Temperature and Voltage Coupling to TRPM8 Channel Opening

CONFERENCE PAPER *in* BIOPHYSICAL JOURNAL · FEBRUARY 2015

Impact Factor: 3.97 · DOI: 10.1016/j.bpj.2014.11.233

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178-Plat
High Temperature Sensitivity is Intrinsic to Voltage-Gated Potassium Channels
Fan Yang, Jie Zheng.
University of California, Davis, Davis, CA, USA.
Temperature-sensitive transient receptor potential (TRP) ion channels are members of the large tetrameric cation channels superfamily but are considered to be uniquely sensitive to heat, which has been presumed to be due to the existence of an unidentified temperature-sensing domain. We find that the homologous voltage-gated potassium (Kv) channels also exhibit high temperature sensitivity comparable to that of TRPV1, which is detectable under specific conditions when the voltage sensor is functionally decoupled from the activation gate through either intrinsic mechanisms or mutations. Interestingly, mutations could tune Shaker channel to be either heat-activated or heat-deactivated. Therefore, high temperature sensitivity is intrinsic to both TRP and Kv channels. Our findings suggest important physiological roles of heat-induced variation in Kv channel activities. Mechanistically our findings indicate that temperature-sensing TRP channels may not contain a specialized heat-sensor domain; instead, non-obligatory allosteric gating permits the intrinsic heat sensitivity to drive channel activation, allowing temperature-sensitive TRP channels to function as polymodal nociceptors.

179-Plat
Permeation and Dynamics of an Open-Activated TRPV1 Channel
Carmen Domene[1], Leonardo Darre[1], Simone Furini[2].
[1]Chemistry, King's College London, London, United Kingdom,
[2]Department of Medical Biotechnologies, University of Siena, Siena, Italy.
Transient receptor potential (TRP) ion channels compose a large and diverse protein family, found in yeast and widespread in the animal kingdom. TRP channels work as sensors for a wide range of cellular and environmental signals. Understanding how these channels respond to physical and chemical stimuli has been hindered by the limited structural information available until now. The three-dimensional structure of the vanilloid receptor 1 (TRPV1) was recently determined by single particle electron cryo-microscopy, offering for the first time the opportunity to explore ionic conduction in TRP channels at atomic detail. In this study, we present molecular dynamics simulations of the open-activated pore-domain of TRPV1 in the presence of three cationic species: Na^+, $Ca2^+$ and K^+. The dynamics of these ions while interacting with the channel pore allowed us to rationalize their permeation mechanism in terms of a pathway involving three binding sites at the intracellular cavity, and the extracellular and intracellular entrance of the selectivity filter. Furthermore, conformational analysis of the pore in the presence of these ions reveals specific ion-mediated structural changes in the selectivity filter, which influences the permeability properties of the TRPV1 channel.

180-Plat
The L596-W733 Bond between S4-S5 Linker and TRP Domain Maintains Basal Activity and Enables Inactivation of TRPV4
Jinfeng Teng[1], Stephen Loukin[1], Andriy Anishkin[2], Ching Kung[1,3].
[1]Laboratory of Molecular Biology, University of Wisconsin, Madison, WI, USA, [2]Department of Biology, University of Maryland, College Park, MD, USA, [3]Department of Genetics, University of Wisconsin, Madison, WI, USA.
Unlike other cation channels, all TRP (Transient Receptor Potential) channel subunits have a TRP-domain helix immediately trailing S6 that bears the gate. The role(s) of TRP-domain helix is unclear. Recent cryo-EM TRPV1 structures revealed that this helix forms a bond with the beginning of the S4-S5 linker. By homology modeling, we identified the corresponding L596-W733 bond in TRPV4 (Vanilloid type 4). L596P, likely a gain-of-function (GOF) mutation, causes bone-developmental blockage of the spondylometaphyseal dysplasia Kozlowski type (SMDK) in human. Our previous screen also isolated W733R as a GOF that suppresses grows of yeast expressing TRPV4. Here we show, when expressed in Xenopus oocytes, TRPV4 with L596P or W733R mutation displays normal depolarization-induced activation and outward rectification. However, as expected from their biological GOF phenotypes, these mutant channels indeed have higher basal open probabilities and limited responses to the strong agonist GSK1016790A. In addition, W733R current also fails to inactivate after activation during depolarization. Systematic substitutions of W733 with amino acids of different properties produce similar electrophysiological defects. The results can be consistently interpreted in the context of the homology model of TRPV4 that we have developed. Our results indicate that the TRP domain stabilizes various functional conformations by bonding to other structures, especially to the S4-S5 linker.

