Characterizing Conformational Changes of ASIC1a During Gating and Peptide Modulation

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Transient Receptor Potential (TRP) channels have evolved in mammals and other higher organisms to control various cellular functions in response to a wide variety of chemical and physical stimuli. This large and diverse family of eukaryotic channels first emerged in fungi where they are mainly responsible for osmoregulation and are considered to be mechanosensitive. The Saccharomyces cerevisiae vacuolar transient receptor potential yeast 1 (TRP1) is the most studied TRP channel from fungi, but the molecular details of its modulation remains elusive so far. Here, we describe the full-length cryo-electron microscopy (cryo-EM) structure of TRP1 at 3.1 Å resolution. The structure reveals a unique architecture for TRP1 among all eukaryotic TRP channels with an evolutionarily conserved and archetypical transmembrane domain, but distinct structural folds created by cytoplasmic N- and C-termini. We identified the inhibitory phosphatidylinositol 3-phosphate (PI(3)P) lipid binding site, which shed light into the lipid modulation of TRP1 in vacuolar membrane. We also elucidated two Ca2+ binding sites: one in the cytoplasmic side, implicated in activation and the other in the vacuolar lumen side, involved in channel inhibition. These findings together with data from molecular dynamics simulations provide structural insights into understanding the basis of TRP1 channel modulation by Ca2+ and lipids.

**Posters: Ligand-gated Channels II**

**1630-Pos**

**Molecular Determinants of Neuropeptide Potency at FMRFamide Gated Sodium Channels from the DEG/ENaC Family**

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Members of the deepsea/eukaryotic sodium channel (DEG/ENaC) superfamily show varying means of activation, from protons and peptide ligands to mechanical stimuli. Structural architecture is highly conserved throughout the family, and the differences between members could shed light on mechanisms of activation and pharmacological modulation. We sought to identify structure/activity relationships underlying neuropeptide potency at a peptide-gated DEG/ENaC channel, the FMRF-amide gated sodium channel (FaNaC) from the gastropod *Aplysia kurodai* (AKFaNaC). We used 12 FMRFa derivatives containing single residue substitutions, site-directed mutagenesis targeting the channel, and two-electrode voltage clamp (TEVC). Whereas, for example, (cylohexylalanine)MRFa (EC50 = 7.9 ± 0.6 μM), FLRFa (13.6 ± 0.9 μM), FMKFa (12.6 ± 1 μM), FMRFa (25.5 ± 3.5 μM), and FMRF-OMe (7.5 ± 0.2 μM) retained similar activity to FMRFa (3.4 ± 0.4 μM) at AKFaNaC, no currents were activated by LMRFa and FKRFa or FMQFa at concentrations up to 100 μM. Whereas, for example, (cyclohexylalanine)MRFa (EC50 = 7.9 ± 0.6 μM), FLRFa (13.6 ± 0.9 μM), FMKFa (12.6 ± 1 μM), FMRFa (25.5 ± 3.5 μM), and FMRF-Ome (7.5 ± 0.2 μM) retained similar activity to FMRFa (3.4 ± 0.4 μM) at AKFaNaC, no currents were activated by LMRFa and FKRFa or FMQFa at concentrations up to 100 μM. Thus, the cyclohexyl moiety of FL, the hydrophobicity of M2, and the positive charge of R3 appear to contribute most significantly to potency. We also used phylogenetics and TEVC to explore the diversity of FMRFa gated channels from various animals to determine the conserved molecular features that could underlie peptide-induced gating. FaNaC had previously been characterized exclusively in gastropod mollusks, but we also identified and characterized FMRFa gated channels from the bivalve mollusk *Crassostrea gigas* and the polychaete annelid *Capitella teleta*. Armed with new knowledge on contributions of peptide residues to potency and sequence similarity throughout a broader FaNaC sub-family, further experiments are on-going in dissecting the mechanism of FaNaC activation by neuropeptides.

