



ABSTRACTS & POSTER ASSIGNMENTS

**78TH ANNUAL MEETING OF
THE SOCIETY OF GENERAL
PHYSIOLOGISTS**

**Tracing the Path of Chloride:
From Ion Channels and
Transporters to Physiology
and Therapeutics**

**3-7 September 2025, MBL, Woods
Hole, Massachusetts, USA**

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POSTER SESSION A: Even-Numbered Abstracts

Thursday, 4 September, 4:30 pm–6:00 pm, Swope Center 2nd Floor

#	Presenter	Title
10	Venkata Mandala (Rockefeller Univ.)	Molecular contacts in self-assembling clusters of membrane proteins
12	Shelby Phelps (University of Texas at Dallas)	In-cell directed evolution of a fluorescent ratiometric indicator for chloride
16	Elena Lehmann (University of Zurich)	Insight into activation and selectivity of volume regulated heteromeric LRRC8A/D channels
18	Ioanna Maria Vynichaki (Univ. of Crete)	Regulation of CFTR by the membrane phospholipid PIP2
20	Jennifer Miller (Univ. of Pittsburgh)	Metal modulation of TMEM16A channels
22	Ana Elena López-Romero (UASLP)	PI(4,5)P2 regulates TMEM16A open probability via two coupled sites
24	Swati Pant (Weill Cornell/MSKCC)	The pentameric chloride channel BEST1 is activated by extracellular GABA
28	Chia-Yu Kang (University of Michigan)	Structural determination and export mechanism of the eukaryotic fluoride exporter (FEX) proteins
32	Tharaka Wijerathne (Western Univ.)	Functional heteromerization of PIEZO1 and PIEZO2 channels
34	Shwetha Srinivasan (Stanford Univ.)	A structural view of gating and conductance in CLC-0
38	Sangyun Lee (Weill Cornell Medicine)	Structural basis of uncoupling in a CLC transporter
40	Carla Marie Peralta (Univ. of Michigan)	A role for a PII-like protein in fluoride ion transport
42	Eric Sefah (NIH)	Phosphatidylinositol 3,5-bisphosphate binding dynamics regulates CLC-7 function
46	Toshiki Yamada (Vanderbilt University)	A conserved mechanism of LRRC8 channel inhibition by three distinct drugs
48	Tara Sweet (SUNY Geneseo)	Evaluating zebrafish Ano1 function
50	Joshua Rosenthal (MBL)	Light-sensitivity in squid chromatophores: A functional study
54	Amy Xiang (Univ. of Maryland Balt.)	TMEM16A channels in capillary thin-strand pericytes control brain blood flow
56	Sara Granados (University of Chicago)	Volume-regulated anion channel blockers alter intracellular calcium signaling
58	Irene Lu (Nanion Technologies)	Activation of TMEM16A/Anoctamin1 through intracellular solution exchange using automated patch lamp systems
60	Jason Rengo (University of Vermont)	G-protein coupled receptors regulate mouse urinary bladder phasic smooth muscle contractions, potentially through potassium channels and calcium-activated chloride channels

POSTER SESSION B: Odd-numbered Abstracts

Friday, 5 September, 4:30 pm–6:00 pm, Swope Center 2nd Floor

13	Olivér Závoti (Semmelweis University)	Molecular and pharmacological evaluation of rare cystic fibrosis causing missense mutations of the CFTR channel
15	Jasmine Tutol (Univ. of Texas Dallas)	An all-optical approach to probe chloride transport with a bright ChlorON
19	Dongun Lee (NIH/NIDCR)	The ER localized lipid scramblase anoctamin 8 determine ER/PM junction PI(4)P and PI(4,5)P2 to control STIM1-Orai1 interaction and current
21	Crystal Lara Santos (Univ of Pittsburgh)	Delineating the structural features of polyunsaturated fatty acids required to inhibit the calcium-activated chloride channel TMEM16A
25	Tatsuya Hagino (Johns Hopkins Univs.)	Structural analysis of volume-regulated anion channel SWELL1 in complex with a novel auxiliary subunit PSA
27	Olive Burata (Nanion Technologies)	An assay technology for kinetic studies and drug development on ClCs: Solid supported membrane-based electrophysiology
29	Kaho Leung (Univ. of South Carolina)	Quantitative subcellular imaging tools for investigating chloride pathophysiology
31	Pietro Scaduto (Sophion Bioscience)	TMEM16A on Qube 384: A robust assay with low data spread and high success rate
35	James Charlick (University of Bristol)	Acute co-treatment with elexacaftor and ivacaftor restores wild-type-like activity to single elexacaftor-tezacaftor-ivacaftor-rescued F508del-CFTR channels
37	Paolo Zuccolini (NIH)	New disease-inducing mutations of the CLC-7 lysosomal transporter in mutant homodimers and heterodimers
41	Veronica Loyo Celis (Ohio State Univ.)	Molecular determinants of pH sensing in the chloride intracellular channel 3 (CLIC3)
43	Chris Peters (Univ. of Illinois Chicago)	Predicting conformational substrates of activation gating in the TMEM16A calcium-activated chloride channel
45	Jyosthna Lunavath (UCL)	High-content dual-fluorescence assay to quantify CFTR biogenesis and chloride vs. bicarbonate ion-channel function
47	Jortan Tun (University of Utah)	Cell type-specific distribution of functional nicotinic receptors in the mouse dorsal root ganglia
49	Paramita Sarkar (NIH/NIDCR)	Ca ²⁺ and cAMP signaling cross-talks at membrane contact sites regulates epithelial HCO ₃ ⁻ transporters CFTR and NBCe1-B defined by extended synaptotagmin 3
51	Wei-Yin Lin (NIH/NIDCR)	Multiple cAMP/PKA complexes at the STIM1 ER/PM junction, specified by E-Syt1 and E-Syt2, reciprocally gates ANO1 (TMEM16A) via Ca ²⁺
57	Patricia Pérez-Cornejo (UASLP)	TMEM16A expression directly modulates the actin cytoskeleton in HEK-293 cells
59	Michael Voldsgaard Clausen (NMD Pharma)	ClC-1 inhibitor compound (NMD670) enhances neuromuscular transmission and restores skeletal muscle function in a rat model of myasthenia gravis

Molecular contacts in self-assembling clusters of membrane proteins

Authors

Venkata Shiva Mandala - United States - Rockefeller University

Submission ID 10

Abstract

Many proteins function not just in isolation but also by interacting with other proteins as part of signaling pathways in living cells. Motivated by recent data pointing to the existence of homo-oligomeric assemblies of membrane proteins called higher-order transient structures (HOTS), and their apparent role in connecting components of membrane signal pathways, we examine by cryo-electron microscopy some of the protein-protein interactions that occur in cluster formation. Metabotropic glutamate receptors and HCN ion channels inside clusters contact their neighbors through structured extracellular and intracellular domains, respectively. Other ion channels, including Kv2.1 and Slo1 appear to form clusters through prominent intrinsically disordered sequences in the cytoplasm. These distinct modes of interaction are associated with clusters exhibiting varying degrees of compactness and order. We conclude that nature utilizes a variety of ways to form connections between membrane proteins in self-assembled clusters.

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In-cell directed evolution of a fluorescent ratiometric indicator for chloride

Authors

Shelby Phelps - United States - The University of Texas at Dallas

Submission ID 12

Abstract

Cells maintain dynamic ion gradients to provide spatial and temporal cues that orchestrate a broad range of cellular processes. While cation gradients have been extensively characterized, chloride gradients are equally critical and contribute to physiological functions such as circadian rhythm, muscle contraction, and digestion. To probe chloride in such contexts, genetically encoded fluorescent indicators can provide a rapid and scalable readout of chloride in living cells. However, these remain limited in scope and applicability.

To this end, our laboratory has developed the ChlorONs, a series of fluorescent protein indicators derived from mNeonGreen with a turn-on emission response to increasing concentrations of chloride. These indicators enable direct monitoring of intracellular chloride dynamics using fluorescence microscopy. To monitor and measure chloride within and across cell types, next-generation ChlorONs with ratiometric features and large dynamic ranges are needed. In this presentation, we will describe the development of a high-throughput, in-cell protein engineering workflow to unlock this function.

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Jasmine N Tutol - The University of Texas at Dallas

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Molecular and pharmacological evaluation of rare cystic fibrosis causing missense mutations of the CFTR channel

Authors

Olivér Závoti - Hungary - Department of Biochemistry, Semmelweis University, Budapest, Hungary

Submission ID 13

Abstract

Cystic fibrosis (CF) is a life-threatening disease caused by the pathogenic mutations of the CFTR anion channel. For patients carrying frequent mutant alleles, modulator therapy combining corrector and potentiator drugs mitigates the symptoms of the disease. Still, for many rare variants, no such treatment is available. Recently, five rare, previously unobserved, cystic fibrosis-causing missense mutations (G126D, I336K, T465I, T582I, D984V) were identified in the Hungarian CF population. Our goal was to better understand the functional consequences of these mutations. Channel expression and maturation were examined by Western blot using crude membrane preps of transiently transfected HEK-293 cell cultures. The mutant channels were also expressed in *Xenopus laevis* oocytes, and channel gating was studied using the inside-out patch clamp technique. Channel glycosylation was impaired for each mutant construct. While G126D and T582I produced a slight decrease in the amount of fully glycosylated CFTR, I336K, T465I, and D984V markedly affected maturation. Treating the cell cultures with corrector drugs (Vx-445 and Vx-661) completely restored glycosylation to wild-type levels. Our earlier results pointed out that G126D, T582I and D984V all affect channel gating differently. Consequently, pharmacological studies were conducted, which showed that the three constructs respond to potentiator drugs (Vx-770, Vx-445) with multifold increases in their currents. The potentiators were now also tested on the I336K and T465I variants. Upon exposure to the potentiators, the two mutants exhibited even more robust increases in their currents, revealing that these constructs heavily impact channel gating.