181-Plat
Comparative Sequence Analysis Suggests a Unified Gating Mechanism for TRP Channels
Vincenzo Carnevale[1], Eugene Palovcak[2], Lucie Delemotte[1], Michael Klein[1].
[1]Temple University, Philadelphia, PA, USA, [2]University of California San Francisco, San Francisco, CA, USA.
The transient receptor potential (TRP) channel superfamily plays a central role in the transduction of diverse stimuli in eukaryotes. Their transmebrane regions assemble in tetramer, similarly to voltage-gated potassium (Kv) channels. Given the degree of structural similarity between the two superfamilies, an intriguing question concerns the sequence determinants of such highly divergent activation mechanisms. To provide insight into this question and to investigate the fascinating hypothesis of a conserved allosteric activation mechanism shared amongst TRP channels, we have performed comparative sequence analysis on large, comprehensive ensembles of TRP and Kv channel sequences. We observe sequence features throughout the TRP channel TM core that are not shared with Kv channels. When interpreted in light of the recently resolved TRPV1 structures, our results suggest a novel, unified model of TRP channel gating.

182-Plat
Effects of Inactivation of TRPM7 Kinase Activity on its Channel Activity in Mice
Taku Kaitsuka[1], Chiaki Katagiri[2], Pavani Beesetty[3], Kenji Nakamura[4], Siham Hourani[3], Kazuhito Tomizawa[5], **J. Ashot Kozak**[3], Masayuki Matsushita[2].
[1]Dept. of Molecular Physiology, Kumamoto University, Kumamoto, Japan, [2]University of the Ryukyus, Okinawa, Japan, [3]Wright State University, Dayton, OH, USA, [4]Mitsubishi Kagaku Inst. of Life Sci., Tokyo, Japan, [5]Kumamoto University, Kumamoto, Japan.
Transient receptor potential (TRP) family channels are involved in sensory pathways and are activated by various environmental stimuli. Among the members of this family, TRPM7 is a unique fusion of an ion channel and a C-terminus kinase domain that is ubiquitously expressed. TRPM7 is a key membrane protein governing cellular $Mg2^+$ homeostasis in mammals since its channel pore is permeable to $Mg2^+$ ions and can act as a $Mg2^+$ influx pathway. Moreover, TRPM7 channel activity is inhibited by intracellular $Mg2^+$. Mechanistic links between its kinase activity and channel function have remained uncertain, partly due to embryonic lethality of TRPM7 gene deletion in mice. In this study, we generated kinase inactive knock-in mutant mice by mutagenesis of a key lysine residue involved in $Mg2^+$-ATP binding. K1646R mutant mice were normal in development and general locomotor activity. In peritoneal macrophages isolated from adult animals the basal activity of TRPM7 channels prior to cytoplasmic $Mg2^+$ depletion was significantly potentiated, while maximal current densities measured after $Mg2^+$ depletion were unchanged in the absence of detectable kinase function. The inhibition of TRPM7 channel currents by 300 uM intracellular $Mg2^+$ or spermine was similar in WT and K1646R macrophages. Serum total $Ca2^+$ and $Mg2^+$ levels were not significantly altered in kinase-dead mutant mice either. Our findings suggest that 1) abolishing TRPM7 kinase activity does not impair its channel activity, but rather, potentiates basal current magnitudes; 2) kinase activity is not essential for regulation of mammalian $Mg2^+$ homeostasis.

183-Plat
Temperature and Voltage Coupling to TRPM8 Channel Opening
Natalia Raddatz[1,2], **Juan P. Castillo**[1,2], Carlos Gonzalez[1], Osvaldo Alvarez[3], Ramon Latorre[1].
[1]CINV, Universidad de Valparaiso, Valparaiso, Chile, [2]equal contribution, Valparaiso, Chile, [3]Universidad de Chile, Santiago, Chile.
Expressed in somatosensory neurons of the dorsal root and trigeminal ganglion, the transient receptor potential melastatin 8 (TRPM8) channel is a Ca2+-permeable cation channel activated by cold, voltage, PIP2 and menthol. Although TRPM8 channel gating has been characterized at the single channel and macroscopic current levels, there is currently no consensus regarding the extent to which temperature and voltage sensors couple to the conduction gate. In the present study we extended the range of voltages at which the TRPM8-induced ionic currents were measured and made careful measurements of the maximum open probability the channel can attain at different temperatures by means of fluctuation analysis. The first direct measurements of TRPM8 channel temperature-driven conformational rearrangements provided here suggest that temperature alone is able to open the channel and that the opening reaction is voltage-independent. Voltage is a partial activator of TRPM8 channels, since absolute open probability values measured with fully activated voltage sensors are less than 1 and they decrease as temperature rises. By unveiling the fast temperature-dependent deactivation process, we show

