uncoupling between the S4S5 linker and the S6 gate. We used two different chimeric approaches to further study the structural determinants of Kv4 channel closed-state inactivation: First, chimeric swapping of S4S5 linker and distal S6 sequences between N-terminally truncated Kv4.2*delta*2-40 A-type channels and non-inactivating *Shaker*IR channels; Second, chimeric insertion of the Kv4.3 cytoplasmic C-terminus or the Kv4.3 T1S1 linker in Kv4.1. The first approach was pursued to possibly prevent inactivation in Kv4.2*delta*2-40 or introduce inactivation in *Shaker*IR. The second approach was pursued to possibly transfer slow Kv4.3 inactivation kinetics to Kv4.1. By two-electrode voltage-clamp on cRNA-injected *Xenopus* oocytes and kinetic analysis of the recorded currents we found that *Shaker* sequences slowed Kv4.2*delta*2-40 inactivation, and that Kv4.2 sequences introduced a novel form of inactivation in *Shaker*IR. Furthermore, we found that, rather than Kv4.3 C-terminal sequences, chimeric introduction of Kv4.3 T1S1 linker sequences made Kv4.1 channels inactivate slower. Our data confirm a model of temporary uncoubling between S4S5 and S6 as a mechanism involved in closed-state inactivation. Furthermore, our data suggest that the T1S1 linker region plays a role in closed-state inactivation.

2704-Pos

Differential Integration of DPLP Protein Variants Regulates Inactivation Kinetics of Neuronal A-Type Current

Henry H. Jerng, Paul J. Pfaffinger.
Baylor College of Medicine, Houston, TX, USA.

Voltage-sensitive potassium channel complexes consisting of Kv4 pore-forming subunits and modulatory subunits mediate the neuronal subthreshold A-type current (I_{SA}), a regulator of membrane excitability and action potential firing patterns. Both the KChIP (Kv channel-interacting proteins) and DPLP (dipeptidyl peptidase-like protein) modulatory subunits are expressed as N-terminal variants, and two DPLP variants (DPP10a, DPP6a) possess the unusual property of inducing similar fast inactivation kinetics of Kv4 channels in reconstitution studies. To investigate whether their effects are similar on native I_{SA}, we characterized I_{SA} from DPP10a-expressing cortical layer II/III pyramidal neurons (CtxPN) and DPP6a-expressing cerebellar granule neurons (CbGN) in rat brain slices. Surprisingly, CtxPN I_{SA} and CbGN I_{SA} differ significantly in their inactivation kinetics. CtxPN I_{SA} undergo mono-exponential decay (τ = ~8 ms); CbGN I_{SA}, bi-exponential decay (at +36 mV: τ-1 = ~11 ms, τ-2 = ~120 ms). While CtxPN I_{SA} resembles heterologously expressed Kv4.2+KChIP3a+DPP10a current, CbGN I_{SA} is not recapitulated by the Kv4.2+KChIP3a+DPP6a current. Since CbGN reportedly also express robust levels of other DPP6 variants competing for Kv4 channels, we quantitated the levels of different DPP6 isoforms by qRT-PCR and determined their percentage contributions: DPP6a (DPP6-E) = ~32%, DPP6b (DPP6-K) = ~42%, DPP6c (DPP6-L) = ~8%, and DPP6d (DPP6-S) = ~18%. Since DPP6a and DPP6b variants together constitute the majority of the CbGN DPP6 species, we performed coexpression studies to investigate whether CbGN I_{SA} is mediated by ternary complex channels with these DPP6 variants. We propose that relative ratios of DPLP auxiliary subunit isoforms likely contribute to the variability of I_{SA} inactivation kinetics between neuronal populations.

2705-Pos

Pyridine Nucleotide Dependence of Kv Beta - Induced Kv Inactivation: Role of Kv Alpha C-Terminus

Oleg A. Barski, Xiao-Ping Li, Aruni Bhatnagar, Srinivas M. Tipparaju.
University of Louisville, Louisville, KY, USA.