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OLIVÉR ZÁVOTI, LÁSZLÓ CSANÁDY *Department of Biochemistry, Semmelweis University, Budapest, Hungary*

An all-optical approach to probe chloride transport with a bright ChlorON

Authors

Jasmine Tutol - United States - The University of Texas at Dallas

Submission ID 15

Abstract

Chloride is the most abundant anion in the body and accounts for approximately 70% of the total negative charge in the extracellular fluid. It is essential in regulating cellular processes such as osmotic pressure, acid-base balance, muscle contraction, and neuronal excitability via tightly regulated transport mechanisms through a diverse array of chloride channels. Despite its importance, chloride transport and dynamics in live cells remain poorly understood. Current approaches to study chloride include electrophysiology, radiolabeling, and fluorescence microscopy with chloride-sensitive indicators. Of these, fluorescence microscopy is particularly attractive because it is minimally invasive and enables real-time, spatially resolved imaging of cellular chloride. However, the development of chloride indicators presents unique challenges, necessitating indicators with high sensitivity, large dynamic range, and bright fluorescence at physiological conditions.

To date, GFP-based chloride indicators have found a strong foothold in cellular imaging applications but have been limited to a fluorescence quenching mechanism, resulting in the loss of signal and limited sensitivity. In this presentation, we report the development of the ChlorONs, a family of genetically encoded, turn-on fluorescent chloride indicators derived from the cephalochordate *Branchiostoma lanceolatum*. A single point mutation near the chromophore has resulted in a second-generation biosensor – ChlorON-1-PRO – with enhanced brightness and chloride affinity at physiological pH. Further, fluorescence microscopy in live osteosarcoma cells demonstrates the utility of ChlorON-1-PRO as a robust chloride biosensor that can be used to identify modulators of chloride transport, offering a new avenue to dissect and therapeutically target chloride in health and disease.

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Insight into activation and selectivity of volume regulated heteromeric LRRC8A/D channels

Authors

Elena Lehmann - Switzerland - University of Zurich

Submission ID 16

Abstract

Upon osmotic swelling, volume regulated anion channels (VRACs) are activated to mediate the release of anions and small osmolytes in vertebrate cells. VRACs are multimeric assemblies of leucine rich repeat containing 8 (LRRC8) proteins, a family encompassing five paralogues (LRRC8A-E) in mammals. Endogenous VRACs form heteromeric assemblies consisting of the essential LRRC8A subunit and at least one other LRRC8 paralogue. Recent studies have highlighted the role of cytoplasmic LRR-domains in channel activation, as observed for the allosteric regulation of VRACs by synthetic nanobodies (sybodies), and the structural properties of heteromeric LRRC8A/C channels. Still, the mechanism of channel gating and structural basis of substrate selectivity has remained elusive. In contrast to LRRC8A/C, LRRC8A/D channels mediate the release of larger substrates (e.g. amino acids and osmolytes) and exhibit similar activation properties as endogenous VRAC channels. We determined the subunit stoichiometry and structure of heteromeric LRRC8A/D channels by cryoEM. We observed a stable 2:1 LRRC8A:LRRC8D subunit ratio as well as a similar pairwise clustering of A and D subunits in hexameric protein assemblies as in heteromeric LRRC8A/C channels. In complex with an inhibitory sybody, clustered LRRC8A subunits reside in an equivalent conformation observed in homomeric LRRC8A channels, whereas the LRR domains of LRRC8D subunits are less well resolved highlighting their increased flexibility in heteromeric assemblies. These findings suggest perturbation of the tight LRRC8A subunit packing by the introduction of LRRC8D subunits as previously described for heteromeric LRRC8A/C channels. Furthermore, the structure of LRRC8A/D channels in complex with potentiating sybodies under activating conditions showed a pronounced increase of the mobility of all LRR domains as presumable mechanism associated with channel gating. In summary, our study allowed us to deepen our understanding on the organization of heteromeric VRAC channels associated with release of larger substrates and the conformational changes associated with their activation.

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Regulation of CFTR by the membrane phospholipid PIP₂

Authors

Ioanna Maria Vynichaki - Greece - Medical School, University of Crete, Greece

Submission ID 18

Abstract

Cystic Fibrosis is a disease that arises from the dysfunction or absence of the Cystic Fibrosis **Transmembrane Conductance Regulator or CFTR**. This protein is the single ion channel member of the ABC superfamily and conducts Cl⁻ and bicarbonate ions across the apical plasma membrane of epithelial cells. Its structure contains two transmembrane domains (TMD1, TMD2) that form the channel pore, two nucleotide-binding domains (NBD1, NBD2) and a unique Regulatory Domain (RD). The opening and closing of the channel (gating) is regulated by ATP binding to the NBDs and the phosphorylation of the RD.

Phosphatidylinositol 4,5-bisphosphate or PIP₂ is a membrane phospholipid that regulates ion channel activity through electrostatic interactions. In 2004 PIP₂ was reported to regulate gating of the unphosphorylated CFTR, while experiments using PIP₂ on the phosphorylated channel remained inconclusive. No other reports on regulation of CFTR activity by PIP₂ have been published. Here, we present data that elaborate on the CFTR-PIP₂ interaction at distinct stages of the CFTR activation cycle. In inside-out patch clamp recordings from *HEK293T cells*, superfusion of CFTR with the water-soluble short-chain dioctanoyl-PI(4,5)P₂ (diC₈-PIP₂) in the presence of MgATP activated the unphosphorylated channel, as well as the phosphorylated CFTR, both during and after phosphorylation by Protein Kinase A (PKA). Additionally, the PIP₂ analogue significantly accelerated CFTR activation kinetics by PKA. To examine the effect on CFTR of depleting membrane PIP₂ levels, we utilized a powerful optogenetic tool in whole-cell recordings to specifically remove the P4 and P5 phosphates of the phospholipid. This intervention inhibited CFTR activity. Overall, our data suggest that CFTR gating is strongly modulated by PIP₂ and hint at an interplay between channel, kinase and PIP₂.

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The ER localized lipid scramblase anoctamin 8 determine ER/PM junction PI(4)P and PI(4,5)P₂ to control STIM1-Orai1 interaction and current

Authors

Dongun Lee - United States - Epithelial signaling & Transport Section, NIDCR, NIH, Bethesda MD

Submission ID 19

Abstract

The anoctamins (ANOs) are encoded by the TMEM16 gene family and function as Ca²⁺-activated Cl⁻ channels. Some members, like the plasma membrane ANO6 and the ER ANO10 also function as lipid scramblases, primarily PtdSer. ANO8 functions as a tether at the ER/PM junctions and activates store-operated Ca²⁺ entry (SOCE) by facilitation interaction of STIM1 with Orai1. The transmembrane domains of ANO8 that may transfer or scramble lipids localize at the ER and thus, whether the ANO8 affect phospholipids at the ER/PM junctions and the role of the lipids in ANO8 control of STIM1-Orai1 are unknown. In the present work, we demonstrate that phospholipids transfer by ANO8 is essential for the ANO8-induced activation of STIM1-Orai1 current. ANO8 markedly enhances PIs turnover and increases junctional PI(4)P and PI(4,5)P₂ levels. Inhibition of PIs turnover by Li⁺, the PI(4)P kinase GSK-3 α and the PI(5)P kinase UNC-3230 had minimal effect on basal STIM1-Orai1 current but inhibited the ANO8-enhanced lipids level and STIM1-Orai1 current. Furthermore, mutation of residues in predicted ANO8 EM-localized lipid transfer domain affected PI(4)P and PI(4,5)P₂ levels and STIM1-Orai1 current. These functional changes were reflected in STIM1 translocation and clustering at the junction. Notable effects of ANO8 on junctional lipids required the PI(4)P/PtdSer exchanger ORP8 and PI(4)P/PtdSer exchange by ORP8 required ANO8. These findings reveal the role of the ER localized ANO8 as a lipid transfer protein that acts with ORP8 and likely other junctional lipid transfer proteins to determine ER/PM junctions lipid composition to regulate STIM1/Orai1-mediated current. Further studies are being conducted to further establish the role of ANO8 as a lipid transfer protein and its physiological role.

