having a >10-fold range in E_2 (1.35 kcal/mol) were: δ (18′, 19′, 20′, 21′ and 23′) and β (21′, 24′ and 27′). These all showed approximately the same Φ value: 0.30. Comparing this value to those in the 'cap' region of other subunits, the Φ-order is $\alpha > \epsilon > \delta = \beta$. The high Φ and large energy changes are apparently only in the α subunit M2-cap, which indicates that domain plays a special role in AChR gating. NIH (NS 23513, 064969)

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Fourier Transform Coupled Tryptophan Scanning Mutagenesis of the Lipid Exposed DM3 And DM4 Transmembrane Domains of the Torpedo Californica Acetylcholine Receptor

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The lipid-protein interface is an important domain of the acetylcholine receptor (AChR) that has recently garnered increasing relevance. Several studies have made significant advances toward determining the structure and dynamics of the lipid-exposed domains of the AChR. However, there is still a need to identify and gain insight into the mechanism through which lipid-protein interactions regulate AChR function and dynamics. In this study, we extend the Fourier Transform coupled Tryptophan Scanning Mutagenesis (FT-TrpScanM) approach to monitor the conformational changes experienced by the δM3 and δM4 transmembrane domains of the *Torpedo californica* AChR, and to identify which lipid-exposed positions on these domains are potentially linked to the regulation of ion channel kinetics. The perturbations produced by periodic tryptohan substitutions along the δM3 and δM4 transmembrane domains were characterized by two-electrode voltage clamp and ^{125}I-labeled α-bungarotoxin binding assays. The periodicity profiles and Fourier Transform spectra of these domains revealed a thinner-elongated helical structure for the closed-channel state and a thicker-shrunken helical structure for the open-channel state. The difference in oscillation patterns between the closed- and open-channel states shows a substantial conformational change along these domains as a consequence of channel activation. These results support the recently proposed spring model for the αM3 transmembrane domain of the *Mus musculus* AChR. Furthermore, the present data demonstrates that the lipid-protein interface of the AChR plays an important role in the propagation of the conformational wave needed for channel gating.

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The Nicotinic Pharmacophore - Binding Interactions in the Neuronal α4β2 Receptor

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The α4β2 nicotinic acetylcholine receptor is a pentameric, neuronal, ligand-gated ion channel that binds nicotine, acetylcholine and structurally related agonists. Pharmacophore models for nicotinic agonists have been proposed since 1970. Central to each model is the presence of a cationic nitrogen and a hydrogen bond acceptor. We have identified the binding partners for both components. Binding of the cationic nitrogen of nicotine is mediated through a cation- π interaction at αW154 in loop β of the extracellular domain as well as a hydrogen bond to the backbone carbonyl of αW154. The hydrogen bond acceptor moiety (the pyridine nitrogen of nicotine) makes a hydrogen bond to the backbone NH of L119 of the complementary subunit. These interactions were also shown to be relevant for other nicotinic agonists at both receptor stoichiometries, $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$. Taken together, these data represent a completed nicotinic pharmacophore and offer insight into the design of new therapeutic agents that selectively target these receptors.

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Energy Changes for Small Molecules at the Acetylcholine Receptor-Channel Transmitter Binding Site

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Acetylcholine receptors (AChRs) are ligand-gated ion channels involved in vertebrate neuromuscular transmission. Binding of acetylcholine (ACh, the endogenous neurotransmitter) increases the equilibrium constant of the 'gating' isomerization and the probability that ion channel domain adopts an ion-permeable conformation. The ratio of the isomerization equilibrium constants with two and zero bound agonist molecules (E_2/E_0) is equal to the ratio of agonist affinities in the inactive vs. active conformations of the protein, at two identical binding sites [$(Kd/Jd)^2$]. We define R=Kd/Jd. Different agonists have different efficacies because they have different R values (E_0=6.5E-7). For ACh (=100 mV, 23 °C, mouse $\alpha_2\beta\delta\epsilon$), E_2=28.2 and R=6500. For choline, E_2=0.05 and R=270. For tetramethylammonium and carbachol, E_2=6.8 and R=3300. As part of a larger project to engineer the AChR transmitter binding site, we have measured E_2 and R values for a series of small molecular fragments. The goal is to generate an atomic map of the isomerization-induced energy changes at the ligand [kcal/mol=0.59ln(R)]. We first studied a series of five- and six- membered N-containing rings (fragments of nicotine). In the nicotine-bound AChBP structure, the pyrrolidine ring of the ligand is at the center of an aromatic 'box'. Systematic substitutions of the atoms of this ring are being made and the corresponding R-values are being determined. Preliminary work suggests that the following small molecules activate wild-type AChRs: 1,1-Dimethyl pyrrolidin-1-ium (E_2~0.26, R~630), 1,1-Dimethyl Thiazolidin-1-ium (E_2~0.68, R~1016) and 4,4-Dimethyl morpholinium (E_2~0.3, R~675).

