874-Pos Board B753

Identification Of Channel-lining Residues In The Prokaryotic Proton-gated Cys-loop Receptor Ion Channel From *Gloeobacter Violaceus*

Rishi Parikh, Moez Bali, Myles H. Akabas.
Albert Einstein College of Medicine, Bronx, NY, USA.

The Cys-loop family of neurotransmitter-gated ion channels mediate excitatory and inhibitory transmission at fast chemical synapses. These proteins form pentameric assemblies with an extracellular ligand-binding domain, a transmembrane ion channel formed from 4 α-helices per subunit, and a large cytoplasmic globular domain between the M3 and M4 segments. The homopentameric proton-gated cation channel from the prokaryote *Gloeobacter violaceus* (Glvi) has been identified as a putative homologue but lacks some classical features of the eukaryotic family members, including the large cytoplasmic loop and the signature disulfide linkage. To gain insight into the structure of Glvi, we engineered 30 individual cysteine substitutions that align with positions -2' - 27' of the M2 segment in eukaryotic family members. Each mutant was expressed in *Xenopus* oocytes and evaluated for the accessibility of the engineered cysteine to *p*-chloromercuribenzenesulfonate (pCMBS$^-$) in the closed state (pH 7.5) or in the sub-maximally open state (pH 5). Of the 30 Cys mutants, in 5 the proton-induced currents were not significantly different than those in water-injected oocytes, and the 8' and 9' mutants showed aberrant gating properties. Of the mutants tested from -2' to 9', E220C (-2') and T224C (2') were reactive at both pH 7.5 and 5.0, and T229C (7') was reactive only at pH 5.0, presumably in the open state. However, from 10' to 27' each mutant tested was modified by pCMBS$^-$ except V240C (19'). This suggests that the M2 helix is tightly associated with the adjacent transmembrane helices on the channel's intracellular side but is loosely packed from 10′ to the extracellular end. Examination of the relative reaction rates may distinguish the channel-lining residues that are more extracellular than 10′.

875-Pos Board B754

Oligomeric Size of the M2 Muscarinic Receptor in the Plasma Membrane of Live Cells as Determined by Quantitative FRET

Luca F. Pisterzi[1], David B. Jansma[1], John Georgiou[2], Michael J. Woodside[3], Judy Tai-Chieh Chou[1], Stéphane Angers[1], Valerică Raicu[4], James W. Wells[1].
[1]University of Toronto, Toronto, ON, Canada, [2]Samuel Lunenfeld Research Institute, Toronto, ON, Canada, [3]Imaging Facility, Toronto, ON, Canada, [4]University of Wisconsin-Milwaukee, Milwaukee, WI, USA.

Only a minimum generally can be placed on the size of oligomers formed by G protein-coupled receptors. Attempts to obtain an explicit estimate have not been persuasive, owing in part to their reliance on questionable models. We therefore have developed a quantitative method based on fluorescence resonance energy transfer (FRET), which we have applied to oligomers of M_2 receptors tagged at the *N*-terminus with enhanced green or yellow fluorescent protein (eGFP-M_2 or eYFP-M_2) and co-expressed in Chinese hamster ovary cells. The approach is based on the relationship between apparent FRET efficiency and the 'pair-wise' FRET efficiency (E) between a single donor and a single acceptor in an oligomer of specified size (n). Emission spectra were analyzed by spectral deconvolution, and apparent efficiencies were measured by donor-dequenching and acceptor-sensitized emission at different ratios of eYFP-M_2 to eGFP-M_2. The data were interpreted in terms of a model that considers all combinations of donor and acceptor within the oligomer to obtain fitted values of E as follows: n=2, 0.473 ± 0.011; n=4, 0.206 ± 0.009; n=6, 0.132 ± 0.005; n=8, 0.100 ± 0.002. The pair-wise FRET efficiency determined independently by fluorescence lifetime imaging was 0.206. The M_2 receptor therefore can be identified as a tetramer, in agreement with the results of a parallel study in which the oligomeric size has been estimated from FRET efficiencies measured at the level of single pixels.

876-Pos Board B755

Oligomeric Size of the M2 Muscarinic Receptor in Live Cells as Determined from FRET Efficiencies at the Level of Single Pixels

Luca F. Pisterzi[1], Michael R. Stoneman[2], James W. Wells[1], Valerică Raicu[2].
[1]University of Toronto, Toronto, ON, Canada, [2]University of Wisconsin-Milwaukee, Milwaukee, WI, USA.

