The molecular details of ion channel regulation by G proteins remain unknown. A first step in this direction is to define the characteristics of interacting proteins of known structure in isolation and in complex form. The three-dimensional structure of a GIRK1-chimera determined by single particle electron microscopy at 25Å is consistent with the crystal structure (Nishida et al., 2007, EMBO J 26:4005-15). We have functionally reconstituted this GIRK1-chimera into a 1:1 ratio of phosphatidylethanolamine to phosphatidylserine planar lipid bilayers. The GIRK1-chimera produces a conductance of approximately 23 pS that shows Mg^{2+}-dependent inwardly rectifying K^+ currents and an absolute requirement on the presence of phosphatidylinositol-4,5-bisphosphate for activation. These currents are blocked by PIP_2 antibody and poly-lysine applied from the cis but not the trans side. Moreover, the channel shows a high affinity for diC8-PIP_2 (EC_{50} ~ 7.5 μM). GIRK1-chimera channel currents are blocked by Ba^{2+} and the GIRK peptide blocker tertiapin when applied from the trans but not the cis side. Interestingly, Gβγ applied from the cis side inhibits GIRK1-chimera currents and shifts phosphoinositide sensitivity by decreasing the apparent affinity to PIP_2. This is in contrast to the Gβγ effects on full-length GIRK1* channels assayed in *Xenopus* oocytes or planar lipid bilayers.

3649-Pos

Expression and Purification of Recombinant Human Inward Rectifier K^+ (KCNJ) Channel

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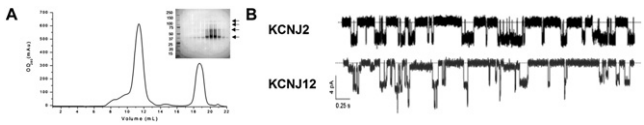
Inward rectifier potassium (KCNJ) channels regulate vital cellular processes including cell volume, electrical excitability, and insulin secretion. Dysfunction of different isoforms has been linked to numerous diseases including Bartter's, Andersen-Tawil, Smith-Magenis Syndromes, diabetes, and epilepsy. We succeeded in expressing 10 of 11 human KCNJ channels tested in *Saccharomyces cerevisiae* under Gal1-inducible promotion. GFP-fusion proteins are located in the plasma-membrane, suggesting the protein is correctly folded and trafficked. Following large scale expression of Kir2.x family members, a 2-step purification process can be used to isolate protein to >95% in a mono-dispersed form (Fig.1A). $^{86}Rb^+$ flux assays and patch clamp analysis on reconstituted proteins confirm the functionality of the purified proteins as inward rectifier potassium channels. For KCNJ2 (Kir2.1) and KCNJ12 (Kir2.2) channels, the unitary conductance in 150mM symmetrical [K^+] (~33pS and ~40.5pS, respectively, Fig.1B at −100mV), sensitivity to spermine block, and activation by $PIP(4,5)_2$ resemble those observed in eukaryotic membranes. The high-level purification and reconstitution of these proteins makes feasible not only ongoing biochemical and structural analysis of eukaryotic KCNJ channels, but also the analysis of channel function in the absence of modulator proteins, and in membranes of defined composition.



3650-Pos

Purified Hetero-Tetramers of the Potassium Channel Kcv Revealing Independent Subunit Contribution to the Tea Block

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Tetraethylammonium (TEA) is a common molecular probe in detecting potassium channel blocking. The external TEA binding affinity has been proposed to be highly related to the aromatic residue located at the outer mouth of the potassium channels such as Y82 in KcsA and Y449 in Shaker, probably due to the π-cation interaction between TEA and the aromatic side chain. In this report, we identified the highly sensitive TEA block for the chlorella virus-encoded Kcv, a miniature model K+ channel that only consists of 94 amino acids with two transmembrane domains and a conservative selectivity filter. By mutagenesis screening at Leu70 of Kcv, which is equivalent to the TEA site Y82 in KcsA, we found substitution of Leu70 to all other amino acids including Tyr, Phe and His will reduce the TEA affinity, suggesting a more complicated mechanism beyond cation-π interaction involved in TEA blocking. We further developed a novel functional stoichiometric approach to exploring how each individual subunit contributes to the TEA binding. We co-expressed the mutant Kcv and a mass-tagged wild-type Kcv, to form hetero-tetramers that can be electrophoretically separated. Because Kcv is able to retain the channel-forming function in detergent SDS [*FEBS Lett.* 581, 1027-1034 (2007)], we can purify all types of hetero-channels directly from the SDS gel, and subject to single channel recording. Through this approach, we established a linear correlation between the free energy for TEA blocking and the number of mutant subunits in a tetramer, which infers that each subunit independently interacts with one ethyl group of TEA and contributes equal energy to the overall TEA affinity. The functional stoichiometric approach we developed with purified hetero-channels can be applied to the mechanism study of many K+ channel drugs and inhibitors.