that TRPM8 channel deactivation is well described by a double exponential time course. The fast and slow deactivation processes are temperature-dependent with enthalpy changes of 27.2 kcalmol-1 and 30.8 kcalmol-1. The overall Q10 for the closing reaction is about 33. A three-tiered allosteric model containing four voltage sensors and four temperature sensors can account for the complex deactivation kinetics and coupling between voltage and temperature sensor activation and channel opening.

184-Plat
TRPM8 is an Ionotropic Testosterone Receptor
Swapna Asuthkar[1], Lusine Demirkhanyan[1], Xiaohui Sun[1], Pia Elustondo[2], Vivek Krishnan[3], Padmamalini Baskaran[3], Kiran Kumar Velpula[1], Baskaran Thyagarajan[3], Evgeny Pavlov[4], **Eleonora Zakharian**[1].
[1]Cancer Biology & Pharmacology, University of Illinois, Peoria, IL, USA, [2]Physiology & Biophysics, Dalhousie University, Halifax, NS, Canada, [3]University of Wyoming, Laramie, WY, USA, [4]New York University, New York, NY, USA.
Testosterone is a key steroid hormone in the development of male reproductive tissues and in the regulation of the central nervous system. Rapid signaling mechanism induced by testosterone affects numerous behavioral traits, including sexual behavior, aggressiveness, or fear conditioning. However, currently identified testosterone receptor(s) are not believed to underlie the fast signaling. Here we report that an ion channel from the transient receptor potential family, TRPM8, commonly known as the cold and menthol receptor is the major component of testosterone-induced rapid actions.
TRPM8 is highly expressed in the prostate epithelial cells. Using an immunohistochemistry approach, we detected a colocalization pattern of TRPM8 with endogenous androgens on human prostate tissues obtained from healthy individuals and patients with prostate cancer. Co-immunoprecipitation experiments performed on cultured prostate epithelial cells, prostate cancer cells, and HEK-293 cells stably expressing TRPM8, further confirmed direct binding of testosterone to TRPM8.
Using cultured and primary cell lines and the purified TRPM8 protein we demonstrate that testosterone can directly activate TRPM8 channel at low picomolar range. Specifically, testosterone induced TRPM8 responses in primary human prostate cells, prostate cancer cells PC3, dorsal root ganglion (DRG) neurons, and hippocampus neurons. Picomolar concentrations of testosterone resulted in full openings of the purified TRPM8 channel in planar lipid bilayers. Furthermore, acute applications of testosterone on human skin elicited cooling sensation, implying testosterone-induced activation of TRPM8. Additionally, animal studies showed altered sniffing behavior and increased blood concentration of testosterone in the TRPM8 knockout mice, suggesting the existence of a feedback loop mechanism in the absence of the receptor. Our data demonstrate that testosterone is an endogenous and highly potent agonist of TRPM8, suggesting a role of TRPM8 channels well beyond their well-established function in somatosensory neurons. This discovery may further imply TRPM8 channel function in testosterone-dependent behavioral traits.

Platform: Protein Lipid Interactions I

185-Plat
Experimental and Computational Studies of Pulmonary Surfactant Protein SP-B Interacting with Lipid Bilayers
Mohammad Hassan Khatami, Ivan Saika-Voivod, Valerie Booth.
Memorial University of Newfounland, St. John's, NL, Canada.
Pulmonary lung surfactant protein B (SP-B) is a 79 residue hydrophobic protein, from the Saposin superfamily. Saposin super family proteins share common features including 3 intra-chain disulfide bonds and 4-5 helical regions. SP-B appears to carry out its essential functions in respiration by binding to and modifying the structures of phospholipid bilayers and monolayers at the air-water interface. Due to difficulties arising from SP-B's extreme hydrophobicity, the 3D structure of full SP-B is not yet known. Thus we are using computational methods in combination with solid-state NMR to investigate the structure of SP-B. Our approach is to generate candidate structures via computational methods, predict the 15N spectra for the computed configurations and then compare them to the experimental NMR spectra.
For the computational studies, we employ Mini-b, a construct of SP-B with known structure, and add the rest of residues to make a full length SP-B. We use several positions of SP-B in a POPC bilayer as initial structures. Calculations are carried out using GROMACS, with OPLS-aa, an all-atom force field, as well as with PACE, a hybrid model force field. Simulation methods include Molecular Dynamics (MD) and Replica Exchange Molecular Dynamics (REMD). For the experimental part, we use recombinantly expressed 15N labeled SP-B in mechanically oriented POPC bilayers to collect 1D 15N solid-state NMR spectra.

