeostasis can be tuned.



1682-Pos Board B412

Rational Design of Peptide Modulators Bi-Directionally Tuning $Ca_V1.3$ Channels

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Ca^{2+} signaling via L-type voltage-gated Ca^{2+} channels (LTCCs or Ca_V1 channels) has been suggested to play prominent roles in diverse physiological processes. In this study, we designed and tested a series of peptides targeting $Ca_V1.3$ channels, inspired by the competitive mechanism between calmodulin (CaM) and distal carboxyl tail (DCT) of LTCCs (Liu, X., *et al.*, Nature, 2010). These peptide modulators when co-expressed with $Ca_V1.3$ containing full-length variant of DCT were able to bi-directionally tune the regulatory mechanisms of actual Ca^{2+} flux through the channel, e.g., inhibit/enhance calcium dependent inactivation (CDI); meanwhile, other biophysical properties of $Ca_V1.3$ channels, such as half-activation and maximum conductance were also simultaneously down- or up- regulated (Liu X., *et al.*, Biophysical J. Suppl., 2008). According to the direction of the tuning, such peptides are termed as iCaMp (inhibitors of CaM pre-association) or eCaMp (enhancers of CaM pre-association). Single-residue mutations on iCaMp/eCaMp substantially attenuated tuning capabilities as well as binding affinities to the channel, indicated by patch-clamp, FRET and Co-IP assays. The key motifs of the peptides were further identified with structural modeling and mutagenesis, and then optimized peptides were synthesized and acutely applied, producing modulation profiles similar to those of co-expressed peptides.

In summary, by way of rational design, we successfully developed a series of peptide modulators, specifically targeting and bi-directionally tuning $Ca_V1.3$ channels, which not only provides an innovative set of genetically-encoded modulators facilitating $Ca_V1.3$ biophysics, but also paves the way to dissect the pathophysiological roles of $Ca_V1.3$ channels, such as in neurogenesis and neurodegeneration.

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Super-Resolution Imaging of $Ca_V1.2$ Channel Clusters

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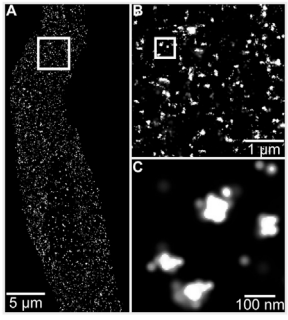
L-type $Ca_V1.2$ channels regulate multiple processes in neurons, muscle and endocrine cells, including action potential duration, excitation-contraction coupling, gene expression, neurotransmitter and insulin release. The amplitude and functional impact of local Ca^{2+} signals ("$Ca_V1.2$ sparklets") depends on the spatial distribution of $Ca_V1.2$ channels in the surface membrane. Here, we used TIRF microscopy in combination with STORM and GSD super-resolution imaging to determine the organization of $Ca_V1.2$ channels in the surface membrane of arterial myocytes and tsA-201 cells. The spatial resolution of our super-resolution maps was ~30 nm. The localization of $Ca_V1.2$ was determined based on immunofluorescence or EGFP fluorescence. As shown in the super-resolution map in Fig. 1, we found that in arterial myocytes, $Ca_V1.2$ channels were expressed in clusters broadly throughout the cell membrane. However, the size and geometry of these clusters varied, suggesting that number of $Ca_V1.2$ channels within clusters was dissimilar. The average size of the clusters was ~3 μm^2. Up to 12 channels could occupy clusters of this size assuming a monomer occupies ~240 nm^2. Similar findings were obtained from tsA-201 cells expressing $Ca_V1.2$-EGFP or immuno-labeled $Ca_V1.2$ channels. Data will be presented on the functional implications of these findings.



1684-Pos Board B414

Is There a Contribution of Both Cav1.4 and Cav1.3 L-Type Calcium Channels to Retinal Synaptic Transmission?