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Metal modulation of TMEM16A channels

Authors

Jennifer Miller - United States - University of Pittsburgh

Submission ID 20

Abstract

TMEM16A is a Cl⁻ channel regulated by intracellular Ca²⁺ and membrane PIP₂. TMEM16-family proteins share a unique Ca²⁺ binding site, which is accessible from the intracellular surface, is located within the plasma membrane, and can accommodate two Ca²⁺ ions. Typically, Ca²⁺ binding sites such as these do not have the capacity to bind metals such as Ni²⁺. However, in addition to Ca²⁺, divalent metals such as Sr²⁺ and Ni²⁺, have been shown to activate TMEM16A currents. This study sought to better understand how Ca²⁺ interacts with TMEM16A by discerning additional metal regulators of TMEM16A. To test this, we performed patch clamping on *Xenopus laevis* oocytes, which naturally and abundantly express a TMEM16A isoform that shares 86% identity with the human channel. In addition to Sr²⁺ and Ni²⁺, here we report that the channel is also activated by mM concentrations of Co²⁺ and Cu²⁺. However, no currents were obtained with the addition of Mg²⁺ or Zn²⁺. We used 1PBC, a potent pore blocker of TMEM16A channels to verify that applied metals activated TMEM16A specifically. Additionally, activating currents of applied metals were similarly depleted with neomycin, suggesting a common PIP₂-dependent regulatory mechanism.

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Jennifer M. Miller (*University of Pittsburgh*), Crystal Lara-Santos (*University of Pittsburgh*), Anne E. Carlson (*University of Pittsburgh*)

Delineating the structural features of polyunsaturated fatty acids required to inhibit the calcium-activated chloride channel TMEM16A

Authors

Crystal Lara Santos - United States - University of Pittsburgh

Submission ID 21

Abstract

Transmembrane protein 16A (TMEM16A) is a widely expressed calcium-activated chloride channel that regulates critical physiological processes, including inflammation, coronary blood flow, and vascular tone. Despite strong evidence for its functional importance, the molecular mechanisms that regulate TMEM16A remain poorly understood. Here, we investigated whether polyunsaturated fatty acids (PUFAs), known lipid signaling molecules, modulate TMEM16A activity. We used excised inside-out patches from *Xenopus laevis* oocytes, which endogenously express a TMEM16A isoform sharing 86% identity with the human channel. Applying various PUFAs with distinct structural properties, we assessed their impact on TMEM16A-conducted currents. We found that PUFAs inhibit TMEM16A activity in a structure-dependent manner. Arachidonic acid (AA; 20:4) at 10 μ M reduced TMEM16A currents by 50% within 44 ± 13 seconds (N=8), while stearic acid (18:0), a saturated fatty acid, had no effect (N=7), indicating that unsaturated bonds are required for inhibition. To test the role of tail length, we applied stearidonic acid (18:4), which has the same number of double bonds as AA, but a shorter carbon chain. It inhibited TMEM16A more slowly, taking 266 ± 22 seconds to achieve 50% inhibition (N=6). Among the PUFAs tested, AA produced the most rapid inhibition. To explore the role of double bond position, we tested a synthetic analog, AA ω -3, with shifted double bonds. AA ω -3 inhibited 50% of the current in 87 ± 5 seconds (N=5), suggesting that while unsaturation is essential, the precise location of double bonds is not critical. These findings suggest that PUFA structure governs TMEM16A inhibition. Understanding how PUFAs modulate TMEM16A may provide mechanistic insights into its regulation and the health benefits associated with PUFA-rich diets.

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PI(4,5)P₂ regulates TMEM16A open probability via two coupled sites

Authors

Ana Elena López-Romero - Mexico - Physics Institute, Universidad Autónoma de San Luis Potosí, San Luis Potosí, México

Submission ID 22

Abstract

The Ca²⁺-activated chloride channel TMEM16A regulates essential physiological processes; however, its overexpression and dysregulation can lead to severe pathologies. Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) is a critical regulator of TMEM16A. Presumably, the binding of PI(4,5)P₂ to four putative Sites (Site1 – Site4) maintains TMEM16A in a conductive state. Whereas Site1 appears essential, the role of Sites 2-4 in TMEM16A function remains undetermined. Here, we applied site-directed mutagenesis, patch clamp electrophysiology, and molecular dynamics (MD) simulations to determine whether the juxtaposed Sites 1 and 3 are functionally coupled to transduce PI(4,5)P₂ regulatory actions on TMEM16A. We expressed in HEK293 cells TMEM16A alone or with the *Ciona intestinalis* voltage-sensitive phosphatase (Ci-VSP) to deplete membrane PI(4,5)P₂. We found that PI(4,5)P₂ depletion diminishes the TMEM16A current density, likely due to a loss of voltage sensitivity and open probability. Notably, this effect was independent of Ca²⁺-dependent activation. It has been proposed that PI(4,5)P₂ depletion induces TMEM16A pore collapse; however, neither the anion selectivity sequence nor the potency of pore blockers A9C and 1PBC was altered by PI(4,5)P₂ depletion, thus ruling out pore collapse. On the other hand, a triple mutant at Site 1 (Site1-3M) exhibited the same current density and voltage activation as WT channels in the absence of PI(4,5)P₂ (+Ci-VSP). However, Site 3-3M channels displayed control WT-like current density (-Ci-VSP), but their voltage activation was the same as WT + Ci-VSP or Site1-3M. MD simulations showed that PI(4,5)P₂-bound to Site 1 interacts with R484 from Site 3, thus suggesting an interaction between Sites 1 and 3. A double-mutant cycle analysis confirmed that R484 and R486 (Site 1) are coupled in a PI(4,5)P₂-dependent manner. Thus, PI(4,5)P₂ regulates TMEM16A open probability through coupled Sites 1-3 by maintaining voltage sensitivity within a physiological voltage range. Supported by COPOCYT.

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The pentameric chloride channel BEST1 is activated by extracellular GABA

Authors

Swati Pant - United States - Memorial Sloan Kettering Cancer Center/Weill Cornell Graduate School of Medical Sciences

Submission ID 24

Abstract

Bestrophin 1 (BEST1) is a calcium activated chloride channel expressed in the eye and other tissues in the body. A link between BEST1 and the principal inhibitory neurotransmitter gamma- aminobutyric acid (GABA) has been proposed. Extracellular GABA is detected by GABA_B G-protein coupled receptors and the pentameric GABA_A chloride channels, both of which have fundamental roles in the central nervous system. Here, we demonstrate that BEST1 is directly activated by extracellular GABA. Through functional and structural studies of human and chicken BEST1, we identify a GABA binding site and determine the mechanism by which GABA binding induces opening of the channel's gate. This gate also undergoes conformational changes in response to intracellular calcium for activation or inactivation of BEST1. This indicates that BEST1 is controlled by ligands from both sides of the membrane. These studies demonstrate that BEST1, which shares no structural homology with GABA_A, is a GABA-activated chloride channel. The physiological implications of this finding remain to be studied.

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Structural analysis of volume-regulated anion channel SWELL1 in complex with a novel auxiliary subunit PSA

Authors

Tatsuya Hagino - United States - Department of Physiology, Johns Hopkins University School of Medicine

Submission ID 25

Abstract

Volume-regulated anion channels (VRACs) are large-pore channels present in nearly all vertebrate cells, playing key roles in cell volume regulation and autocrine/paracrine signaling. Here, we identify the ubiquitously expressed puromycin-sensitive aminopeptidase (PSA) as a binding partner of the obligatory VRAC subunit SWELL1 (also known as LRRC8A) and show the cryo-electron microscopy structure of the SWELL1–PSA complex. Three PSA molecules associate with a single SWELL1 hexamer, coupling adjacent leucine-rich repeat (LRR) domains into local dimers. Functionally, PSA overexpression suppresses VRAC activation, whereas its deletion results in elevated basal channel activity. Notably, PSA's regulatory role on VRACs is independent of its aminopeptidase activity. Our findings identify PSA as the first auxiliary subunit of VRACs, highlight the role of intracellular LRR domains in allosteric channel gating, and propose a new strategy for modulating VRAC function in diverse physiological contexts, including cGAMP transport and STING signaling.

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An assay technology for kinetic studies and drug development on CICs: Solid supported membrane-based electrophysiology

Authors

Olive Burata - Germany - Nanion Technologies

Submission ID 27

Abstract

High-resolution functional assays are essential for understanding ion-coupled transporters and accelerating discovery of their modulators. The SURFE²R platform, based on solid-supported membrane electrophysiology (SSME), enables label-free, time-resolved measurement of electrogenic transport from purified proteins reconstituted into liposomes or native membranes. Using the bacterial H⁺/Cl⁻ antiporter ecCIC as a model, we demonstrate the platform's ability to resolve distinct influx and efflux kinetics, quantify pH-gradient effects, assess lipid-dependent activity, and evaluate inhibition by small molecules such as OADS. These examples illustrate how SSME provides precise kinetic parameters, mechanistic insights, and pharmacological profiling in a single, flexible assay format. The approach is broadly applicable to CICs, SLC transporters, and other electrogenic systems, offering a robust bridge between basic mechanistic studies and early-stage drug screening.

