receptor X were transfected into CHO cells. The CHO transfectants were characterized by FACS and then scaled up for KinExA binding studies. KinExA has been used to measure binding affinity of Adnectin-A to the cell surface expressed receptor X to measure the effect of avidity of the multivalent adnectin binding to receptor clusters. As controls for the functional activity of the Adnectin-A and the affinity of the monovalent interaction, the same KinExa assay was used, substituting the soluble receptor X extracellular domain for the transfected CHO cells. The binding avidity measured by KinExA for CHO expressed receptor is 14 pM for both species of receptor X. However, the affinity of Adnectin-A for monovalent soluble Receptor X was quite different between the species suggesting that avidity due to receptor clustering equilizes the functional avidity at the cell surface.

Platform: Voltage-gated K Channels: Activation/ Inactivation Mechanisms

92-Plat

A-Type Kv4 Channel Closed-State Inactivation is Modulated by the Tetramerization Domain Interacting with Auxilary KChIP4a

Yi-Quan Tang[1], Fan Yang[2], Jingheng Zhou[1], Jie Zheng[2], **KeWei Wang**[1].
[1]Peking University, Beijing, China, [2]University of California at Davis, Davis, CA, USA.

A-type Kv4 potassium channels undergo a conformational change towards a non-conductive state at negative membrane potentials, a dynamic process known as closed-state inactivation (CSI). CSI causes inhibition of channel activity without prerequisite of channel opening, thus providing a dynamic regulation of neuronal excitability, dendritic signal integration and synaptic plasticity. However, the structural determinants underlying Kv4 CSI remain largely unknown. We have recently demonstrated that auxiliary KChIP4a subunit contains an N-terminal Kv4 inhibitory domain (KID) that directly interacts with Kv4.3 channels to enhance CSI. In this study, we utilized the FRET two-hybrid mapping and BiFC-based screening combined with electrophysiology, and identified the intracellular tetramerization (T1) domain that functions to suppress CSI and serves as a receptor for the binding of KID. Disrupting Kv4.3 T1-T1 interaction by mutating C110A within the C3H1 motif of T1 domain facilitated CSI, and ablated the KID-mediated enhancement of CSI. Furthermore, replacing the characteristic C3H1 motif of Kv4.3 T1 domain with the T1 domain from Kv1.4 without the C3H1 motif or Kv2.1 with the C3H1 motif resulted in channels functioning with enhanced or suppressed CSI, respectively. Taken together, our findings reveal a novel role of the T1 domain in suppressing Kv4 CSI with the C3H1 motif functioning to stabilize the channel activation gate; and KChIP4a KID directly interacts with the T1 domain to relieve the stabilization, leading to facilitation of CSI and inhibition of channel function.

93-Plat

Two-in-One: Activation and Inactivation at the Intracellular Gate of a Kv Channel

Manuel Covarrubias[1], Jeffrey D. Fineberg[2].
[1]Neuroscience, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA, USA, [2]Physiology and Molecular Biophysics, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA, USA.

N-type and P/C-type inactivation are firmly established mechanisms of inactivation in voltage-gated K^+ (Kv channels). However, Kv4.x channel complexes, which undergo fast preferential closed-state inactivation (CSI; Fineberg et al., 2012, *JGP* 140.5:513-527), appear to use a distinct but unknown inactivation mechanism. Previously, we hypothesized that a weak interaction between the voltage sensing domain and the intracellular activation gate underlies CSI (Bähring & Covarrubias, 2011, J Physiol 589:461-79). Thus, CSI is essentially governed by the intracellular activation gate, which fails to open and adopts an inactivated conformation. To directly test this hypothesis, we investigated the heterologously expressed Kv4.1 ternary channel complex including accessory subunits KChIP1 and DPP6, and exploited the ''trap-door'' paradigm of the activation gate. The results show that Kv4.1 inactivation traps intracellularly applied quaternary ammonium blockers (bTBuA and TBuA) inside the channel's pore. The trapped blockers can only escape if the channels are opened again by subsequent depolarizations. By contrast, inactivation cannot trap TEA, whose binding kinetics is faster than that of channel gating. Moreover, under identical conditions, a Shaker Kv channel (ShB-T449K) known to exhibit fast P/C-type inactivation cannot trap bTBuA. These findings conclusively suggest that the intracellular activation gate of the Kv4.1 ternary channel complex plays a novel dual role, controlling both activation and inactivation. Supported in part by NIH grant R01 NS032337 (MC).