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Cav1.3 and Cav1.4 L-type calcium channels were previously both shown to be expressed in the retina. Whereas Cav1.4 channels are predominantly expressed in the outer plexiform layer (OPL) at photoreceptor ribbon synapses, reports on the distribution pattern of Cav1.3 channels in the retina are controversial. One study reported the uniform expression across all retinal cell layers, and others showed accumulation in photoreceptor inner segments or the OPL or the inner nuclear layer or the ganglion cell (GC) layer of the retina. Mutations in the pore-forming α1-subunit, found in patients diagnosed with Congenital Stationary Night Blindness type 2 (CSNB2), result in impaired signaling between photoreceptor cells and second-order neurons. Exemplary, we report the functional consequences of the novel $Ca_V1.4$ mutation GV found in an Austrian family. Biophysical analysis of GV channels in whole-cell patch-clamp experiments revealed a reduced current density ([pA/pF]: wt: 12.8 ± 1.4, n=18; GV: 3.7 ± 1.0, n=7; $p<0.001$, 15 mM Ca^{2+} used as charge carrier) due to decreased surface expression of functional channels, expected to lead to impaired retinal signaling. In contrast, the contribution of Cav1.3 channels to synaptic transmission is rather ambiguous. We therefore performed multi electrode (MEA) analyses of light-dark evoked GC activity in Cav1.3-/- mice retinas. Retinas were excised from adult wildtype (wt) and $Cav1.3^{-/-}$ mice (red-light, carbogen-equilibrated Ames) and mounted ganglion-cell-side-down (via nitrocellulose-menbrane) on a MEA array. Preliminary data showed a prolongation of GC response latencies in $Cav1.3^{-/-}$ compared to wt supporting the idea that Cav1.3 channels also contribute to synaptic transmission. However qRT-PCR analysis of Cav1.4-/- retinas showed a significant upregulation of Cav1.3-mRNA, thus an alternative role for signal transduction might also be suggested. Support: Austrian Science Funds (FWF), P22526:AK; SFB F44 (F44020): JS, AK, (F44060): GJO, Medical University Vienna.

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Knockout of the $\alpha_2\delta$-1 Calcium Channel Subunit Alters Calcium Homeostasis and Electrical Activity in Pancreatic Islet Cells

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Mouse pancreatic islets contain approximately 80% β-cells, 15% α-cells and 5% δ- and F-cells. High voltage-gated calcium channels (HVCC) regulate the biosynthesis and release of pancreatic hormones. Based on their pharmacological properties L-type calcium channels (LTCC) (Cav1.2 or Cav1.3) represent the predominant current component in islet cells. Insulin release depends primarily on LTCC and Cav2.3, whereas glucagon release relies on Cav2.1 or Cav2.2. All these α1 subunits form functional complexes with auxiliary channel subunits likely including $\alpha_2\delta$-1. Here we investigate the role of the $\alpha_2\delta$-1 subunit in calcium homeostasis and electrical activity of pancreatic α- and β-cells using $\alpha_2\delta$-1 knockout mice. Voltage clamp experiments of freshly dissociated α- and β-cells show dramatically (2.5-fold) decreased calcium currents in $\alpha_2\delta$-1-/- compared to wild-type cells. Previously we demonstrated that Cav1.2 lacking $\alpha_2\delta$-1 subunits displays left-shifted voltage-dependence of activation and slowed calcium current kinetics. α- and β-cells of $\alpha_2\delta$-1-/- mice showed little to no changes in the voltage-dependence of activation or kinetic properties of calcium influx compared to wild-type cells. Interestingly, the remaining current is almost completely blocked by the LTCC antagonist isradipine (2μM). Fluorescent calcium transients in response to high glucose (16.7 mM) showed a decreased amplitude and increased frequency of oscillations in isolated islets of $\alpha_2\delta$-1-/- mice compared to wild-type. Analysis of low glucose-induced calcium oscillations in α-cells, and of the electrical activity of both cell types in intact islets are currently under way. Together, our results indicate that $\alpha_2\delta$-1 is critical for membrane incorporation of calcium channels but does not affect LTCC gating properties in pancreatic endocrine cells. The reduced calcium current