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Structural determination and export mechanism of the eukaryotic fluoride exporter (FEX) proteins

Authors

Chia-Yu Kang - United States - University of Michigan

Submission ID 28

Abstract

Microorganisms have fluoride-specific efflux systems to prevent fluoride toxicity. While bacterial fluoride exporters have been studied in detail, the eukaryotic FEX exporters have not yet been biochemically or structurally characterized. Here, we use electrophysiology, structural studies, and molecular dynamics simulations to reveal the molecular mechanism of fluoride export by eukaryotic FEX proteins from yeasts and plants. We show that FEX acts as a passive fluoride channel, and the fluoride export rate varies among species. FEX activity is sodium-dependent, suggesting that sodium might play a role in channel gating. In contrast, Li^+ , with an ionic radius 0.26 Å smaller than Na^+ , inhibits FEX activity, indicating that cation binding and channel activity are specifically related. The cryo-EM structure of FEX from *Candida albicans*, along with mutagenesis studies, reveals a fluoride permeation mechanism through a single pore lined with many phenylalanine residues. Furthermore, we identified an electronegative vestibule at the external side of FEX that contributes to a sodium binding site. We propose that this feature prevents extracellular fluoride accumulation and might contribute to sodium binding during membrane depolarization. Together, these results provide molecular insight into fluoride transport in eukaryotes.

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Quantitative subcellular imaging tools for investigating chloride pathophysiology

Authors

Ka Ho Leung - United States - University of South Carolina

Submission ID 29

Abstract

As the most abundant anions in cells, chloride ions play crucial roles in numerous cellular functions, with intracellular chloride concentrations ranging from 10 mM to 120 mM across different organelles. Dysregulation of chloride homeostasis has been implicated in a variety of diseases, collectively contributing to a substantial healthcare burden. In this presentation, I will discuss our recent advancements in the development of subcellular imaging tools that enable the precise visualization of the intracellular chloride landscape and dynamics. These imaging tools have significantly enhanced our understanding of chloride pathophysiology, including investigations into the physiological role of chloride in the cGAS–STING pathway, which is a critical component of the innate immune system driving aging-related inflammation and neurodegeneration.

TMEM16A on Qube 384: A robust assay with low data spread and high success rate

Authors

Pietro Scaduto - United States - Sophion Bioscience Inc

Submission ID 31

Abstract

TMEM16A (ANO1) is a Ca^{2+} -activated Cl^- channel (CaCC) implicated in numerous physiological and pathophysiological processes. The channel has been proposed as a therapeutic target for asthma, secretory diarrheas, and hypertension. TMEM16A gating uniquely depends on both membrane voltage and cytosolic Ca^{2+} in a synergistic manner (Scudieri et al. 2012). Development of a robust, high-throughput electrophysiology assay for TMEM16A has been challenging, as many automated patch clamp (APC) platforms rely on fluoride in the internal solution to enhance seal formation. The poor solubility of calcium fluoride results in precipitation, limiting precise control over internal calcium concentration and hindering assay performance. Here, we present a novel approach to assess pharmacological inhibition of TMEM16A using the Qube 384 (Sophion Bioscience) APC platform. Our method consistently achieves: (1) high success rates (>80% of wells with valid recordings), (2) excellent pharmacological reproducibility across experiments, and (3) minimal current rundown and low variability in IC_{50} values. This assay overcomes prior limitations by enabling reliable Ca^{2+} control without compromising data quality, thus providing a robust platform for TMEM16A screening in drug discovery.

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Functional heteromerization of PIEZO1 and PIEZO2 channels

Authors

Tharaka Wijerathne - United States - Western University of Health Sciences

Submission ID 32

Abstract

Homotrimeric PIEZO1 and PIEZO2 channels, despite their distinct mechanosensory properties, possess a high degree of sequence and structural similarities and are co-expressed in a discrete number of cells, including chondrocytes, some sensory neurons, and vascular endothelial and smooth muscle cells. We hypothesized that these channels could form functional heterotrimers, a mechanism that would diversify their physiological roles. To test this, we used split GFP-based bimolecular fluorescence complementation and FRET to demonstrate that PIEZO1 and PIEZO2 can indeed form heterotrimers in heterologously expressed in HEK293T cells at the membrane level, revealing the formation of both PIEZO1:PIEZO1:PIEZO2 and PIEZO1:PIEZO2:PIEZO2 stoichiometries. To understand the functional implications of heteromerization, we first characterized the pore properties of homomeric PIEZO channels using the exclusion volume method. Our results shows that homomeric PIEZO1 and PIEZO2 channels possess a similar minimal pore radius of approximately 4 Å. While PIEZO channels are primarily known for cation permeability, we also discovered that both PIEZO1 and PIEZO2 channels outwardly rectify chloride currents at a peak conductance of 10-13 [JL1] pS. We next used a functional bimolecular fluorescence complementation approach using a split GCaMP to show that heteromeric PIEZO channels are capable of eliciting calcium influx in response to a mechanical stimulus. These findings collectively demonstrate that PIEZO1 and PIEZO2 share similar pore properties and can form functional heteromeric channels.

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A structural view of gating and conductance in CLC-0

Authors

Shwetha Srinivasan - United States - Stanford University

Submission ID 34

Abstract

The CLC family of proteins comprises Cl⁻ channels and Cl⁻/H⁺ antiporters that are ubiquitously expressed across diverse tissues, where they regulate processes ranging from electrical signaling in nerves and muscles to acidification of intracellular organelles. The dual functionality, fast ion conduction by channels and coupled ion exchange by transporters, enables the family to support a wide range of physiological roles (Jentsch and Pusch. 2018. *Physiol. Rev.* 98:1493–1590). Mutations in CLC genes cause a spectrum of human diseases, including myotonia congenita, epilepsy, and osteopetrosis. Despite their functional diversity, all CLC proteins share a characteristic structural foundation – a homodimeric architecture in which each subunit houses an independently gated ion pathway (Ludewig, Pusch and Jentsch. 1996. *Nature* 383:340–343; Middleton, Pheasant and Miller. 1996. *Nature* 383:337–340). In addition to the independent gating mechanism, a “common gating” mechanism inactivates both CLC pores cooperatively. Common gating involves a large conformational change (Bykova, Zhang, Chen and Zheng. 2006. *Nat. Struct. Mol. Biol.* 13:1115–1119), but the molecular details of this change have remained uncertain. To address this gap, we determined high resolution structures of CLC-0, the original “double barreled” chloride channel, for which common gating has been most thoroughly characterized (Miller. 1982. *Philos. Trans. R. Soc. London. B, Bio. Sci.* 299:401–411). The structures reveal a massive, concerted conformational change, unprecedented within the CLC family and consistent with CLC-0’s pronounced temperature dependence (Pusch, Ludewig and Jentsch. 1997. *J. Gen. Physiol.* 109:105-116). Building on these insights, we used structure-guided mutagenesis and electrophysiological assays to investigate the molecular basis of CLC-0’s exceptionally high single-channel conductance. Together, these findings transform the structural framework for understanding conduction and gating across the CLC family.

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Acute co-treatment with elexacaftor and ivacaftor restores wild-type-like activity to single elexacaftor-tezacaftor-ivacaftor-rescued F508del-CFTR channels

Authors

James Charlick - United Kingdom - University of Bristol

Submission ID 35

Abstract

Mutations in the anion channel, cystic fibrosis transmembrane conductance regulator (CFTR) cause the genetic disease cystic fibrosis (CF). The treatment of people with CF and the predominant disease-causing variant, F508del, which impairs CFTR trafficking, gating, and stability, has been transformed by the triple-combination therapy elexacaftor-tezacaftor-ivacaftor (ETI). Here, we report the single-channel behaviour at 37 °C in excised inside-out membrane patches of heterologously expressed F508del-CFTR rescued by three methods: i) low temperature; ii) chronic E (2 μ M) T (3 μ M) I (1 μ M) treatment and iii) chronic ETI treatment with acutely added potentiators, E (2 μ M) and I (100 nM), quantifying mean values for open probability (P_o), burst duration (MBD) and interburst interval (IBI) $\pm$ SD. First, we demonstrated that the activity of ETI-rescued F508del-CFTR channels in the acute presence of EI was strictly dependent upon phosphorylation by PKA. When compared with wild-type CFTR (P_o , 0.4 ± 0.2 , $n = 6$; MBD, 97 ± 20 ms; IBI, 186 ± 65 ms, $n = 4$), the activity of low temperature-rescued F508del-CFTR was greatly reduced as a result of brief, infrequent openings (P_o , 0.07 ± 0.05 , $n = 3$; MBD, 58 ± 7.8 ms; IBI, 1172 ± 1095 ms, $n = 3$). ETI-rescued F508del-CFTR exhibited prolonged MBD, but wild-type-like P_o was not restored because IBI was only modestly reduced (P_o , 0.17 ± 0.08 , $n = 13$; MBD, 129 ± 86 ms; IBI, 593 ± 252 ms, $n = 5$). Crucially, wild-type-like P_o was restored to ETI-rescued F508del-CFTR channels by acute treatment with E and I (P_o , 0.6 ± 0.2 , $n = 8$). Underlying this activity was a strikingly long MBD (678 ± 220 ms, $n = 6$), while IBI was unaffected (455 ± 365 ms, $n = 6$). We conclude that E and I restore wild-type-like P_o to ETI-rescued F508del-CFTR by stabilising channels in the open state. These findings provide an explanation for the clinical efficacy of the ETI triple-combination therapy. Supported by the Cystic Fibrosis Trust.

