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Linear Free Energy Relationships for Neurotransmitter Binding to a Nicotinic Acetylcholine Receptor
Prasad Purohit, Shaweta Gupta, Iva Bruhova, Anthony Auerbach.
Physiology & Biphysics, Suny at Buffalo, Buffalo, NY, USA.
Agonists turn on receptors because they bind with a higher affinity to the active vs. resting form of their target sites. We measured the correlation between low-affinity (LA) and high-affinity (HA) neurotransmitter binding energies in adult mouse muscle acetylcholine receptors (AChRs) by using single-channel electrophysiology. The energy values were estimated from current interval durations, for different agonists and for mutations of seven different binding site amino acids. The results were analyzed as a linear free energy relationship, the slope of which gives the fraction of the overall binding chemical potential at the point the LA complex is established. All mutations of four binding site alpha-subunit aromatic amino acids (Y93, W149, Y190 and Y198) and alphaG147 produced fold-decreases in LA binding (relative to ACh, wild-type) that were about half those at in HA binding. For most residues (and agonists) this slope was ~0.5, but mutations of two binding site residues had a significantly higher (0.90; alphaG153) or lower (0.25; epsilon P121) slope. The sequence of energy changes in the binding process appears to be alphaG153>(aromatics and alphaG147) >epsilonP121.

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Energy Maps of Acetylcholine Receptor Gating
Prasad Purohit, Shaweta Gupta, Snehal Jadey, **Anthony Auerbach**.
Physiology & Biphysics, Suny at Buffalo, Buffalo, NY, USA.
Occupancy of an AChR binding site by an agonist (A) shifts a pre-existing equilibrium from the Closed-channel towards the Open-channel conformation. We estimated energy changes in adult mouse $A_2C \leftrightarrow A_2O$ gating (adult mouse) from single-channels. Mutations mainly cause local energy changes that can be mapped onto structures of related proteins. We made maps showing residue reaction progress (phi values) and approximate extents of energy changes in the channel-opening isomerization. Phi values range from 1 to 0, are approximately constant for each position and have a modal distribution (n=5 populations). Residues having similar phi values are clustered, with phi decreasing approximately longitudinally between the binding site and the gate. Two separated regions in the alpha subunits - the transmitter binding sites and M2-M3 linkers in the membrane domain - have the highest phi values (~0.95), followed by the rest of the extracellular domain (~0.8), most of the membrane domain (~0.6) and the gate region of the pore (0.3). Residues at the extracellular-transmembrane domain interface undergo large energy changes in $C \rightarrow O$. Largest energy changes occur at the binding sites, and in the alpha subunit at the M2-M3 linker, M1 and the gate. All of these energy changes, including those associated with the affinity change for the agonist, appear to arise from local 'resettling' events that do not transfer significant energy over distance. Expansion and flexure of the M2-M3 linkers in the alpha subunit appears to trigger the global allosteric transition, and the hydrophobic gate appears to unlock in three steps. The local character of side chain energy changes and the similar phi values of the binding sites and distant M2-M3 linkers suggest that the gating transition is not strictly a mechanical process.

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Gating Phi Values in the Muscle Acetylcholine Receptor αM2-αM3 Linker with vs. without Agonists
Shaweta Gupta, Anthony Auerbach.
Physiology and Biophysics, University at Buffalo, Buffalo, NY, USA.
Acetylcholine receptors are ligand gated ion channels that transduce a chemical signal into an electrical signal at the neuromuscular junction ($\alpha_2\beta\delta\varepsilon$ subunits arranged around a central pore). A global, reversible allosteric transition between stable closed- and open-channel states ('gating') takes place either in the presence or absence of agonists. Phi (the slope a log-log plot of channel opening rate constant vs. equilibrium constant for a series of mutation of one residue) gives the extent of the perturbed residue's progress in the transition state of the gating reaction, on a scale 1 to 0. Previously-estimated diliganded gating phi values in the α subunit decrease approximately linearly between the transmitter binding site (~0.95, same as for the agonist), through the extracellular domain (0.8), through M2 (0.6) to the gate region (~0.3). Some residues away from the binding site have the same phi value without or without agonists (Purohit and Auerbach, PNAS, 2007). However, unliganded phi values for eight binding site residues (αY93, αW149, αY190, αY198, αG147, αG153, εP121, δP123; 0.77 ± 0.023) are lower than the corresponding diliganded values (0.94 ± 0.017). We estimated di- and un-liganded phi gating values for four positions in the αM2-αM3 linker (αI260, αE262, αP265, αS268), another hi-phi domain that is apparently not connected by a high-phi pathway to the binding sites. The unliganded phi values for these four positions (0.88 ± 0.022) were about the same as in diliganded gating (0.90 ± 0.031). So far the mutability in phi appears to pertain only to the binding site, perhaps because the affinity change for the agonist contributes to the gating energy only when ligands are present.

