stability and function of the solubilized and purified receptor is preserved in lipid-analog detergents that have acyl chains similar to the most abundant lipid (16:0, 18:0, 16:1 16:0) in the endogenous *Torpedo* lipid environment, providing a suitable set of detergents for future studies. Supported by NIH grants, RISE, FIPI, 2RO1GM56371-12 and 2U54NS43011.

688-Pos

Oligomeric Size and Configuration of the M_2 Muscarinic and β_2 Adrenergic Receptors in Live Cells as Determined by Pixel-Level FRET

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G protein-coupled receptors are known to form oligomers, but estimates of their size range from dimers to large arrays. We therefore have determined the oligomeric size of fluorophore-tagged M_2 and β_2 receptors in the plasma membrane of Chinese hamster ovary cells by examining the distribution of FRET efficiencies measured at the level of single pixels. Each receptor was fused at its *N*-terminus to enhanced green or yellow fluorescent protein and co-expressed as the complementary pair ($EGFP^2$-M_2 and EYFP-M_2, or $EGFP^2$-β_2 and EYFP-β_2). Pixel-level emission spectra were recorded from images captured in a single plane; the relative contribution of each fluorophore then was determined by spectral deconvolution and used to calculate the corresponding FRET efficiency. The distribution of efficiencies from each cell was analyzed as a sum of Gaussians. The number of Gaussians and the numeric relationship among the corresponding means (E) is determined by the number of combinatorial arrangements of FRET-productive pairs within a two-dimensional oligomer as predicted by the binomial theorem. A dimer will reveal a single efficiency or Gaussian, and a triangular trimer will reveal two; a square tetramer will reveal three efficiencies, and a rhombus will reveal five. Both the M_2 receptor and the β_2 receptor required five Gaussians to describe the distributions of efficiencies from several cells taken together. In each case, an efficiency (E) identified as the pair-wise efficiency (E_p) was related to the other four efficiencies in the manner predicted for a rhombus.

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Anesthetic Binding Sites in the Neuronal N-Acetylcholine Receptor Transmembrane Domain

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Neuronal nicotinic acetylcholine receptors (nAChRs) are sensitive to general anesthetics at physiologically relevant concentrations, but the locations and mechanisms of relevant anesthetic interactions are unknown. The interactions of the anesthetics halothane, isoflurane, and ketamine with the transmembrane domains of the human nAChR α_4 and β_2 subunits were studied using fluorescence spectroscopy and high-resolution solution NMR. The isolated transmembrane domains, with the extracellular and intracellular domains removed by mutagenesis, were expressed in E. coli and purified into detergent micelles. Multi-angle light scattering experiments on a mixture of α_4 and β_2 subunits demonstrated their interaction in detergent micelles. Anesthetics quenched the intrinsic tryptophan fluorescence of these proteins in a manner consistent with specific binding. Direct interaction with anesthetic halothane, isoflurane, and ketamine was confirmed by NMR saturation transfer difference spectroscopy. The anesthetics induced chemical shift changes for specific resonances in the NMR spectra of the α_4 and β_2 transmembrane domains. Two specific interaction sites for the volatile anesthetics halothane and isoflurane have been identified at the extracellular and intracellular interfaces near the ends of the transmembrane domain in the α_4 subunit. Differing from halothane and isoflurane, the intravenous anesthetic ketamine affected a set of residues at a more interior location near the extracellular interface. Changes in NMR peak intensity and line width indicated modulations of protein motion by all three anesthetics. This study demonstrated that general anesthetics interact at specific sites within the isolated transmembrane domain of nAChR and affect protein dynamics on a time scale consistent with protein function. This work was supported by NIH grants: R01GM066358, R01GM056257, R37GM049202, and R01GM069766.