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New disease-inducing mutations of the CLC-7 lysosomal transporter in mutant homodimers and heterodimers

Authors

Paolo Zuccolini - United States - National Institutes of Health

Submission ID 37

Abstract

CLC-7 is a lysosomal transporter composed of two identical protomers, each displaying an independent transport pathway. Each cycle moves one H⁺ out of the lysosome and two Cl⁻ into the lumen to counterbalance the transmembrane voltage generated by V-ATPase mediated influx of H⁺. Previous work, in which CLC-7 was trafficked to the plasma membrane, have shown that the transporter is active only at voltages higher than ~90 mV. A common voltage-dependent gating mechanisms known as common gate affects both protomers at the same time. Here we present the characterization of three CLC-7 mutations from new patients with a mild form of HOD syndrome. These mutations are localized in different parts of the protein, but they all cause an acceleration of CLC-7 activation kinetics. To evaluate their impact on the coordination between the two subunits, we investigated if and how these mutations could alter the gating of a neighbor WT subunit. We created three double mutants (DMs) by inserting each mutation in the transport deficient (TD) CLC-7 E314A and we tested if such DMs were TD before co-transfecting them in HEK cells with WT (4:1 ratio). In fact, if DM/DM homodimers are non-conductive, currents measured in co-transfected cells must be mediated by the WT halves of WT/DM couples. Of the three analyzed mutations, one was so impactful that DM/DM homodimers were conductive despite the removal of E314. The other two were able to accelerate the gating of WT protomer but to different extents, depending on their localization. Our data confirm the acceleration of CLC-7 gating as underlying factor in many diseases and reinforce the concept of coordinated conformational change in the two subunits. The localization of a gain-of function mutations within the protein also determines to which extent they alter the common gate mechanism.

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Structural basis of uncoupling in a CLC transporter

Authors

Sangyun Lee - United States - Weill Cornell Medicine College

Submission ID 38

Abstract

CLC Cl⁻ channels and Cl⁻/H⁺ transporters are vital for human physiology and their mutations can cause genetic disorders. In transporters, the Cl⁻ and H⁺ pathways intersect at a conserved glutamic acid, E_{gate}, which serves as the Cl⁻ gate. Rotation of the E_{gate} sidechain between the two pathways enables H⁺ movement, and its mutations abolish H⁺ coupling. However, the structural basis of coupled Cl⁻:H⁺ exchange remains poorly understood. To address this question, we take advantage of the finding that large polyatomic anions, such as NO₃⁻ and SCN⁻, impair H⁺ coupling in transporters. CryoEM imaging of CLC-ec1, the CLC exchanger from *E. coli*, with Cl⁻ and the uncoupling anions shows that in Cl⁻, E_{gate} (E148 in CLC-ec1) and conserved F357 can adopt two rotameric states in a pH-dependent manner: R1, favored at high pH, where E148 lines the Cl⁻ pathway and F357 is 'down' state that renders the pathway more hydrophobic, thus preventing H⁺ transport, and R2 at low pH, where E148 lines the H⁺ pathway and F357 in an 'up' state which favors H⁺ pathway hydration. In contrast, the uncoupling anions favor the H⁺-transport incompetent state R1 only, pH independently. MD simulations show that with Cl⁻ at the central binding site, S_{cen}, E148 and F357 can adopt R1 and R2, and the H⁺ pathway is more hydrated in R2, likely facilitating H⁺ transport. This suggests that F357 acts as a gate for the H⁺ pathway and that its movements are energetically coupled to those of E148. In contrast, SCN⁻ binding at S_{cen} decouples the two gating residues, while F357 exclusively adopts a down conformation, independent of the state of E148, and H⁺ pathway remains dehydrated. These findings explain how SCN⁻ specifically inhibits H⁺ movement in CLC-ec1 while leaving anion transport intact, and reveal the precise molecular steps underlying coupled exchange in CLCs.

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A role for a PII-like protein in fluoride ion transport

Authors

Carla Marie Peralta - United States - University of Michigan

Submission ID 40

Abstract

The Fluc fluoride channels defend bacteria against fluoride toxicity. Understanding mechanisms of fluoride transport is important for applications ranging from developing treatments for oral pathogens, to PFAS degradation by microbes, where the breakdown of fluorinated compounds can lead to increased intracellular fluoride. Recently, using bioinformatic analysis, we identified a protein of unknown function, DUF190, that is genetically associated with Fluc transporters across bacterial phyla. DUF190 is a member of the PII-like signaling protein family, whose members regulate a variety of cellular processes by sensing the cellular energy state through binding of metabolic effectors such as nucleotides. We hypothesized that DUF190, or Fluf (Fluoride-associated protein of unknown function), might regulate fluoride transport in response to intracellular nucleotide levels. Thermal stabilization assays indicate that Fluf binds with Mg-ATP, Mg-ADP, or Mg-cAMP. To test whether Fluc and Fluf interact, we use Biolayer Interferometry binding assays, which show that Fluf interacts with Fluc in the presence of ADP and cAMP, but not ATP. This nucleotide-dependent interaction has functional consequences for fluoride transport: using Solid-Supported Membrane Electrophysiology, we show that Fluc transport is reduced or abolished in the presence of Mg-ADP and Mg-cAMP, but not in the presence of Mg-ATP. Together, these results suggest that Fluf regulates fluoride transport by Fluc in response to cellular energy levels. We hypothesize this Fluc-blocking mechanism works to prevent fluoride ions from entering the cell from the outside in high fluoride environments under low energy conditions when membrane potential is dissipated. Crystal structures of Fluf bound to Mg²⁺, ATP, and cAMP reveal nucleotide-dependent changes within the T-loop region of the protein, which is likely involved in binding to Fluc. Future directions include obtaining the ADP-bound structure of Fluf, as well as the entire complex including Fluc, and mutagenesis experiments on residues that may facilitate binding of Fluf to Fluc.

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CARLA MARIE PERALTA, *University of Michigan*

Molecular determinants of pH sensing in the chloride intracellular channel 3 (CLIC3)

Authors

Veronica Loyo Celis - United States - ¹Department of Physiology and Cell Biology, College of Medicine, The Ohio State University, Columbus, Ohio, USA.

Submission ID 41

Abstract

Chloride intracellular channels (CLICs) are a family of proteins capable of existing in both soluble and transmembrane forms. While pH dependence is a well-characterized feature of the soluble form, this property has only been described in the transmembrane forms of CLIC1, CLIC2, and CLIC6. Here, we report for the first time the pH regulation of transmembrane CLIC3 and identify a key residue implicated in this intrinsic property.

CLIC3 was ectopically expressed in HEK-293 cells, where it localized to the plasma membrane. pH-dependent activation of CLIC3 was assessed using whole-cell patch-clamp and single-channel recordings. The recording solution is composed of (in [mM] pipette:130 NMDG-Cl, 1 CaCl₂, 1 MgCl₂, 10 glucose, 5 EGTA, 10 Hepes, pH 7.2; and bath:135 NMDG-Cl, 1 CaCl₂, 10 glucose, 10 Hepes, pH 7.2 and 6.2). The pH was changed by the addition of CsOH in the bath solution. The current density and single-channel conductance increased by 110% and 130%, respectively, when the pH in the bath solution was changed from 7.2 to 6.2. The precise mechanism of pH regulation of CLIC3 is not known. Here, through comprehensive mutagenesis and electrophysiological studies, we identified a critical histidine residue (H21) in the N-terminus as a potential pH sensor for CLIC3. The H21A mutation abolished any changes in current density and conductance when pH was changed from 7.2 to 6.2. However, H181, which is present in the C-terminus and is conserved in all CLIC proteins, does not play any role in pH sensitivity.

In summary, we demonstrate that histidine at position 21 (H21) in the N-terminal domain is critical for both conductance and pH-dependent activation of transmembrane CLIC3.

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Phosphatidylinositol 3,5-bisphosphate binding dynamics regulates CIC-7 function

Authors

Eric Sefah - United States - National Institutes of Health

Submission ID 42

Abstract

Lysosomal acidification is essential for cellular degradation, nutrient recycling, and the maintenance of organelle homeostasis. The lysosomal chloride/proton antiporter CIC-7, in complex with its β -subunit OSTM1, provides the chloride conductance required to sustain the proton gradient generated by the V-ATPase, and disruption of this acidification process underlies several lysosomal storage diseases (LSDs). Emerging evidence reveals that phosphatidylinositol 3,5-bisphosphate PI(3,5)P₂ directly binds CIC-7, promoting a compact association between its cytosolic and transmembrane domains^{1,2}. Loss of PI(3,5)P₂ binding—whether through lipid depletion or disease-causing mutations—disrupts this association, leading to cytosolic domain dissociation, the adoption of novel transmembrane conformations, and transporter activation³. While this model hints at a large-scale structural remodeling, the mechanistic link between PI(3,5)P₂ inhibition and altered Cl⁻ transport remains unresolved.