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Functional Asymmetry of Agonist Binding in Fetal and Adult Muscle Acetylcholine Receptors
Tapan K. Nayak, Shaweta Gupta, Anthony Auerbach.
Physiology and Biophysics, SUNY @ Buffalo, Buffalo, NY, USA.
Neuromuscular acetylcholine receptors (AChRs) have two agonist-binding sites at the a-d and either a-g (fetal) or a-e (adult) subunit interfaces. The g-subunit of fetal AChRs is indispensable for the proper development of neuromuscular junctions (NMJ) and muscle differentiation. g- is replaced by the e-subunit at the adult synapses. We are carrying out a single-channel study of binding and gating in fetal and adult AChRs activated by ACh, carbamylcholine (CCh), trimethylammonium (TMA), nicotine (Nic) or choline (Cho). The net agonist binding energy (the log of the high/low affinity ratio) for both receptor types was ACh>CCh>TMA>Nic>-Cho. However, for all ligands these energies for g-AChRs were ~-2.0 kcal/mol more favorable than in e-AChRs. By using AChRs having only one functional binding site (knockout and hybrid receptors), we dissected the independent contribution of each of the sites separately. For CCh, Nic and Cho the a-g binding site provides more agonist energy (~-2.0 kcal/mol) for gating compared to a-d and a-e. In e-AChRs the two binding sites are, however, symmetrical for ACh. Functional asymmetry is a manifestation of the differences in structure at the binding sites and we are investigating residues in the g-subunit that may provide the extra binding energy. Cho is a critical nutrient for fetal brain development and Nic exposure during development results in long-term abnormalities in the adult nervous system. We hope that understanding the biophysical properties of agonists in different AChRs subtypes will provide insight into these physiological conditions.

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Acidic Side-Chain Rotamers and their Impact on Ion Conduction through the Nicotinic Acetylcholine Receptor
Tyler J. Harpole[1], Claudio Grosman[2].
[1]Center for Biophysics and Computational Biology, University of Illinois, Urbana, IL, USA, [2]Molecular and Integrative Physiology, Center for Biophysics Computational Biology, University of Illinois, Urbana, IL, USA.
It has long been recognized that the glutamates at the intracellular mouth of the nicotinic acetylcholine-receptor (AChR) pore provide most of the stabilization to the passing cations. Moreover, it has recently been found that these ''intermediate-ring'' glutamates contribute asymmetrically to the single-channel conductance of the muscle receptor, with only two of the side chains being sufficient to achieve a wild-type value (Cymes and Grosman, 2012). Further single-channel recordings suggested that the difference between the two glutamates that contribute to the conductance and those that do not lies in the different conformations adopted by these side chains. To test these ideas in a framework of stereochemical rigor, we performed molecular simulations on a homology model of the open-channel muscle AChR that corrects for a register error in the original structural model. Using Brownian Dynamics, we found that different glutamate rotamers can account for dramatic changes in the computed single-channel conductance, and using Molecular Dynamics, we found that two of the four glutamates project their carboxylate oxygens into the pore while the other two glutamates project them away from the pore. In addition, using Brownian Dynamics simulations, we found that pore-facing glutamates are responsible for the majority of the single-channel conductance, whereas non-pore facing glutamates have a negligible impact on it. Overall, this configuration of glutamate side chains in the intermediate ring of charge (which is in remarkable agreement with the one we surmised on the basis of single-channel recordings) points to an unusual arrangement of acidic groups that may perhaps explain their bulk-like pKa values.