Binding of ancillary β-subunits (Kvβ) to the N-terminal T1 domain of Kv1 and Kv4 regulates channel function and localization. The β subunits of Kv channels belong to the aldo-keto reductase superfamily (AKR6). These proteins bind NAD(P)(H) with high affinity, but the mechanisms by which nucleotides regulate channel gating are unclear. Herein we report that when coexpressed with Kv1.5 in COS-7 cells, Kvβ3 shifts the half-activation potential and imparts inactivation to slowly inactivating Kv1.5 current. Addition of NAD(P)H to the patch pipette increased rate and extent of inactivation, whereas $NAD(P)^+$ reduced inactivation. These results conform to a model assuming that NAD(P)(H) binding regulates rate and extent of inactivation synergistically by altering the number of Kvβ monomers involved in inactivation. Deletion of 56 C-terminal amino acids of Kv1.5 (KvΔC56) did not significantly affect Kv association with Kvβ or Kvβ-mediated inactivation. KvΔC56 did not, however, respond to changes in intracellular pyridine nucleotide concentration when co-expressed with Kvβ3 and neither NAPDH nor $NADP^+$ altered rate or extent of inactivation. Glutathione-S-transferase (GST) fusion protein containing peptides from the last 38 (Ile565-Leu602) and 60 (Arg543-Leu602), but not 19 (Asp584-Leu602), amino acids of Kv1.5 C-terminus precipitated Kvβ2 and Kvβ3 in pull-down assays from lysates of transformed bacteria. The C-terminal peptide (GST-C60) also precipitated Kvβ1 and Kvβ2 from mouse brain extracts. The GST-C60 construct did not bind to apoKvβ2, and it displayed higher affinity for Kvβ2:NADPH than for the Kvβ2:$NADP^+$ binary complex. These results suggest that nucleotide binding provides an efficient mechanism to adjust potassium flux in response to metabolic changes. The C-terminal domain of Kv1.5 from Arg543-Asp584 interacts with Kvβ and this interaction may be involved in sensing different conformational states of Kvβ bound to either reduced or oxidized pyridine nucleotides.

2706-Pos

Depenedence of Ependence 6β-Acetoxyl-7α-Hydroxyroyleanone Block of Kv1.2 Channels on C-Type Inactivation

Yuk M. Leung[1], Kar-Lok Leung[2], Chia-Huei Lina[1], Chia-Chia Chaoa[1], Chun-Hsiao Choua[1], Li-Yun Changc[3], Shao-Wei Chend[4], Tzu-Hurng Chengd[4], Yue-Hsiung Kuoe[5].
[1]Graduate Institute of Neural and Cognitive Sciences, China Medical University, Taichung, Taiwan, [2]Department of Anesthesia, China Medical University and Hospital, Taichung, Taiwan, [3]Graduate Institute of Molecular Systems Biomedicine, China Medical University, Taichung, Taiwan, [4]Department of Biological Science and Technology, China Medical University, Taichung, Taiwan, [5]Tsuzuki Institute for Traditional Medicine, China Medical University, Taichung, Taiwan.

Voltage-gated K^+ (Kv) channels repolarize excitable cells by providing a pathway for K^+ efflux. Kv channels activate when the membrane is depolarized, and subsequently exhibit slow inactivation (C-type inactivation) during continuous depolarization. A selective pharmacological agent targeting C-type inactivation is so far lacking. Here we reported that 6β-acetoxy-7α-hydroxyroyleanone (AHR), a diterpenoid compound isolated from *Taiwania cryptomerioides* Hayata, could selectively modify C-type inactivation of Kv1.2 channels. Extracellular, but not intracellular, AHR (50 μM) dramatically speeded up the slow decay of Kv currents and left-shifted the steady-state inactivation curve. AHR blocked steady-state Kv currents with an IC_{50} of 17.7 μM and the effects of AHR were completely reversible. AHR did not affect at all the kinetics and voltage-dependence of Kv1.2 channel activation. The degree of block of Kv currents by AHR was independent of the intracellular K^+ concentration. In addition, effect of AHR was much attenuated in a Kv1.2 V370G mutant defective in C-type inactivation. Furthermore, ATP-sensitive K^+ (K_{ATP}) channel, which does not display C-type inactivation, was not affected by AHR. Therefore, block of Kv1.2 channel by AHR did not appear to involve direct occlusion of the outer pore but may depend on the C-type inactivation gate. AHR could thus be a pharmacological tool targeting the C-type inactivation gate of Kv channels.

Ligand-gated Channels-Glutamate Receptors

2707-Pos

TARP Modulation of AMPA Receptor Pharmacology: Polyamine Block and Competitive Antagonism

David MacLean, Derek Bowie.
McGill, Montreal, QC, Canada.