To address this, we performed short- and long-timescale molecular dynamics simulations, combined with cluster analysis, to map dominant conformational states and potential long-range allosteric communication between the PI(3,5)P₂-binding pocket and residues critical for Cl⁻ transport. Our results show that PI(3,5)P₂ binding stabilizes the loop region between helix F and G (FGL) and the cystathionine beta synthase 2 (CBS2) linker, maintaining a compact conformation. Upon PI(3,5)P₂ removal, this region becomes dynamic, propagating flexibility along helix F to the gating glutamate E247. This rearrangement remodels the Cl⁻ binding site, resulting in increased Cl⁻ dynamics between the external and central binding sites, including an instance of Cl⁻ release into the lumen. These findings provide a direct structural mechanism linking PI(3,5)P₂ regulation to CIC-7 activity and offer insight into how lipid dysregulation can contribute to LSD-related lysosomal acidification defects.

References

1. Schrecker, Korobenko, and Hite. 2020. eLife 9:e59555.
2. Leray, Hilton, Nwangwu, Becerril, Mikusevic, Fitzgerald, Amin, Weston, and Mindell. 2022. eLife 11:e74136.
3. Hilton, Lin, Sefah, Deme, Parker, Langton, Grabe, Lea, Newstead, Mindell. Unpublished.

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Predicting conformational substrates of activation gating in the TMEM16A calcium-activated chloride channel

Authors

Christian Peters - United States - University of Illinois Chicago

Submission ID 43

Abstract

TMEM16A is a calcium-activated chloride channel with wide-ranging physiological importance, including intestinal motility, nociception and smooth muscle contraction. Each subunit of a TMEM16A dimer contains 10 membrane-spanning helices and is activated by direct binding of calcium at a pocket formed by helices TM6-8. Increasing calcium concentration causes the channel to shift from a voltage-dependent current exhibiting outward rectification to an Ohmic current. However, despite the availability of multiple high resolution cryo-EM structures, a *bona fide* open structure (exhibiting a pore of sufficient diameter to pass a chloride ion) remains elusive, as does a comprehensive explanation for the apparent shift in channel gating with increasing calcium exposure. Here, we leveraged a published structure of TMEM16A, PDB# 7ZK3 (Lam et al. 2022. Nat Commun. 13:2798) in complex with the pore blocker 1PBC (Peters et al. 2015. PNAS. 112:3547-52) as a starting point to identify conformational changes associated with opening the channel. Using atomistic MD simulations, we observe several stable structural clusters with a pore size sufficient to permeate chloride ions, through which we demonstrate *in silico* chloride permeation. Among these structures, we identify a network of hydrophobic amino acids from TM3-4 at the outer pore region which are retained from 7ZK3 as the pore dilates. Site directed mutagenesis at these sites caused significant shifts in gating of the protein, likely owing to accelerated channel deactivation. Taken together, these findings provide important insight into the structural basis of TMEM16A permeation and gating function.

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High-content dual-fluorescence assay to quantify CFTR biogenesis and chloride vs. bicarbonate ion-channel function

Authors

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Submission ID 45

Abstract

Accurate functional characterization of rare CFTR variants is essential for understanding CFTR-related disorders. We developed a high-content, image-based dual-fluorescence assay to quantify CFTR membrane proximity and anion channel activity, while monitoring intracellular pH. HEK293 cells expressed a bicistronic plasmid encoding iodide-sensitive YFP fused to CFTR and the pH-sensitive red fluorophore pHuji. pHuji monitored cytosolic pH and enabled image segmentation for estimating YFP-CFTR membrane proximity. Following CFTR activation, extracellular iodide triggered iodide/chloride or iodide/bicarbonate exchange, quenching YFP fluorescence to report anion conductance. Experiments were performed in chloride- or bicarbonate-based buffers after ellexacaftor/tezacaftor/ivacaftor (ETI) or vehicle (DMSO) chronic treatment.

WT-CFTR exhibited enhanced anion exchange upon forskolin (Area Above YFP quenching Curve, AAC₇, in RFU*s, mean±SEM, n>5: I⁻/Cl⁻: 2.54 ± 0.12 vs. 1.34 ± 0.09, p < 0.001; I⁻/HCO₃⁻: 1.52 ± 0.09 vs. 0.86 ± 0.05, p < 0.01) or ETI treatment (I⁻/Cl⁻: 3.37 ± 0.11, p < 0.001; I⁻/HCO₃⁻: 1.50 ± 0.06, p < 0.01), serving as benchmark for normal function and biogenesis. F508del-CFTR showed low baseline membrane proximity and conductance (I⁻/Cl⁻: 0.69 ± 0.03; I⁻/HCO₃⁻: 0.43 ± 0.02), consistent with the severe trafficking and gating defect of this cystic fibrosis-causing mutant. ETI partially rescued localization and function (I⁻/Cl⁻: 2.09 ± 0.12, p < 0.01; I⁻/HCO₃⁻: 1.09 ± 0.07, p < 0.05), but responses remained low, particularly in bicarbonate.

Patient-derived I556V-CFTR, associated with recurrent pancreatitis, showed mild trafficking and associated anion exchange defects (I⁻/Cl⁻: 1.05 ± 0.04; I⁻/HCO₃⁻: 0.63 ± 0.03). ETI improved localization and function (I⁻/Cl⁻: 2.78 ± 0.10, p < 0.001; I⁻/HCO₃⁻: 1.12 ± 0.06, p < 0.01) to near-WT levels, indicating no selective bicarbonate impairment. In all cases, pHuji timelines indicated minimal cytosolic pH changes during anion fluxes. This scalable assay simultaneously assesses CFTR trafficking and anion transport while controlling for pH artifacts.

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A conserved mechanism of LRRC8 channel inhibition by three distinct drugs

Authors

Toshiki Yamada - United States - Anesthesiology, Vanderbilt University Medical Center, Nashville, TN, USA

Submission ID 46

Abstract

Volume-regulated anion channels (VRACs) are comprised of Leucine Rich Repeat Containing 8 (LRRC8) proteins and participate in essential physiological processes. Recent studies have uncovered new and potential “druggable” roles of LRRC8 channels in cardiovascular, immunological, metabolic, and neurological disorders. However, the molecular pharmacology of LRRC8 anion channels is poorly developed. We employed a structurally defined homomeric LRRC8 channel chimera (8C-8A(IL1²⁵)) and heteromeric LRRC8A/LRRC8C (8A/8C) channels, to investigate the mechanism of action of three structurally distinct LRRC8 inhibitors: zafirlukast, pranlukast, and DCPIB. Molecular dynamics (MD) simulations identified putative zafirlukast binding sites in 8C-8A(IL1²⁵) comprising the amino (N)-terminal domain (NTD) and plasmalemmal inter-subunit fenestrae between transmembrane (TM) helices 1 and 2. Pranlukast and DCPIB also clusters in fenestrae albeit closer to the external pore of the channel. In support of the zafirlukast MD models, patch clamp analysis revealed that mutations in NTD, TM1, and TM2 alter 8C-8A(IL1²⁵) and 8A/8C sensitivity to zafirlukast. Surprisingly, the same mutations also enhanced 8C-8A(IL1²⁵) and 8A/8C sensitivity to pranlukast and DCPIB, suggesting a common mechanism of action. The correlation between voltage-dependent inactivation induced by mutations or low-pH and inhibitor sensitivity supports a model in which the drugs disrupt TM helix-lipid interactions and destabilize the conduction pore.

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Cell type-specific distribution of functional nicotinic receptors in mouse dorsal root ganglia

Authors

Jortan Tun - United States - University of Utah

Submission ID 47

Abstract

Sensory neurons express a diverse repertoire of ion channels and receptors, yet how individual receptors shape the function of specific cell types remains largely unknown. In the dorsal root ganglia (DRG), this challenge is amplified by the diversity of heterometric ion channel isoforms, each with distinct physiological roles. The nicotinic acetylcholine receptor (nAChR) family exemplifies this complexity, existing in multiple heteropentameric subtypes with unresolved cell type-specific functions.

To address that, we developed a functional cell type atlas of nAChR distribution in the mouse DRG using Constellation Pharmacology, a calcium-imaging-based profiling approach that integrates neurochemical markers with pharmacological responses to TRP channel agonists. Across the different spinal levels (thoraco-lumbar-sacral), we identified at least 19 functional phenotypes or cell types, mapped them to known transcriptomic classes, and resolved the selective expression of five receptor subtypes ($\alpha 6$, $\alpha 3\beta 4^*$, $\alpha 7^*$, $\alpha 4\beta 2^*$, and mAChR). Some subtypes displayed striking sexual dimorphism, revealing important molecular differences between the male and female sensory systems.

These findings establish a functional map of nAChR subtypes in sensory neurons and provide a broadly applicable framework for dissecting ion channel and receptor function across different tissues.

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Evaluating zebrafish Ano1 function

Authors

Tara Sweet - United States - SUNY Geneseo

Submission ID 48

Abstract

Ano1 is a calcium-activated chloride channel that plays a critical role in various physiological functions, including chloride and fluid secretion in lung and intestinal epithelial cells (Schroeder et al., 2008, *Cell*, 134(6):1019-29). We are interested in studying the physiological role of Ano1 in zebrafish. As a first step, we investigated the molecular function of zebrafish Ano1. We cloned the zebrafish Ano1 gene, expressed it in a mammalian system, and assessed its halide transport function using a colorimetric iodine flux assay (Tang and Wildey, 2004, *J Biomol Screen*, 9(7):607-13). Our results show that zebrafish Ano1 functions as a calcium-activated anion channel. This work sets the stage for future studies on the physiological contributions of Ano1 in zebrafish.

