both the N-terminus and the C-linker was significantly different from zero at all possible combinations of intracellular regions. In contrast, at all possible combinations of intracellular regions no significant interaction energy was observed for the S4-S5 linker and the CNBD. It is concluded that in CNG channels the S4-S5 linker cooperates with both N-terminus and C-linker in the process of translating ligand binding to the pore opening.

3678-Pos

Structural Insight into the Ion Selectivity and Ca^{2+} Blockage in Cyclic Nucleotide Gated Channels

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Cyclic nucleotide-gated (CNG) channels are non-selective cation channels that play crucial roles in visual and olfactory signal transduction. They conduct all alkali metal and some alkaline earth metal ions, most notably Ca^{2+}. In this study the non-selective bacterial channel NaK is used as a model to study the structural basis of ion selectivity in CNG channels. Though NaK is non-selective and bears some similarities with CNG channels, there is divergence in some of the most critical properties of CNG channels. Moreover, the protein sequence at the C-terminal end of the selectivity filter in NaK differs markedly from CNG channels both in amino acid composition and sequence length. As a result, wild-type NaK may not be a viable structural model for CNG channel selectivity. So, within the NaK selectivity filter (^{63}TVGDGNFS^{70}) the DGNFS sequence was replaced with ETPP to represent most CNG channels, DTPP to mimic an E◇D mutation and NTPP to represent a neutral residue mutant. We present high resolution crystal structures and single channel recordings of of these NaK channel mutants. These mutants share several striking functional similarities in ion selectivity with eukaryotic CNG channels: they are non-selective and permeate Na^+ and K^+ equally well; externally added Ca^{2+} serves as a permeating blocker, with the conserved acidic residue in the filter mediating Ca^{2+} binding. The structures of these CNG-mimicking mutant channels in complex with various cations reveal a unique selectivity filter architecture containing three contiguous ion binding sites different from both wild type NaK and K^+ channels. Taking into account identical selectivity filter sequences, these structures are believed to serve as accurate working models for CNG channel pores and yield novel insights into the structural basis of their ion selectivity and Ca^{2+} blockage properties.

3679-Pos

Subunit-Specific Regulation of Photoreceptor CNG Channels by Phosphoinositides

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Cyclic nucleotide gated (CNG) channels in retinal photoreceptor cells play a key role in vertebrate phototransduction. The ligand sensitivity of photoreceptor CNG channels is adjusted during adaptation and in response to paracrine signals, but the mechanisms involved in channel regulation are only partly understood. Heteromeric A3+B3 (cone) and A1+B1 (rod) channels are sensitive to regulation by PIP_3 or PIP_2, demonstrating a decrease in apparent affinity for cGMP. To determine what subunit types are necessary for PIP_3 sensitivity, we generated heteromeric channels by co-expression of PIP_3-insensitive A2ΔN (Brady et al., 2006) with B3 or B1 subunits. Using patch-clamp recording in the inside-out configuration, we found that both channel types were insensitive to PIP_3 regulation, suggesting that A3 or A1, but not B3 or B1 subunits, confer phosphoinositide sensitivity to heteromeric channels. Consistent with this idea, co-expression of A3 with B1 formed channels that were sensitive to PIP_3 regulation. Unlike homomeric A1 or A2 channels, A3-only channels paradoxically did not show a decrease in apparent affinity for cGMP after PIP_3. However, PIP_3 induced a nearly three-fold increase in cAMP efficacy for A3 channels, an effect that was reversed by poly-lysine application. The PIP_3-dependent increase in cAMP efficacy for A3 channels was abolished by mutation of a critical ligand-discrimination residue (D609K) or by truncation of the channel distal to the cyclic nucleotide-binding domain (613stop). Furthermore, the apparent cGMP affinity of A3-613stop channels was reduced three-fold by PIP_3; this change in cGMP sensitivity also was reversed by poly-lysine. Together, these results suggest that regulation of A3 subunits by PIP_3 exhibits two components, one of which is unmasked either by assembly with B3 subunits or by deletion of the C-terminal region of A3. (Supported by NIH EY012836)

