Tandem pore domain (K2P) potassium channels play a crucial role in modulating cellular resting membrane potential, thereby controlling cellular excitability. TREK1, a member of the mechanosensitive K2P subfamily, is modulated by a wide array of stimuli that include heat, stretch, pH, and volatile anesthetics, but TREK1 lacks specific and high affinity pharmacology. In this study, we leveraged the high affinity binding of the immunomodulatory drug Rapamycin to the FKBP Rapamycin Binding (FRB) domain of mTOR, bioengineer a TREK1/FRB fusion protein sensitive to rapamycin. We designed multiple unique fusion constructs by inserting the FRB protein into the TM2/TM3 loop of TREK1 at a variety of alternative fusion sites. We then determined the functional behavior of these TREK1/FRB fusion proteins via two-electrode voltage clamp (TEVC) electrophysiology, characterizing the sensitivity of the TREK1/FRB fusions to gating by temperature. Nearly all of the TREK1/FRB fusions yielded functional potassium channels, though altering the FRB fusion site led to varied basal channel activities and temperature sensitivities. Application of rapamycin to this panel of TREK1/FRB channels resulted in up to15-fold potentiation of TREK1 activity in the most sensitive fusion construct, with dose response studies demonstrating EC50 values as low as 63 nanomolar. By introducing a well characterized T2098L mutation into the FRB portion of the TREK1/FRB fusion protein, we were also able to activate the TREK1/FRB channel using an analog of rapamycin (AP21967) that lacks the immunomodulary activity of rapamycin. This work yields a highly specific genetically encodable tool that can specifically activate the TREK1 channel for future in-vivo physiological studies.

1465-Pos

Three new toxins from the south American spider *Pamphobeteus verdolaga* inhibit calcium and potassium channel currents of murine cardiomyocytes

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The spider species *Pamphobeteus verdolaga* was recently discovered in the Aburra's Valley, Colombia. A bioinformatics analysis of the venom gland's transcriptome identified numerous peptides with potential effects on ion channels. Of those, three were then synthetized through Fmoc solid-phase methods (referred to as *vrdg* peptides). Since *vrdg172* and *vrdg183* possess two disulfide bridges, we hypothesize that they block ion channels, compared with *vrdg66,* which is a linear peptide. In this study, we evaluated the effects of *vrdg66, vrdg172* and *vrdg183* on Ca^{2+} (I_{CaL}) and K^+ (I_K) currents in mouse cardiomyocytes using the whole-cell configuration of the patch-clamp technique. When used at a concentration of 1 μM, *vrdg66* showed a minor inhibitory effect on the peak amplitude of I_{CaL} ($-16.3 \pm 10.5\%$; n=6) and I_K ($-24.4 \pm 4.4\%$; n=5), compared with the control current. At the same concentration, *vrdg172* similarly blocked I_{CaL} ($-38.8 \pm 15.6\%$; n=4) and I_K ($-43.9 \pm 5.8\%$; n=3). Interestingly, *vrdg183* demonstrated a weak inhibitory effect on the peak I_K ($-6.2 \pm 24.6\%$; n=3) and a sizeable inhibitory effect on I_{CaL} ($-66.7 \pm 6.5\%$; n=4). In a further characterization of *vrdg183*, the blocking effect on I_{CaL} was confirmed in isoproterenol-stimulated cardiomyocytes. Finally, a concentration-effect curve (10, 100, 300, 1000 and 10000 nM; n=4 for each experimental point) allowed us to calculate an IC_{50} for *vrdg183* of 858.28 nM. The activation and inactivation kinetics were not affected by any of the concentrations tested. In summary, we report novel spider toxins that inhibit mammalian voltage-gated ion channels, likely acting as pore blockers. The *vrdg183* toxin exhibits a selective inhibitory effect on I_{CaL} over I_K, with moderate affinity (Grant 111577757673, Minciencias, Colombia).