G protein-coupled receptors are known to form oligomers, but their size (n) is unclear. We therefore have developed an approach whereby an explicit value of n can be obtained from the efficiency of fluorescence resonance energy transfer (FRET) measured between fluorophore-tagged receptors at the level of single pixels. That approach has been applied to M_2 receptors fused at the *N*-terminus to enhanced green or yellow fluorescent protein (EGFP^2-M_2 or EYFP-M_2) and coexpressed in Chinese hamster ovary cells. Pixel-level emission spectra were recorded from images captured in a single plane, and the pair-wise FRET efficiency (E) was calculated from the relative contributions of EGFP^2-M_2 and EYFP-M_2 as determined by spectral deconvolution. The number of efficiencies obtained for an oligomer of given size is expected to depend upon the number of combinatorial arrangements of FRET-productive pairs as predicted by the binomial theorem. A dimer will reveal a single efficiency, for example, and a square tetramer will reveal at least three. A sum of three Gaussians was required to describe the distribution of efficiencies obtained from cells coexpressing EGFP^2-M_2 and EYFP-M_2. Also, differences among values of E in an oligomer of specified size can be predicted on the basis of a model in which the apparent FRET efficiency is determined by the pair-wise FRET efficiencies for all combinations of donors and acceptors. With the present data, the observed differences in the mean values of E agree with the predicted differences when n is 4. The M_2 receptor therefore can be identified as a tetramer, in agreement with the results of a parallel study in which the oligomeric size has been estimated from intensity-based FRET and fluorescence lifetimes.

877-Pos Board B756

Gating Current Measurements Reveal Ligand-Selective Conformational Changes in the M2 Muscarinic Receptor

Ricardo Navarro-Polanco[1], Eloy Moreno-Galindo[1], Tania Ferrer-Villada[1], Marcelo Arias[2], J. Ryan Rigby[2], Jose Sánchez-Chapula[1], Martin Tristani-Firouzi[2].
[1]University of Colima, Colima, Mexico, [2]University of Utah, Salt Lake City, UT, USA.

In addition to external stimuli, membrane voltage modulates the activity of several G-protein-coupled receptors (GPCRs). For example, membrane depolarization reduces the affinity of the M2 muscarinic receptor (M2R) for acetylcholine (ACh). The intrinsic capacity of muscarinic receptors to "sense" transmembrane potential was confirmed by the recording of "gating" currents that reflect reorientation of charges within the receptor in response to changes in voltage. We studied the effects of membrane voltage on agonist activation of M2R in atrial myocytes and on gating currents in oocytes heterologously expressing M2R. Depolarization decreased the apparent affinity of M2R for ACh, but increased the apparent affinity and efficacy of pilocarpine (PILO), as measured by acetylcholine-activated K^+ current, I_{KACh}. Voltage-induced conformational changes in M2R (as measured by gating currents) were modified in a ligand-selective manner. For example, ACh reduced gating charge displacement while PILO increased the amount of charge displaced. Thus, these ligands manifest opposite voltage-dependent I_{KACh} modulation and exert opposite effects on M2R gating charge displacement. Finally, mutations in the putative ligand binding site perturbed the movement of the M2R voltage sensor and confirmed a link between voltage sensing and changes in agonist affinity. Our data suggest that voltage modulates the conformation of the ligand binding site, while ligand binding induces ligand-specific conformational changes in the receptor. Voltage-dependent GPCR modulation has important implications for cellular signaling in excitable tissues. Gating current measurement allows for the tracking of subtle conformational changes in the receptor that accompany ligand binding and changes in membrane voltage.

878-Pos Board B757

Site-specific Fluorescence Reveals GABA-induced Structural Movements Near the Extracellular End of the Pore-lining M2 α-helices of the GABA-A Receptor

Cassandra M. Theusch, Meyer B. Jackson, Cynthia Czajkowski.
University of Wisconsin - Madison, Madison, WI, USA.

Binding of the neurotransmitter γ-aminobutyric acid (GABA) to the extracellular domain of the heteropentameric GABA-A receptor induces a rapid cascade of structural rearrangements which ultimately opens a transmembrane chloride-conducting channel. The conformational movements associated with channel opening, however, are largely unclear. Here, we used voltage clamp fluorometry to monitor GABA-induced movements near the extracellular mouth of the channel. Residues at the C-termini of the α_1, β_2 and γ_2 channel-lining M2 α-helices (19′ - 28′) were individually mutated to cysteine and co-expressed with wild-type subunits in *Xenopus* oocytes to form $\alpha_1\beta_2\gamma_2$ GABA-A receptors. The cysteine mutant subunits all formed functional receptors that were activated by GABA. We labeled the cysteine mutant receptors with the sulfhydryl-reactive, environmentally-sensitive fluorescent probe tetrametylrhodamine-6-maleimide (TMRM). Combined voltage clamp and fluorometry monitored GABA-induced channel activity and local protein movements simultaneously in real time. GABA induced decreases in fluorescence from TMRM tethered to three residues in α_1M2 (N274C (20′), P277C (23′), Y281C (27′)), four residues in β_2M2 (P273C, (23′), K274C (24′), I275C (25′), P276C (26′)) and two residues in γ_2M2 (K285C (20′), P288C (23′)). The decreases in fluorescence at these sites suggest that these residues become more exposed to a hydrophilic or aqueous chemical environment in response to GABA-induced gating transitions. The fluorescence change from TMRM at β_2 K274C (24′) initiates earlier and rises faster than the current response, suggesting that the probe is detecting a movement prior to channel gating. Further study of the fluorescence and current responses in combination with structural modeling will help us understand the role of these movements in GABA-A receptor activation.