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Ca²⁺ and cAMP signaling cross-talks at membrane contact sites regulates epithelial HCO₃⁻ transporters CFTR and NBCe1-B defined by extended synaptotagmin 3

Authors

Paramita Sarkar - United States - Epithelial signaling & Transport Section, NIDCR, NIH, Bethesda MD

Submission ID 49

Abstract

Endoplasmic reticulum (ER)-plasma membrane (PM) contact sites (MCS) are the location of the Ca²⁺ and cAMP signaling complexes. MCS, including the ER-PM junctions, are assembled by tether proteins, among which are the Extended Synaptotagmins (E-Syts), a family of lipid transfer proteins (LTP) that play a pivotal role in mediating intermembrane communications and lipid transfer. The lipid requirement for assembly and transduction by the cAMP and Ca²⁺ signaling pathways that regulate numerous physiologic functions remain elusive. The present study focuses on the role of E-Syt3 in Ca²⁺ and cAMP signaling. E-Syt-3 is a potent inhibitor of the luminal cAMP-activated Cl⁻ channel CFTR and the basolateral NBCe1-B. Here, we report that stimulation of the Ca²⁺ mobilizing muscarinic receptors by carbachol (Cch) relieves the inhibition of the transporters by E-syt3 to promote interaction and activation of CFTR by the cAMP/PKA signaling complex and of NBCe1-B by IRBIT. Reactivated Na⁺-HCO₃⁻ cotransporter NBCe1-B at a phosphatidylserine (PtdSer) nanodomain at the ER/PM junction. The mechanism of relieve of the constitutive inhibition during the HCO₃⁻ secretory state is not known to relieve of the inhibition requires both Ca²⁺ influx by the specific Ca²⁺ influx channel Orai1 and hydrolysis of PI(4,5)P₂. Accordingly, mutating the E-Syt3 Ca²⁺ bind site in the E-Syt3 C2C domain prevent inhibition of the transporters by E-Syt3. These findings revealed an unexpected mutual regulation of the transporters by activating cAMP/PKA signaling pathway and IRBIT and by the Ca²⁺ signaling pathway that controls access of the cAMP/PKA pathway and IRBIT to their substrates.

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Light-sensitivity in squid chromatophores: A functional study

Authors

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Submission ID 50

Abstract

In coleoid cephalopods skin coloration changes when differently pigmented chromatophores expand and contract under efferent neural control, partly directed by the visual system. Observations in octopus, however, also indicate distributed photosensitivity in the skin itself, a claim supported by the detection of mRNA transcripts coding for the same opsin that underlies vision, which expresses in certain dermal sensory neurons. Opsin transcripts were also found in the skin of cuttlefish and squid; in the latter, the protein and its downstream effector, G_q , express widely in dermal tissue, including the cells of the chromatophore. However, physiological evidence of photoresponsiveness, and clues of possible mechanistic underpinnings are lacking. We demonstrated light-triggered constriction and expansion of different subgroups of chromatophores in patches of excised mantle of *D. pelaeii*. To ascertain whether such function depends on local circuits or is intrinsic to the chromatophores, we isolated them by enzymatic and mechanical dissociation of the skin. Stimulation with elevated $[K^+]_o$ or L-glutamate induced radial muscle fiber contraction accompanied by an increase in $[Ca^{2+}]_i$, as reported by fluorescence imaging, confirming that isolated chromatophores remain viable. Video microscopy demonstrated that in quiescent chromatophores viewed with inert deep-red light, sudden epi-illumination with blue light triggered radial muscle contraction and $[Ca^{2+}]_i$ transients. The opsin thought to mediate such capability belongs to the R class; using squid-specific antibodies and confocal microscopy, we immuno-detected the key enzyme of this signaling cascade, PLC β , in the various cell types of the chromatophore. To ascertain its functionality, we showed that the pharmacological activator m-3M3FDC potently elevated $[Ca^{2+}]_i$, an effect largely restricted to the radial muscle fibers that control the expansion of the pigment sac. The results support the notion of autonomous photoresponsiveness of squid chromatophores, which complements the descending control from the CNS and may be implicated in camouflage. HERMES project Nos. 65708, 63827.

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Multiple cAMP/PKA complexes at the STIM1 ER/PM junction, specified by E-Syt1 and E-Syt2, reciprocally gates ANO1 (TMEM16A) via Ca²⁺

Authors

Wei-Yin Lin - United States - The Epithelial Signaling and Transport Section and The National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD

Submission ID 51

Abstract

Anoctamin 1 (TMEM16A, ANO1) is the decision-making Ca²⁺-activated Cl⁻ channel that determines numerous physiological functions, including neuronal, muscle, and epithelial functions. Besides being gated by Ca²⁺, how the channel is regulated is poorly understood. Here, we discovered and characterized a fundamental novel molecular mechanism that dynamically regulates ANO1 surface expression and gating by Ca²⁺, orchestrated by the cAMP/PKA pathway at the STIM1 ER/PM junctions. ANO1 and the scaffold IRBIT (IP₃ receptors binding protein released with IP₃) possess FFAT motifs that interact with the ER localized VAPA (VAMP-associated protein A), directing them to the ER/PM junctions. At the junctions, STIM1 assembles (Adenylyl cyclases) ACs-AKAP-PKA complexes and mediates their interaction with the ANO1-VAPA-IRBIT complexes. The function of the complexes is then determined by the associated E-Syts. E-Syt1 forms the complex ANO1-VAPA-IRBIT-E-Syt1-AC8-AKAP5-PKA, leading to the phosphorylation of ANO1 at S673, which increases ANO1 surface expression and Ca²⁺ affinity. In the complex, the Ca²⁺ and cAMP signaling pathways synergize to markedly enhance ANO1 function. By contrast, E-Syt2 dissociates the ANO1-VAPA interaction, forming an alternate complex of ANO1-IRBIT-E-Syt2-AC6-AKAP11-PKA that phosphorylates ANO1 at S221, markedly reducing both ANO1 surface expression and Ca²⁺ affinity. Accordingly, deletion of IRBIT in mice profoundly impairs activation of ANO1 in parotid acinar cell that is essential for fluid and electrolyte secretion. The present findings, while revealing a new function of STIM1 in the assembly and transduction of the cAMP signaling pathway, elucidate a novel fundamental mechanism regulating ANO1 surface expression and Ca²⁺ gating. These findings should have significant implications and broadly impact the function of multiple tissues.

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TMEM16A channels in capillary thin-strand pericytes control brain blood flow

Authors

Liuruimin Xiang - United States - University of Maryland Baltimore

Submission ID 54

Abstract

Thin-strand pericytes cover the deepest reaches of the brain's capillary network, and their fine processes are intimately associated with adjacent endothelial cells. Recent work has revealed that these pericytes contribute to electrical signaling throughout the brain's vasculature by generating hyperpolarizing signals that can be transmitted over long distances to relax remote ensheathing pericytes around proximal branches of the capillary bed and smooth muscle cells around arterioles, eliciting blood flow increases. Resetting hyperpolarizing events in thin-strand pericytes must involve the engagement of ion channels that carry depolarizing currents, but the channels that mediate such conductances are unknown. Here, we reveal that thin-strand pericytes of the mouse cortex express functional Ca^{2+} -activated Cl^- channels (CaCCs) encoded by the *Ano1* gene, and we demonstrate that these are controlled by endoplasmic reticulum Ca^{2+} release through ryanodine receptors (RyRs) and IP3 receptors (IP3Rs), and Ca^{2+} entry via L- and T-type voltage-dependent Ca^{2+} channels (VDCCs). T-type VDCC Ca^{2+} entry is amplified via RyRs and IP3Rs, whereas L-type VDCC Ca^{2+} entry activates CaCCs directly. Activation of CaCCs will drive the pericyte membrane potential toward the Cl^- equilibrium potential which plays a key role in counterbalancing K^+ channel mediated hyperpolarizing signaling and shaping capillary blood flow in the brain.