Ligand-gated Channels

3651-Pos

Desensitization Contributes to the Postsynaptic Response of Ionotropic Receptors; A Comparative Study of Cys-Loop, Purinergic, and Glutamate Receptor-Channels

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All naturally-occurring ionotropic receptors desensitize significantly in the continuous presence of a sufficiently high concentration of agonist. However, the neurotransmitter lifetime in the synaptic cleft is limited by diffusion, neurotransmitter reuptake, and/or enzymatic cleavage, to an extent such that desensitization does not appreciably occur during the agonist pulse. Nevertheless, channels remain prone to desensitization during the much longer interpulse intervals while they deactivate. As a result, it is of interest to determine the extent to which ligand-gated ion channels (LGICs) known to participate in fast synaptic transmission undergo desensitization upon ligand removal, since entry into these refractory states would progressively decrease the postsynaptic-current response. To address this problem, we have applied high-frequency trains of brief (approximately 1 ms) agonist pulses to outside-out membrane patches expressing these LGICs; receptors under study include the rat purinergic P2X receptor, the rat AMPA-type glutamate receptor, the rat gamma-aminobutyric acid receptor (GABAR), the human glycine receptor (GlyR), and the human ganglionic (alpha3-beta4) and mouse-muscle nicotinic acetylcholine receptors (nAChRs). Our results indicate that all tested receptors exhibit increasingly reduced peak responses in a train-frequency- and receptor-dependent manner, consistent with the notion that the extent of desensitization upon deactivation is substantial. These findings suggest that a) receptor desensitization may contribute to limit the in-vivo postsynaptic response mediated not only by glutamate receptors (which has been proposed earlier), but also by all of the other ionotropic receptors studied here, and b) that the occurrence of desensitization cannot be neglected (as it often is) in attempts to characterize the kinetic behavior of these channels.

3652-Pos

Effects of Protons on Macroscopic and Single-Channel Currents Mediated by the Human P2X7 Receptor

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Human P2X7 receptors (hP2X7Rs) belong to the P2X family, which opens an intrinsic cation channel when challenged by extracellular ATP. hP2X7Rs are expressed in cells of the inflammatory and immune system. During inflammation, ATP and protons are secreted into the interstitial fluid. Therefore, we investigated the effect of protons on the activation of hP2X7Rs. hP2X7Rs were expressed in *Xenopus laevis* oocytes and activated by the agonists ATP or benzoyl-benzoyl-ATP (BzATP) at different *pH* values. The protons reduced the hP2X7R-dependent cation current amplitude and slowed the current deactivation depending on the type and concentration of the agonist used. These effects can be explained by (i) the protonation of ATP, which reduces the effective concentration of the genuine agonist, free ATP^{4-}, at the high- and low-affinity ATP activation site of the hP2XR, and (ii) direct allosteric inhibition of the hP2X7R channel opening that follows ATP binding to the low-affinity activation site. Due to the hampered activation via the low-affinity activation site, a low *pH* (as observed in inflamed tissues) leads to a relative increase in the contribution of the high-affinity activation site for hP2X7R channel opening.

3653-Pos

P2X7 Receptor-Mediated Disruption of the Plasma Membrane and Endoplasmic Reticulum Morphology and Cell Survival

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The cation-conducting P2X7 receptor channel (P2X7R) operates as a cytolytic and apoptotic nucleotide receptor but also controls sustained cellular responses, including cell growth and proliferation. However, it has not been clarified how