quenching results of halothane (Q_{max}~ 85%, K_d ~ 2 mM^{-1}) and thiopental (Q_{max}~ 100%, K_d ~ 0.1 mM^{-1}) suggest these two anesthetics bind to almost all TRPs. However, ketamine and etomidate only quenched about 20 ~ 30% with nonspecific binding, indicating other non-TRP binding sites may exist. X-ray structure of GLIC (PDB: 3EAM) reveals three tryptophan (TRP) sites: A) W47 and W72 in the extracellular domain (ECD); B) W160 at the interface between ECD and transmembrane domain (TMD); and C) W213 and W217 in the TMD. Fluorescence quenching of three site-directed mutants, where A, B, or C site was singly remained, demonstrated similar binding affinities to all three sites for both halothane (Q_{max}: 84 ~ 90%, K_d : 1.3 ~ 2.1 mM^{-1}) and thiopental (Q_{max}: 94 ~ 100%, K_d : 0.07 ~ 0.1 mM^{-1}). Of these three sites, halothane prefers W47 and W72 ($Q_{max} = 90 \pm 2\%$, $K_d = 1.3 \pm 0.2$ mM^{-1}) while thiopental prefers W160 ($Q_{max} = 94 \pm 2\%$, $K_d = 0.067 \pm 0.005$ mM^{-1}). In consistent with fluorescence quenching experiments, our theoretical study also finds that both anesthetics bind to all TRPs though both prefer W160. Furthermore, non-TRP sites are discovered by our theoretical study: one near the TM2-3 loop and the other near D86 in the ECD. Collectively, This investigation will elucidate where volatile and intravenous anesthetics interact with pLGICs and reveal how the binding may lead channel functional changes. (Supported by NIH R01GM056257, R01GM069766, R37GM049202, R01GM066358).

3659-Pos

Mutational Analysis of the Prokaryotic Pentameric Ligand-Gated Ion Channel GLIC

Borna Ghosh, Susan Hanson, Cynthia Czajkowski.
University of Wisconsin, Madison, Madison, WI, USA.

For pentameric ligand-gated ion channels (pLGICs), the movements involved in coupling neurotransmitter binding to channel activation are unclear. The crystal structures of the prokaryotic pLGICs homologs from *E. chrysanthemi* (ELIC) and *G. violaceus* (GLIC) in presumed closed and open channel conformations provide unparalleled opportunities to explore how ligand binding triggers channel opening. GLIC is a proton-gated channel. To test the hypothesis that the proton binding site in GLIC is located in an analogous position as the agonist binding sites of eukaryotic pLGICs and is formed, in part, by acidic amino acids, we neutralized acidic residues in Loops A (E74Q), C (E176Q, D177N, E180Q), and F (D144N, E146Q). Two-electrode voltage clamp recordings from wild-type and mutant GLIC channels expressed in *Xenopus* oocytes showed that the mutations had no effect on proton-mediated channel gating (pH_{50}, pH_{max}) suggesting that titration of these carboxylates is not involved in the proton-dependent gating of GLIC and that the proton binding site is located elsewhere.

We are using fluorescence recording of site-specific labels in GLIC expressed in *Xenopus* oocytes combined with two-electrode voltage clamping to monitor the motions in GLIC associated with channel opening.We made cysteine mutations in a cys-less GLIC (C26A) background in loop 2 (K32C), loop 9 (T157C), M2 helix (E242C, T243C, N244C) and M2-M3 loop (K247C, P249C). The cysteine mutant receptors were labeled with the sulfhydryl-reactive, environmentally-sensitive fluorescent probe Alexafluor 546 C_5-maleimide. Combined voltage clamp and fluorometry monitor proton-induced channel activity and local protein movements simultaneously. Application of pH 5 buffer decreased fluorescence at T157C, K247C and P249C suggesting that these residues become more exposed to a hydrophilic or aqueous environment consistent with a movement of the M2-M3 loop upwards and outward on channel activation.

3660-Pos

Molecular Dynamics Investigation of Anesthetic Halothane Interactions with the Proton-Gated Ion Channel GLIC

Mary Hongying Cheng, Yan Xu, Rob D Coalson, **Pei Tang***.
University of Pittsburgh, Pittsburgh, PA, USA.