**1631-Pos**

**Validation of an ASIC1a Ligand-Gated Assay on an Automated Patch Clamp Platform and its Use for Novel Ligand Screening**

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There is growing interest in automated patch clamp assays for ligand-gated targets, many of which are expressed throughout the nervous system and underlie complex neurological diseases. The Acid-Sensing Ion Channel (ASIC) family comprises combinations of ASIC1-4 proteins that form cation-selective channels in the central and peripheral nervous system. ASIC1a proteins are involved in synaptic plasticity, and implicated in stroke, ischemia and pain. Small molecules and toxins modulate ASIC1a channels, and fragments can bind to ASIC3 channels (Wolkenberg et al., 2010), so we screened a diverse low molecular weight library for novel ASIC1a modulators. All data was generated on the gigaseal QPatch48 system (Sophhon) using coated metal pipetting tips and a CHO cell line stably expressing human ASIC1a. Currents were elicited from a holding potential of −60 mV by rapid application of saline or compound-containing solutions of varied pH. EC50 of pH activation and IC50 of test compound inhibition were determined by cumulative applications to the same cell. A proprietary low MW library was tested at 200 μM (N=2) and active hits were confirmed by cumulative IC50 testing (N=3). We validated the pharmacology of the hASIC1a cell line using toxins (e.g. IC50 of 120 nM for Methylbiguanin-3), small molecules (Benzamil IC50 of 4.0 μM), and clinical development compounds, which paved the way to screen a proprietary library of low molecular weight compounds in an effort to find chemical building blocks for the design of novel and efficacious ASIC1a modulators. A hit rate of 3.0% was achieved which included several novel scaffolds with low μM potency, which can be used in biophysical binding assays and structure-based drug design to develop ligand-efficient modulators of the human ASIC1a channel.

**1632-Pos**

**Determining the Topology of the Acid-Sensing Ion Channel Intracellular Domains**

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Acid-sensing ion channels (ASICs) are trimeric cation-selective channels activated by decreases in extracellular pH. The intracellular N and C terminals of ASIC1 variously influence channel gating, ion selectivity, channel trafficking, and signaling in ischemic cell death. While there are numerous x-ray and cryo-EM structures of the extracellular and transmembrane segments of ASIC1, these important intracellular tails remain unresolved. Here we set out to map the coarse topology of these intracellular domains using the voltage-sensitive dipicrylamine as a dark acceptor for fluorescent proteins inserted into various positions of chicken ASIC1 tails. With this patch clamp FRET approach, we find that the N-terminus of the channel undergoes an axial motion between the resting and desensitized states of the channel, while no movement of the C-tail is apparent. Further, when labeling both tails with pH-insensitive FRET donor-acceptor pairs, we find that the tails of the channel to not appear to separate as the channel transitions between the resting and active/desensitized states. Together, these data allow us to build a topological model of the intracellular tails of ASIC1 that will be a foundation for working hypotheses in future experiments.

**1633-Pos**

**Characterizing Conformational Changes of ASIC1a During Gating and Peptide Modulation**


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Acid-sensing ion channels (ASICs) are ligand-gated cation channels that open in response to proton binding and contribute to synaptic transmission, as well as the initiation of pain and neuronal death during ischemia. Although some peptide inhibitors have shown to reduce pain and ischemic injury via ASIC1a, no drugs are currently available that specifically target these channels under pathological conditions. Advances have, in part, been hampered by the limited understanding of the complex mechanism of ASIC1a function. Here, we use several approaches to introduce fluorescent labels in different domains of ASIC1a to follow conformational changes during gating and modulation by peptides. Using Cys-conjugated fluorescent labels introduced in the palm and finger domain, along with concatameric channel constructs, we elucidate the binding mechanism and stoichiometry of Psimotoxin-1, a spider venom peptide that increases the apparent proton affinity of ASIC1a. We also show that we can incorporate a fluorophore into the intracellular C-terminal domain via a synthetic peptide using a split intein-based approach. Preliminary data using this method indicate that we can follow intracellular conformational changes with high (millisecond) time resolution using high-sensitivity patch-clamp fluorometry. The importance of this is highlighted by the fact that there is currently little information on the structure and dynamics ofASIC intracellular domains. Together, these approaches promise new insights into understanding the dynamic conformational changes under (patho-)physiologically relevant conditions and could aid in the development of new ASIC1a modulators.