External polyamines have been a valuable tool as pharmacological markers of Ca^{2+}-permeable AMPARs (CP-AMPARs). However, recent work in the developing retina has revealed a population of CP-AMPARs which are unexpectedly insensitive to external polyamines. Because TARPs attenuate internal polyamine block, we hypothesized that TARP association with CP-AMPARs would also diminish external polyamine block, and thus display the phenotype observed in the retina. Similarly, TARPs have also been reported to reduce the potency of another useful pharmacological tool: the competitive antagonist CNQX. We therefore examined the impact of TARPs on CP-AMPARs for both of these antagonists using excised patches. We found that TARPs did attenuate internal block, however external block was not substantially reduced. Indeed, the rate of onset of block was actually accelerated. Moreover, TARPs do not significantly reduce CNQX inhibition of peak responses. In the presence of TARPs, CNQX has been reported to

become a weak partial agonist at AMPA receptors. We report that these CNQX-evoked responses actually desensitize. A kinetic analysis of responses in the presence of CNQX suggests that for AMPA receptors with TARPs, but not AMPA receptors alone, CNQX inhibits channel function in part by desensitizing receptors. These data demonstrate three things, first that internal and external polyamine block, previously thought to occur through a common mechanism, are in fact dissociable processes. Second, that TARPs do not significantly reduce the potency of CNQX as an antagonist and third, that TARPs enable CNQX to inhibit AMPARs by both competitive antagonism and by desensitization. As the effects of TARPs and other auxiliary proteins become known, such re-evaluations of pharmacological tools like polyamines and competitive antagonists will be required, particularly as CP-AMPARs are emerging as therapeutic targets.

2708-Pos

Crystal Structure of KA2-Subtype Ionotropic Glutamate Receptor Amino Terminal Domain

Janesh Kumar, Mark L. Mayer.
Laboratory of Cellular and Molecular Neurophysiology, Porter Neuroscience Research Center, NICHD, NIH, DHHS, Bethesda, MD, USA.

The glutamate receptor ion channels that mediate excitatory synaptic transmission in the mammalian brain have a unique modular architecture distinct from that for other ligand gated ion channels. It took ten years since publication of the first crystal structure of an isolated ligand-binding domain (S1S2) (1) to solve structures of the amino terminal domain (ATD) of GluR6 (2) and GluR2 (3). These structures give insight into the regulation of subtype specific assembly by ATDs. However, structures of ATDs from other subtypes are still unknown. We have now determined high-resolution crystal structures of KA2 ATD in 2 forms at 1.4 Å & 1.6 Å respectively. KA2ATD has a clamshell-like fold similar to GluR6 and GluR2. However, the dimer assembly is significantly different than that for GluR6 & GluR2 where the R1 & R2 domains of both the protomers contribute equally to dimer formation. In KA2, the R2 domains also co-assemble and form close contacts similar to GluR6 & GluR2 ATDs but the R1 domains are separated. This assembly is interesting because the R1 domain has loops that likely specify subtype specific assembly. These loops make no contacts across the dimer interface in KA2 ATD, consistent with obligate co-assembly of KA2 with GluR5-7 for formation of functional ion channels.

References

(1) Armstrong, N., Sun, Y., Chen, G.Q. & Gouaux, E. Structure of a glutamate-receptor ligand-binding core in complex with kainate. Nature 395, 913-917 (1998).
(2)Kumar J, Schuck P, Jin R, Mayer ML (2009) The N-terminal domain of GluR6-subtype glutamate receptor ion channels. Nat Struct Mol Biol. 16, 631 - 638.
(3) Jin R, Singh SK, Gu S, Furukawa H, Sobolevsky AI, Zhou J, Jin Y, Gouaux E (2009) Crystal structure and association behaviour of the GluR2 amino-terminal domain. EMBO J. 28(12):1812-23.

2709-Pos

A Comparative Molecular Dynamics Simulation Study of the Amino Terminal Domain of Ionotropic Glutamate Receptors

Ranjit Vijayan, Philip C. Biggin.
University of Oxford, Oxford, United Kingdom.