Experimental studies found that general anesthetics inhibited the function of GLIC. To investigate the underlying inhibition mechanism, we studied the interaction of halothane with GLIC and determined the potential of mean force (PMF) for transporting a sodium ion across the GLIC channel in the presence and absence of halothane. Multiple halothane binding sites were identified through docking and multi-nanosecond molecular dynamics (MD) simulations; three important ones are: A) inter-subunit site near TM2-3 loop; B) inter-subunit site near D86; and C) intra-subunit site near W160. MD simulations demonstrated that halothane altered GLIC dynamics and electrostatic interactions between critical residues. Halothane near W160 reduced the stability of salt-bridges between D32 and R192, whose homologous electrostatic interaction was suggested to be important for channel gating in the ligand-gated ion channels. To assess potential impact of halothane binding to channel conductance, we calculated PMF using adaptive biased force method. Protonation state of E222 is a determinant for the PMF. PMF profile with five deprotonated E222 residues in the pentamer showed a significantly deeper energy well than that with three deprotonated E222, suggesting that five deprotonated E222 may trap an ion and that partial protonation of E222 is necessary for ion leaving the trap. While halothane near sites A and B introduced comparatively small effects on PMF, a profound PMF change near E222 was observed in the presence of halothane at W160. This change may likely affect the single channel conductance. Taken together, halothane may modulate GLIC function through altering salt bridges crucial for gating, coupled with a breaking of the fivefold symmetry of the pore-lining helices, leading to a change in the energy profile for ion passage through the channel. Supported by NIH (R01GM66358 and R01GM56257) and NCSA through PSC.

3661-Pos

Packing of the Extracellular Domain Hydrophobic Core is Evolutionarily Optimized to Facilitate Pentameric Ligand-Gated Ion Channel Activation

Cosma D. Dellisanti[1], Sonya M. Hanson[2], Lin Chen[3], Cynthia Czajkowski[1].
[1]Dept. of Physiology, University of Wisconsin, Madison, WI, USA, [2]Molecular Physiology and Biophysics Section, NINDS, Bethesda, MD, USA, [3]Molecular & Computational Biology, University of Southern California, Los Angeles, CA, USA.

Protein function depends on protein dynamics as well as structure. Local loose packing of hydrophobic cores is not infrequent in proteins, as the enhanced flexibility likely contributes to their biological function. The crystal structure of the extracellular domain of the nicotinic acetylcholine receptor (nAChR) α1 subunit revealed a hydrophilic water-filled cavity formed by Thr-52 and Ser-126 buried in the hydrophobic core of the protein. Mutation of these residues to bulky hydrophobic amino acids substantially reduced acetylcholine activated channel current suggesting loose-packing of the β-sandwich core is important for nAChR function. Intriguingly, structure-based sequence alignment suggests the presence of loose packing of the hydrophobic β-sandwich core in other pentameric ligand-gated ion channels (pLGICs), whereas tight packing is observed in the crystal structures of the nonchannel homolog, acetylcholine binding protein (AChBP). Here, we examined the generality and importance of this loose packing for pLGIC function using experimental and computational approaches. Mutating aligned residues in the related heteropentameric GABA-A receptor disrupted GABA-mediated currents. Mutations in the β2 subunit had the largest effects suggesting distinct requirements for subunit flexibility in receptor activation. Using FoldX, we examined the energetic cost of mutating residues in the hydrophobic core on protein folding in AChBP, prokaryotic and eukaryotic pLGICs as a measure of protein stability. Interestingly, a loss in protein stability appears correlated to the ability of pLGICs to rapidly switch from closed to open channel states in the presence of ligand. Overall, we suggest that loose packing of the hydrophobic core likely developed as an evolutionary strategy aimed to optimize the specialized allosteric mechanisms of pLGICs.

3662-Pos

Site-Specific Fluorescence Reveals Distinct Structural Changes Induced in the Human ρ1 GABA Receptor by Inhibitory Neurosteroids

Gustav Akk[1], Ping Li[1], Alpa Khatri[2], John Bracamontes[1], David S. Weiss[2], Joe Henry Steinbach[1].
[1]Washington University School of Medicine, St. Louis, MO, USA, [2]University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

The ρ1 GABA receptor is inhibited by a number of neuroactive steroids. A previous study (Li et al., 2007, JPET 323:236-247) focusing on the electrophysiological effects of inhibitory steroids on the ρ1 receptor found that steroid inhibitors could be divided into three major groups based on how mutations to residues in the M2 transmembrane domain modified inhibition. It was proposed that the steroids act through distinct mechanisms. We have selected representatives of the three groups (β-estradiol, pregnanolone and pregnanolone sulfate), and probed how these steroids modify fluorescence changes from the Alexa 546 C5 maleimide fluorophore attached to residues in the extracellular region of the receptor. The results indicate that the steroids have distinct effects on fluorescence changes. Pregnanolone sulfate diminished the fluorescence change produced by GABA at the K217C and S66C positions. The application of β-estradiol reduced the fluorescence change at the L166C and S66C positions. Introduction of the T298F mutation abolished the β-estradiol-induced reduction in fluorescence change at the L166C and S66C sites, and also abolished the functional inhibition produced by the steroid. Pregnanolone did not affect fluorescence changes at any of the sites examined. The findings are consistent with the steroids acting as allosteric inhibitors of the ρ1 GABA receptor, and support the hypothesis that divergent mechanisms underlie the action of inhibitory steroids on the ρ1 GABA receptor.