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Thinking Outside the Box: Residues that Shape the Agonist Binding Site of Nicotinic Acetylcholine Receptors

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Nicotinic acetylcholine receptors (nAChRs) are pentameric neurotransmitter-gated ion channels that mediate rapid synaptic transmission throughout the central and peripheral nervous systems. They are well-established targets for small molecule treatments for Alzheimer's disease, schizophrenia, Parkinson's disease, epilepsy, autism, and smoking cessation. To date, 17 human genes have been identified that code for nAChR subunits, termed α1-α10, β1-β4, γ, δ, and ϵ. nAChRs are subdivided into two main categories: the prototypical muscle-type receptor with a precise stoichiometry of $(\alpha1)_2\beta\delta\gamma$ (fetal form) and the ''neuronal'' nAChRs that are formed from various combinations of α2-α10 and β2-β4 subunits. Crystal structures of the acetylcholine binding protein have indicated that various ligands are positioned into a localized binding pocket formed from a cluster of conserved aromatic residues, termed the ''aromatic box''. Previous work from our lab has indicated that a common component of the neurotransmitter-binding occurs through the cationic center of the ligand and the face of an aromatic amino acid, termed the cation-π interaction, as well as hydrogen bonding interactions. Together, these binding events cause a change in protein structure permitting ion flow through the channel pore. In the immediate vicinity of the agonist binding site, all nAChR subtypes show identical amino acid compositions, yet significant pharmacological variations are seen. Previous work identified a point mutation, G152K, located near but not directly contributing to the agonist binding site in the α7 nAChR that is critical to agonist potency. Interestingly, this residue is a glycine in the low affinity receptors, such as the muscle-type and α7 nAChRs, but a lysine in the high affinity α4β2 nAChR. Here, we investigate the importance of this residue (G or K) in influencing acetylcholine and nicotine binding interactions in the muscle-type, α7 and α4β2 nAChRs.

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Efficient Isolation and Characterization of Nicotinic Acetylcholine Receptor from *Torpedo Californica* using Lipid Analog Detergents

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The effect of detergent solubilization on nicotinic acetylcholine receptor (nAChR) function has been extensively studied by several laboratories with the ultimate goal of characterizing the dynamic detergent-lipid-protein interactions of functional nAChR in both native and reconstituted membranes, as well as the detergent-solubilized state. These studies have provided substantial data on suitable detergents for solubilization, purification and functional reconstitution of the nAChR obtained from the electric organ of *Torpedo californica* electric rays. However, the molecular mechanisms by which particular detergents influence nAChR function remain poorly understood. In the present study, we characterized the effect of detergent solubilization and affinity column purification on *Torpedo* nAChR by using a series of lipid-like detergent with similar acyl composition to the most abundant fatty acid found in the native tissue of *Torpedo* (16:0, 18:0, 16:1 16:0)as well as cholesterol- analog detergents. . Fatty acid analogs included members of the Fos-choline (FC) family of detergents (FC-12, −14, −16 and lyso-FC), while cholesterol analogs were represented by cholate, taurocholate and CHAPS. Each detergent was used to s solubilize and purify the nAChR using established affinity column protocols, followed by analytical size exclusion chromatography (A-SEC) to probe the stability and aggregation state of the nAChR in solution, as well as planar lipid bilayers to probe ion channel function. The overall results showed that the