3680-Pos

Transferring S3-S4 Motif from Kv Channels to the CNG Channel

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Cyclic nucleotide-gate (CNG) channels belong, functionally, to the family of ligand-gated ion channels. They are activated by the binding of cyclic nucleotides, such as cGMP, cIMP or cAMP to an intracellular binding domain within the carboxyl terminal. Structurally, however, CNG channels are grouped with ion channels containing six transmembrane segments, such as voltage-dependent potassium channels. These proteins probably derive from a common evolutionary ancestor, but the properties of the channels are quite different. Whereas the membrane potential controls the activity of voltage-gated K^+ (Kv) channels, CNG channels have little or no inherent voltage dependence, particularly at saturating concentrations of agonist. Even though CNG channels contain an S4 positively-charged segment similar to the S4 voltage sensor in K_V channels, little is known about its relevance in CNG channels. In this work, we transferred the voltage-sensor S3-S4 motif from Kv2.1 and KvAP to bCNGA1 and analyzed the function of these chimeras expressed in *Xenopus* oocytes using excised patch clamp. Our results suggest that the S3-S4 motif of CNG channel can be swapped by the homologous region of Kv channels and, although these chimeras are modulated by voltage, activation remains cGMP-dependent.

3681-Pos

Caesium Permeation Reveals an Unusual Voltage Dependent Gating at the Selectivity Filter of CNGA1 Channels

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CNG channels are permeable to alkali monovalent and divalent cations and to small organic cations. Therefore, CNG channels have a low ionic selectivity, attributed to an intrinsic flexibility of the filter region (Laio & Torre, 1999) mediating the coupling between permeation and gating (Gamel & Torre 2000; Holmgren, 2003; Kush, 2004). We have analysed in more detail the permeation of Cs+ in WT CNGA1 channels. In symmetrical Na+ or K+ conditions and in the presence of saturating cGMP concentrations the ratio between the current at +200 and −200 mV I200/I-200 is 1.3. In contrast, in the presence of symmetrical Cs+ I200/I-200 it is about 0.75. Under these conditions, single channel recordings reveal a surprising behaviour: the single channel conductance for Cs+ ions is about 18 pS at −180 mV, but becomes less than 5 pS at voltages larger than 100 mV. The open probability at −180 mV is about 0.2 and becomes close to 1 at +100 mV. When Thr360 is mutated to alanine, the single channel conductance for Cs+ ions is around 15 pS both at +100 and −100 mV. These results confirm the notion that the pore region of CNGA1 channels is highly flexible and that permeating ions modify and control channel gating. These results show also that the pore region of CNGA1 channels acts as a voltage sensor and modifies their conformation in response to changes of the applied field. The molecular mechanisms involved in these rearrangements are likely to consist in reorientation of electric dipoles, such as those of Thr360.

3682-Pos

Functional Expression and Subcellular Localization of F-Channels in Human Ventricular and hESC-Derived Cardiomyocytes

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Caveolae are specialized lipid rafts in the plasma membrane responsible for the interaction, sublocalization and function of proteins and ion channels. The Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) genes encode for the alpha subunit of f-channel present in cardiac pacemaker cells, in the adult and developing cardiac myocytes (CM). HCN4 is the predominant isoform in the sinoatrial node and, in the rabbit, experimental evidence indicate that it localizes into membrane caveolae, where caveolin-3 (Cav3)-channel interaction regulates current properties and autonomic modulation. HCN4 is abundant in undifferentiated human embryonic stem cells (hESC) and immature hESC-derived CM (hESC-CM). To date, no information is available on i) developmental changes of HCN4 channel localization and function in human CM ii) the relationship with HCN channel expression/function in adult CM.
Confocal analysis showed that HCN4 and Cav3 colocalize in adult human ventricular CM. In the same cells, f-current was consistently recorded upon hyperpolarization (70% cells), with a voltage of half maximal activation (Vh) of −94 mV. Protein and mRNA for Cav3 were not detected in undifferentiated hESC, but expression increased during maturation of hESC-CM. Oppositely, HCN4 was highly expressed in hESC and early hESC-CM, but a 5-fold decrease in mRNA levels occurred in late hESC-CM. In these cells, HCN4 appeared to be associated with Cav3. Activation properties of f-current recorded in late hESC-CM resembled those measured in adult ventricular CM (Vh=-93 mV). Current activation was faster and occurred at more positive potentials in hESC and early CM. In conclusion, cardiac maturation is associated with the recruitment of HCN4 channel and CAV3 into membrane lipid rafts, suggesting that sub-cellular localization of f-channel in lipid rafts is a fundamental step during cardiac maturation.