Posters: Other Channels

1466-Pos

Purification of a novel MgtE homolog and its gating-related structural dynamics in membrane-mimetics

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Magnesium (Mg^{2+}) is the most abundant divalent cation in the cell and is critical for numerous cellular processes. Despite its importance, the mechanisms of intracellular Mg^{2+} transport and its regulation are poorly understood. MgtE is the main Mg^{2+} transport system in almost half of bacterial species, and is an ortholog of mammalian SLC41A1 transporters, which are implicated in neurodegenerative diseases and cancer. Till date, only MgtE from *Thermus thermophilus* ($MgtE_{TT}$) has been extensively characterized mostly in detergent micelles, and gating-related structural dynamics in biologically-relevant membranes are scarce. The MgtE homolog from *Bacillus firmus* ($MgtE_{BF}$) is unique since it lacks the entire Mg^{2+}-sensing N-domain, but has conserved structural motifs in TM-domain for Mg^{2+} transport. In this work, we have successfully purified this novel homolog in a stable and functional form, and ColabFold structure prediction analysis suggests a homodimer. Further, microscale thermophoresis (MST) experiments show that $MgtE_{BF}$binds Mg^{2+} and ATP, similar to $MgtE_{TT}$. Importantly, we show that, despite lacking the N-domain, $MgtE_{BF}$ mediates Mg^{2+} transport function in the presence of an inwardly directed Mg^{2+} gradient in reconstituted proteoliposomes. Furthermore, comparison of the organization and dynamics of Trp residues in TM-domain of $MgtE_{BF}$ in membrane-mimetics, in apo- and Mg^{2+}-bound forms, suggests that the cytoplasmic binding of Mg^{2+} might involve modest gating-related conformational changes at the TM-domain. Overall, our results show that the gating-related structural dynamics (hydration dynamics, conformational heterogeneity) of the full-length $MgtE_{BF}$ is significantly changed in functionally-pertinent membrane environment, emphasizing the importance of lipid-protein interactions in MgtE gating mechanisms.

1467-Pos

Comparing proton transfer pathways in PSII from cyanobacteria and higher plants using Monte Carlo sampling to trace hydrogen bond networks

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Aerobic photosynthesis uses water as the terminal electron donor in its electron transfer chain, known as the Z scheme. The Oxygen Evolving Complex, a Mn_4O_5Ca catalyst in photosystem II (PSII) reduces water to O_2. Water channels bring substrate water into the OEC and remove product protons to the lumen. In thermophilic cyanobacteria, three channels have been well characterized. The program MCCE uses Monte Carlo (MC) sampling to determine the Boltzmann distribution of protonation states, side chain positions, and water molecule orientation and occupancy within the protein. The hydrogen bonds made in each accepted MC microstate are determined. These connections are used to trace proton transfer networks in *Pisum sativa* (Pea) (5xnl) PSII for comparison with earlier studies of *T. vulcanus* PSII (4UB6). Near the OEC the hydrogen-bonded pathways are remarkably conserved in Pea but the end of the three previously characterized channels diverge from those found in *T. vulcanus.* Thus, while the region containing the OEC catalyst is highly conserved PSII may provide evidence of the plasticity of proton transfer paths. Supported by DOE BES DE-SC0001423.

1468-Pos

Connexin hemichannels function as molecule transporters independently of ion conduction

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Connexin hemichannels allow the permeation of both atomic ions and small molecules between the intra- and extracellular environments. The conventional view of hemichannels is that their pores serve as large passive conduits, through which molecules can diffuse simultaneously with ions. In stark contrast to this notion, we demonstrate that the permeation of ions and molecules in connexin hemichannels is uncoupled and relies on different mechanisms. First, while a depolarizing pulse increases the relative open probability and subsequent atomic ion flux, molecule permeation is abolished and occurs only at negative potentials when the connexin-mediated ionic current is undetectable. Second, human mutations that cause diseases, previously recognized as loss-of-function mutations due to the lack of ionic currents, are still capable of transporting molecules with kinetics similar to wild-type channels. Third, permeants do not affect the relative open probability or single-channel conductance, indicating that molecules do not