Glutamate receptors account for the vast majority of excitatory neurotransmission in the vertebrate nervous system. The modular architecture of these membrane receptors consists of an extracellular amino terminal domain (ATD) and ligand binding domain (LBD) as well as a transmembrane ion channel. While numerous water soluble constructs of the LBD have been crystallised, high resolution structures of the ATD have been unavailable till very recently. Multiple long (3 x 50 ns) molecular dynamics simulations of five ATD dimer structures - two GluA2 (AMPA) and three GluK2 (kainate) - were performed to evaluate the stability of the structure and the rigidity of the dimer interface. The dimers remained intact throughout the course of all simulations. Overall, these structures appear to undergo very little motion. It is yet unknown whether the ATD is capable of binding a ligand. Simulations of these ligand-free structures suggest two possible regions with increased flexibility that may lead to a potential binding site. Several water molecules are also conserved across the structures as well as the two families. Electrostatic calculations indicate that the "bottom" of the ATD and the "top" of the LBD exposes complementary charge surfaces, which could explain how the two modular regions may interact in the full-length receptor.

2710-Pos

Structurally Variable Regions of the Ligand-Binding Domain of Ionotropic Glutamate Receptors Link Dynamics and Functional Properties

Rodney E. Versace, Marco Ceruso.
City College of New York, New York, NY, USA.

It has been hypothesized that the behavior of the channel gate is controlled by structural and dynamical events within the bi-lobate ligand binding domain (LBD) of ionotropic glutamate receptors (iGluRs). With the ulterior goal of developing reliable coarse-grained models that would allow probing this hypothesis with μs to ms time-scale simulations, we have characterized the ns time-scale conformational dynamics of the LBD of GluR-2, GluR-6 and NR2A, respectively representing the AMPA, kainate and NMDA receptor class. Each LBD was modeled both using the AMBER SB99 and the GROMOS G43A1 force field. The structural and dynamical properties of the 3 LBDs were obtained from extensive molecular dynamics simulations (at least 3 independents 20 ns run per system and force field). To compare these properties across the three classes of LBD a common structural core was defined based on structural similarity. The results of these computational experiments show that (1) all 3 LBDs possess 2 common rigid body domain motions that modify the degree of twist and hinge bending of the lobes; (2) these motions do not appear to be class specific at this time-scale; instead (3) structural elements outside of the common structural core modulate the conformational space of the core and are responsible for defining class specific dynamics of the LBDs. These structural elements have been implicated previously in the modulation of ligand efficacy and extent of receptor desensitization. Thus, together these observations suggest that these identified structural elements impart class specific dynamics behavior to the LBD of iGluRs and may be responsible for the distinct functional properties of these receptors.

2711-Pos

Theoretical Investigation of Structure and Gating Mechanisms in Glutamate Receptor Ion Channels

Michael J. Yonkunas, Maria Kurnikova.
Carnegie Mellon University, Pittsburgh, PA, USA.

The ionotropic glutamate receptors (iGluRs) are excitatory synaptic transmitters found in the fore-brain. Together with potassium, sodium, and calcium channels they form a tetrameric ligand gated ion channel class of P-loop receptors. The structures of iGluRs are currently not resolved, while functional mechanisms are not well understood. To gain insight into the structure of the iGluR channel region, the KcsA potassium channel pore is used as a working model for its known structural homology with iGluR transmembrane regions. A conserved hydrophobic patch among all P-loop receptors is located along the pore lining M3 helix which spans the lipid-water interface. This patch creates a helical bundle crossing where single residue mutations produce constitutive open channels in mice, suggesting its role in the control of ion conduction. Molecular dynamics simulations and umbrella sampling methods were used to examine the opening of the KcsA M3 bundle crossing starting from the closed KcsA crystal structure (PDB ID: 1k4c). The potential of mean force defining the free energy landscape obtained from the structures of KcsA is then used to abstract the unknown closed and open forms of iGluR transmembrane regions. Assuming the P-loop region comprising the selectivity filter changes little between functional states, energetically defined conformations provide points of refinement for unknown structures of transmembrane regions for proteins such as iGluRs. In order to describe a mechanistic model of iGluR, Ca2+/Mg2+ selectivity is currently under investigation for an NMDA type iGluR. Homology modeling of the transmembrane region based on potassium channels is being used to infer structure; and Ca2+/Mg2+ interactions with organic caging agents are used to parameterize divalent ion selectivity. Understanding how iGluRs control ion conduction together with monovalent versus divalent ion selectivity will aid in describing ion channel structure-function relation.

2712-Pos

Rearrangements at the Heterodimer Interface are Integral to NMDA Receptor Activation

William F. Borschel, Gabriela K. Popescu.
University at Buffalo, Buffalo, NY, USA.