94-Plat

Development and Validation Studies of Universal Pharmacophore Models for hERG Channel Openers

Serdar Durdagi[1,2], Matthew Patterson[2], Sergei Y. Noskov[2].
[1]Department of Biophysics, Bahcesehir University, Faculty of Medicine, Istanbul, Turkey, [2]Biological Sciences, Institute for Biocomplexity and Informatics, University of Calgary, Calgary, AB, Canada.

The intra-cavitary drug blockade of hERG channel, a common off-target for many drugs, have been extensively studied both experimentally and theoretically. Structurally diverse ligands inadvertent blockade of rapid component of delayed rectifying K^+ currents are potentially pro-arrhythmic and may lead to drug-induced long QT syndrome-LQTS. There are a number of natural strategies for rational drug design; one dubbed the ''passive'' approach avoids block of hERG1 whereas the ''proactive'' strategy designs treatments to activate the channel. While ''passive'' approach has been developed for decades, studies of structural mechanisms of hERG channel activation by small molecules are truly novel. Accordingly, design of the hERG openers or current activators may offer a momentum for modern anti-arrhythmia drug development. Significant number of small molecules with capacity for hERG activation was identified in mandatory hERG screens. To establish possible correlation between activators structure and reactivity, we attempted to construct a universal pharmacophore model for hERG channel openers using PHASE protocol. The biochemical data on 38 K^+ channel activators are used in training and test sets. These compounds span a wide range of structurally different chemotypes with ~10^5-fold variances in binding affinity, which is sufficient for statistically sound model. A developed five sites AAHHR (A, hydrogen-bond accepting, H, hydrophobic, R, aromatic) pharmacophore model has showed reasonable high statistical results compared to other constructed models and was selected for steric and electrostatic contour maps analysis. The predictive power of the model was also tested with 6 external test-set (as true unknowns) compounds. Pharmacophore model is also combined with previously developed receptor-based homology model of hERG K channel and novel activators are generated and screened. The developed ligand-based models may serve as a basis for the synthesis of novel potential therapeutic hERG activators.

95-Plat

N-Terminal Regulation of hERG1 K^+ Channel Deactivation

Steven J. Thomson, Angela Hansen, Michael C. Sanguinetti.
Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, UT, USA.

Slow deactivation of hERG1 (Kv 11.1) potassium channels maintains I_{Kr} during final repolarization of the cardiac action potential and opposes asynchronous early depolarization. Inherited point mutations in hERG1 that accelerate deactivation of I_{Kr} cause long QT syndrome (LQTS), a disorder of ventricular repolarization that increases the risk of lethal cardiac arrhythmia.

The intracellular N-terminal domain of hERG1 is known to be essential for slow deactivation. Deletion of the entire (~350 residues) or just the initial 16 residues of the N-terminus accelerates deactivation 10-fold. The same effect is achieved by neutralization of the charged residues, Arg4 or Arg5. How many of the 4 N-termini are required to slow channel deactivation is unknown. hERG1, like other Kv channels, is a homotetramer. By repeatedly linking the C-terminal of one subunit to the N-terminal of the next subunit we constructed concatenated hERG1 tetramers. A variety of homomeric and heteromeric concatenated tetramers were characterized (i.e., WTn/R4A:R5A(4-n); where n = 1 to 4). The concatenated channel containing a single R4A/R5A subunit and 3 wild-type subunits deactivated as fast as the concatenated channel containing only R4A/R5A subunits. The LQTS-associated mutation R56Q, located in the N-terminal of hERG1 was also studied. Again, a concatenated tetramer containing a single mutant subunit deactivated as fast as channels with R56Q mutations in all four subunits. Our results show that all 4 N-termini are required to mediate slow deactivation in wild-type hERG1 channels.

96-Plat

Lipid Affinity to the Voltage-Gated Potassium Channel KvAP

Elise Faure[1], Christine Thompson[2], Rikard Blunck[1,2].
[1]Physiology, Université de Montréal, GÉPROM, Montréal, QC, Canada, [2]Physique, Université de Montréal, GÉPROM, Montréal, QC, Canada.

Voltage-gated potassium channels (KV) are formed by a central conducting pore surrounded by four voltage sensor domains. Functional studies have revealed that biophysical properties of lipid molecules in the channels environment can have strong effects on the activity of KV channels. Here, we investigated the influence of different lipids as well as their affinity to KvAP channels. We carried out electrophysiology measurements by fusing vesicles containing purified channels into planar lipid bilayers with varied lipid compositions. We found that KvAP properties are mainly determined by the lipid