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Effects of Isoflurane Binding in the Pore of a Ligand-Gated Ion Channel

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A high resolution homologue of the eukaryotic nicotinic acetylcholine receptor has been recently crystallized in an apparently open state from the bacteria *Gloeobacter violaceus* (Bocquet *et al*., Nature, 2008). Using the crystal structure of *Gloeobacter violaceus*' ligand-gated ion channel (GLIC) as a starting configuration, extensive molecular dynamics (MD) simulations were performed on the microsecond time scale for both a control and isoflurane-flooded system. This MD data reveal that isoflurane may diffuse from random positions within the water until it binds tightly in the pore to the M2 helices of GLIC. A similar result has also been observed in analogous simulations of the nicotinic receptor. The binding of isoflurane to the M2 helices in GLIC proceeds along a path into the channel via the large opening of the pore on the extracellular domain side. Analysis of the MD trajectory following isoflurane binding shows noticeable dehydration and decreased flexibility of the pore's solvent compared to the control system. Moreover, a close examination of the protein's backbone motions from both MD simulations suggests that presence of isoflurane dramatically increases the dynamics of the protein, in particular the dynamics of the individual residues in the M2-M3 loop. This combined data present a two-fold effect of isoflurane binding in the pore of GLIC: i) a statistically significant change in the dynamics occurs at nearby pore residues, and ii) ion conductance across the membrane decreases due to a change in the dynamical properties of the water in the pore.

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Molecular Dynamics Simulation Reveals a Possible Mechanism of Activation of the Prokaryotic Proton Activated Channel Glic

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Recent bioinformatics and high resolution structural determination revealed prokaryotic ancestors to the superfamily of eukaryotic Cys-loop receptors. One of these channels, called GLIC, is proton-activated and may provide advantages to investigating mechanisms underlying activation of Cys-loop receptors. Here, we used all-atom molecular dynamics simulations to uncover a possible mechanism of activation of the GLIC channel. We started with the x-ray structure of GLIC in the open state and subjected it to simulation under normal conditions. During the 10 ns simulation the channel appeared to close. Then we screened the apparent closed structure for the presence of salt-bridges, and compared the result to salt bridges in the original open x-ray structure. We found 11 salt bridges per subunit that were not present in the open structure. We then protonated the corresponding 11 acidic residues, conducted another 10 ns simulation and found that the channel remained open. We then extracted the final structural frames from the two MD simulations and employed single channel current simulations using the coarse-grained Biology Monte Carlo method. The open structure resulted in a significant ionic current, whereas the closed structure exhibited none. Thus, the 11 acidic residues emerge as likely candidates for mediating proton activation of the GLIC channel.

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The Effect of a Hydration Pocket on the Function Of nAChR Studied by Computational Approach

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The first and solely available crystal structure of the extracellular domain of the nicotinic acetylcholine receptor (nAChR) was published in 2007 (Dellisanti et al. 2007). The intriguing finding of the paper was the existence of a hydration pocket inside the beta sandwich core of the nAChR. A well-ordered water molecule bonded by two hydrophilic residues, Thr52 and Ser126, was found. Based on the patch-clamp experiments of the wild-type and mutant receptors and the fact that the nonchannel homolog acetylcholine binding protein (AChBP) has bulky hydrophobic residues at these positions, authors assumed that hydration pocket might be a key element required for the receptor function as a ligand-gated ion channel. Although this assumption seems quite reasonable, computer simulation is required to provide the energetics of conformational changes.

The application of brute force MD simulations to the study of the above problem and other aspects of the action of nAChRs is problematic. This reflects the fact that the time scale for the gating transition is beyond the range of current computational capability. For example, nanoseconds-long MD simulations performed earlier (Law et al. 2005, Cheng et al. 2007) sample only the local conformational space of the initial channel's state. Thus we explored the origin of the hydrophilic/hydrophobic substitution effect by formulation this problem in terms of the relevant free energy changes and applying a proper thermodynamic cycle plus free energy perturbation calculations. Exploring the effect of the Thr52Val in both the open and closes states of nAChR appeared to provide an interesting microscopic insight on the operation of this system and highlighted the importance of using the proper computational strategies in studying biophysical problems.