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Volume-regulated anion channel blockers alter intracellular calcium signaling

Authors

Sara T Granados - United States - Department of Biochemistry & Molecular Biology, The University of Chicago, Chicago, IL

Submission ID 56

Abstract

Volume-regulated anion channels (VRACs) are composed of LRRC8 proteins found in vertebrate cells. Under hypotonic conditions, VRACs activate to efflux chloride and other osmolytes to recover cell volume. Pharmacological inhibitors such as DCPIB and dicumarol are being used to assess the physiological roles of VRACs in various cell types, including immune cells. DCPIB is a potent VRAC inhibitor commonly used as a standard control to abolish VRAC currents (I_{VRAC}). Here, we demonstrate that VRAC inhibitors not only abolish I_{VRAC} but also interfere with Store-Operated Ca^{2+} Entry (SOCE). While DCPIB and dicumarol deplete intracellular Ca^{2+} stores, this depletion is insufficient to trigger SOCE. On the other hand, endoplasmic reticulum (ER) Ca^{2+} store depletion via thapsigargin or ionomycin activates SOCE, but administration of DCPIB or dicumarol prior to SOCE significantly inhibits Ca^{2+} influx. Mechanistically, we found that these VRAC blockers lead to SOCE inhibition due to depolarization of the mitochondrial membrane potential, and disruption in the interaction between STIM1 and ORAI1 Ca^{2+} -channels, independent of VRAC expression. Notably, the effects of DCPIB and dicumarol are dependent on their ability to permeate the plasma membrane, as albumin in serum completely negate these effects. Interestingly, murine-activated T-cells treated with DCPIB or dicumarol in serum-containing media did not show the expected inhibitory effects observed in buffer, strongly suggesting that the phenotypic changes caused by these drugs may stem from their interactions with intracellular targets. In summary, our results demonstrate that despite being effective VRACs inhibitors in serum-free buffer, both DCPIB and dicumarol exert multiple off-target effects that are VRAC-independent. These effects may not be apparent in serum-containing culture conditions, which could lead to misleading conclusions. We strongly recommend the cautious use of DCPIB and dicumarol and suggest the identification of novel VRAC inhibitors that more accurately reflect the specific role of these channels in physiological settings.

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TMEM16A expression directly modulates the actin cytoskeleton in HEK-293 cells

Authors

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Submission ID 57

Abstract

The calcium-activated chloride channel TMEM16A is expressed in several tissues and has a role in diverse physiological processes, including epithelial secretion, smooth muscle contraction, and cell migration (Arreola et al. 2024. *Cell Calcium*. 121:102891-102905). Scaffolding proteins ezrin, radixin and moesin (ERM) link TMEM16A to the actin cytoskeleton, and together with the cytoskeleton regulate TMEM16A activity (Perez-Cornejo et al. 2012. *Proc. Natl. Acad. Sci. U S A*. 109:10376-10381; Ohshiro et al. 2014. *J. Pharmacol. Sci.* 125: 107-111). Alternatively, it has been shown that chloride transport by TMEM16A is needed for ciliogenesis (Ruppersburg and Hartzell. 2014. *Mol. Biol. Cell*. 25: 1793-1807), and actin polymerization during sperm capacitation by regulating the activity of ERK1/2 and RhoA (Roa-Espitia et al. 2025. *Int. J. Mol. Sci.* 26: 3750-3767). These data suggest that TMEM16A interacts with structural and signaling proteins to organize microtubules and microfilaments. Thus, we investigated whether TMEM16A expression in HEK293 cells directly influences the actin cytoskeleton. HEK293 cells were either transiently transfected with murine TMEM16A(ac) or stably transfected with the TMEM16A(ac)-3XFlag cDNA. Using co-immunoprecipitation (Co-IP), western blot, migration and adhesion assays, pull-down and fluorescence microscopy experiments, we investigated RhoA activation and actin polymerization. Our Co-IP assay revealed a direct interaction between RhoA and TMEM16A in unstimulated cells. Although cells expressing TMEM16A had more active RhoA than non-transfected cells, signaling through the RhoA-ROCK-MLC pathway was not enhanced. When we evaluated the S1P-induced migration, we found no difference between transfected and non-transfected cells, indicating that TMEM16A did not enhance actomyosin-contraction and motility. Instead, we discovered that TMEM16A favoured cell adhesion and increased the amount of F-actin in cells. Hence our data indicate that TMEM16A expression induced changes in RhoA activation that led to actin polymerization and increased cell adhesion, independently of chloride flux. Supported by research grants from COPOCYT and SECIHTI.

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Activation of TMEM16A/Anoctamin1 through intracellular solution exchange using automated patch lamp systems

Authors

Irene Lu - United States - Nanion Technologies

Submission ID 58

Abstract

TMEM16A/Anoctamin1 (Ano1) is a calcium (Ca^{2+})-activated chloride channel (CACC) which has a broad functional spectrum in processes including trans-epithelial ion transport, olfaction, photo-transduction, smooth muscle contraction, nociception, cell proliferation and control of neuronal excitability. The ion channel has been implicated in several diseases and may serve as a therapeutic target in cystic fibrosis, asthma, pain and certain cancers.

TMEM16A/Ano1 is activated by elevated cytosolic Ca^{2+} concentrations. To initiate channel activity in a heterologous cell environment, cytosolic Ca^{2+} can be introduced through different methods, for example, addition of Ca^{2+} in the intracellular solution or intracellular solution exchange from zero Ca^{2+} to Ca^{2+} -containing solutions. These two methods pose their own challenges. Intracellular solution exchange is a highly technical challenging process in conventional patch clamp method and is not available on all automated patch clamp platforms. Automated patch clamp technology often uses fluoride in the intracellular solution to facilitate the sealing between cell membrane and patch substrate. The strong tendency of fluoride precipitates with Ca^{2+} makes it impossible to provide a precise Ca^{2+} concentration for channel activation.

Here, we demonstrate two assays for TMEM16A/Ano1 using fluoride-free intracellular solution and internal perfusion feature across Nanion's automated patch clamp platforms: SyncroPatch 384 PE, Patchliner, and Port-a-Patch. The results show that the intracellular solution can be exchanged in a robust manner to activate TMEM16A/Ano1, providing a reliable approach for pharmacological studies.

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ClC-1 inhibitor compound (NMD670) enhances neuromuscular transmission and restores skeletal muscle function in a rat model of myasthenia gravis

Authors

Michael Voldsgaard Clausen - Denmark - NMD Pharma

Submission ID 59

Abstract

The ClC-1 chloride channel is abundantly expressed in skeletal muscle fibers, where it stabilizes resting membrane potential and accounts for ~80% of resting membrane conductance (G_m). By dampening muscle fiber excitability, the ClC-1 channel plays a key role in maintaining the balance between excitatory and inhibitory currents and thus reliable generation of muscle action potentials. Inhibition of ClC-1 channels prolongs the duration and increases the amplitude of endplate potentials, suggesting that ClC-1 blockade could enhance neuromuscular transmission in disease states with transmission failure, such as myasthenia gravis (MG).

Here, we evaluated the effect of ClC-1 inhibition with the clinical candidate NMD670 in a rat MG model with compromised neuromuscular transmission. Administration of a ClC-1 inhibitor (NMD670) reduced chloride conductance, increased endplate potential amplitude and duration, and significantly improved compound muscle action potential and evoked muscle force. Grip strength and survival were also significantly improved with NMD670. These findings demonstrate that ClC-1 inhibition restores synaptic transmission and muscle contractility, providing a potential therapeutic approach to improve muscle strength and mobility in neuromuscular disorders characterized by transmission failure.

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G-protein coupled receptors regulate mouse urinary bladder phasic smooth muscle contractions, potentially through potassium channels and calcium-activated chloride channels

Authors

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Submission ID 60

Abstract

Detrusor smooth muscle (DSM) of the bladder exhibits intrinsic Ca^{2+} activity that propagates in waves to elicit phasic non-voiding contractions. Phasic contractions produce fluctuations in bladder pressure, called transient pressure events (TPE), and are associated with bursts of afferent nerve activity to the central nervous system that signal filling status (Heppner. 2016. J Gen Physiol.4:323-35). G protein-coupled receptors (GPCR) regulate bladder contraction and relaxation. This study investigates the role of GqPCR on TPE in isolated mouse urinary bladder. We hypothesized GqPCR inhibition reduces Ca^{2+} activity and TPE frequency and amplitude. Urinary bladders were isolated from male and female mice, attached to a syringe pump and inline pressure transducer, and partially filled to induce TPE. Inhibition of GqPCR signaling with YM-254890 (500nM) decreased mean TPE frequency (88%, $p=0.001$), amplitude (95%, $p=0.035$), and intravesical pressure (86%, $p=0.023$) ($n=8$ bladders). Application of the PKC activator, Phorbol 12, 13-dibutyrate (PDBu 100nM), rescued intravesical pressure ($p=0.227$) and produced a modest return in TPE amplitude ($p=0.132$) but not frequency ($p=0.010$) compared to baseline ($n=7$ bladders). Measuring DSM membrane potential with sharp electrodes, YM-254890 appears to elicit a hyperpolarization, while subsequent application of PDBu results in depolarization ($n=1$ cell). This suggests intrinsic GqPCR activity mediates membrane potential in part through PKC activity, likely by inhibiting potassium channels. More experiments are required to confirm this result. Furthermore, the presumptive calcium-activated chloride channel (CaCC) inhibitor Ani9 (5mM) reduces TPE frequency (43%, $p=0.079$), but not amplitude (0%, $p=0.934$) or intravesical pressure (14%, $p=0.314$) ($n=2$ bladders). This suggests intrinsic GqPCR activity and IP_3 mediated Ca^{2+} release may activate CaCC, producing the initial depolarization required to activate L-type Ca^{2+} channels and DSM contraction. Due to off-target effects of many CaCC activators/inactivators, mainly modulating intracellular Ca^{2+} release, future experiments will be conducted with an inducible smooth muscle specific CaCC (TMEM16a) knockout mouse model.

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