186-Plat
Creation of Water-Soluble Integral Membrane Proteins using an Engineered Amphipathic Protein "Shield"
Dario Mizrachi.
Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, USA.
Integral membrane proteins (IMPs) play crucial roles in all cells and represent attractive pharmacological targets. However, functional and structural studies of IMPs are hindered by their hydrophobic nature and the fact that they are generally unstable following extraction from their native membrane environment using detergents. Here, we devised a general strategy for in vivo solubilization of IMPs in a functional conformation without the need for detergents or mutations to the IMP itself. This technique, called SIMPLEx (solubilization of IMPs with high levels of expression), involves creating fusions between an IMP target and truncated apolipoprotein A-I, which serves as an amphipathic proteic "shield" that sequesters the IMP from water and promotes its solubilization.

187-Plat
Validating the Retinal Flip of Rhodopsin using Molecular Dynamics
Jun Feng, **Blake Mertz**.
Chemistry, West Virginia University, Morgantown, WV, USA.
Rhodopsin, the mammalian dim light photoreceptor, is the most well-characterized structural model of a G protein-coupled receptor (GPCR). Photoisomerization of its covalently bound chromophore, retinal, triggers rhodopsin activation. Spectroscopic studies of rhodopsin in the dark [1] and Meta I [2] states have definitively shown that the C9- and C13-methyl groups of retinal are oriented towards the extracellular side of the protein. However, the structures of the active Meta II state [3] and a constitutively active triple mutant [4] had a 180° rotation along the long-axis of the retinal polyene chain, leading to an orientation of the C9- and C13-methyl groups towards the cytoplasmic side of the protein. The biophysical significance of this potential flip and its role in the structural transition during activation is still unknown. We employed molecular dynamics simulations to determine the role of the retinal flip in rhodopsin activation. Rhodopsin was modeled starting with the Meta II crystal structure but in the Meta I protonation state, to favor a deactivation transition. Surprisingly, two of our four simulations produce a reverse flip of the polyene chain on the microsecond timescale. This flip is accompanied by the rotation of the Trp265 side chain, which is implicated in a "transmission switch" common to GPCR activation. A decrease of water within the retinal binding pocket is also observed, along with distinct protein hydration features concurrent with the flipping of retinal. These results provide a bridge between spectroscopic and crystallographic studies, showing that it is possible for a retinal flip to occur from Meta I to Meta II state. [1] Salgado (2004) *Biochemistry* 43:12819; [2] Salgado (2006) *JACS* 128:11067; [3] Choe (2011) *Nature* 471:651; [4] Deupi (2012) *PNAS* 109:119.

188-Plat
HIV gp41-Antibody Interaction at the Viral Membrane Interface Defined by EPR Spectroscopy
Likai Song[1], Zhen-Yu J. Sun[2], Mikyung Kim[2,3], Pavanjeet Kaur[1], Gerhard Wagner[2], Ellis L. Reinherz[2,3].
[1]National High Magnetic Field Laboratory and Florida State University, Tallahassee, FL, USA, [2]Harvard Medical School, Boston, MA, USA, [3]Dana-Farber Cancer Institute, Boston, MA, USA.
HIV enters human T cells through the fusion of viral and host-cell membranes. This fusion process is mediated by a surface protein, gp41, and the platform provided by the cholesterol-rich viral membrane. The membrane proximal ectodomain region (MPER) of gp41 plays a critical role in this fusion process and is a major target of anti-gp41 antibodies and vaccine design. Here, EPR and NMR techniques were used to define MPER structure on the membrane, and how neutralizing anti-gp41 antibodies recognize their membrane-immersed epitopes and disrupt a hinge-related function of the MPER. The analyses of several HIV-1 clade B and clade C MPERs revealed a structurally conserved pair of helices immersed in the viral membrane separated by a flexible hinge, which include critical helix capping residues. Double alanine mutations of the capping residues result in an altered hinge structure with a deeper lipid-buried MPER middle region, as well as