subunit, αCys$^{192/193}$,αTyr^{198} (Loop C), γTrp^{55} (Loop D) and γTyr^{117} (Loop E) of the agonist binding site. [^{3}H]Cytisine efficiently photolabels the agonist binding sites of AChBPs and the α4β2 nAChR (both α4 and β2 subunits are labeled). The sites of [^{3}H]Cytisine labeling in *the Torpedo* nAChR and in AChBPs and the α4β2 nAChR (ongoing experiments), along with results from cytisine docking simulations will be used to compare modes of interaction of α4β2 nAChR-selective and subtype non-selective agonists (e.g. ACh) to nAChRs and AChBPs.

678-Pos

Mutations at Ag153 in Nicotinic Acetylcholine Receptors increase the Un-Liganded Gating Equilibrium Constant

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Rarely, an AChR mutation will increase the affinity of the resting receptor for ACh (reduced K_d). One such example is αG153S, a cause of slow-channel congenital myasthenic syndrome. The presence K vs. G side chain at this position in neuronal α4β2 vs. neuromuscular $(\alpha1)_2\beta\delta\epsilon$ AChRs has been suggested to be the basis for the high affinity of nicotine only to the former. In the present study we examined AChRs having a side chain substitution at αG153(ACDKPRSWY). We measured (single-channels, $(\alpha1)_2\beta\delta\epsilon$; −100 mV, 23 °C, cell-attached) the equilibrium dissociation constant (K_d) for the partial agonist choline for the ADRSK mutants, and the diliganded (E_2) and un-liganded (E_0) isomerization ('gating') equilibrium constants for all mutants. The αG153S mutant (wild-type) parameters were: E_2~0.38 (0.05), E_0~3.5E10-6 (1.2E-7), K_d~540 ∈ μM (4 mM) and J_d ~1.64 ∈ μM (6 ∈ μM). The fold-changes in each of these parameters were: E_2, 7.6; E_0 29; K_d, 7.4; and J_d, 3.7. The largest effect of the S substitution was on E_0. Preliminary results for the other 153 mutants also show even larger changes in E_0. The G153K mutation showed a ~210-fold increase in E_0. It is possible that the above effect of the G153K mutation with regard to nicotine activation is due, al least in part, to the increase in E_0. This alone would reduce the EC_{50} of macroscopic currents and effectively change the appearance of nicotine, from a weak to a strong agonist.

679-Pos

A Transmembrane Binding Site at a Subunit Interface for *Torpedo* Nicotinic Acetylcholine Receptor Potentiators And Inhibitors

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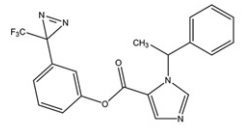
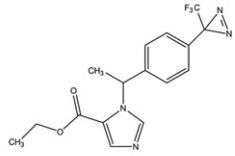
Photoreactive derivatives of the general anesthetic etomidate have been developed to identify their binding sites in $GABA_AR$ and nAChRs. One such drug, [^{3}H]TDBzl-etomidate, acts as a positive allosteric potentiator of *Torpedo* nAChR and binds to a novel site in the transmembrane domain at the γ-α subunit interface (*Nirthanan et al 2008, JBC 283:22053-62*). To extend our understanding of the binding site(s) of nAChR allosteric modulators, we developed [^{3}H]TFD-etomidate containing the photoactivatable trifluromethyldiazirinyl group on etomidate's benzene ring. [^{3}H]TFD-etomidate inhibited ACh-induced currents (IC_{50} = 4 ∈ μM), but inhibited the binding of [^{3}H]phencyclidine to the *Torpedo* nAChR ion channel with IC_{50}s of 2.5 and 0.7 mM in the resting and desensitized states, respectively. In the presence of the ion channel blocker tetracaine, [^{3}H]TFD-etomidate photolabeled amino acids at the lipid interface (αM4 and βM4) and at the γ-α subunit interface, αM2-10, γMet299 and γMet295. In the absence and presence of agonist, [^{3}H]TFD-etomidate photoincorporated at low efficiency within M2 ion channel domain (M2-6, M2-9 and M2-13). These results suggest that the γ-α subunit interface is a binding site for *Torpedo* nAChR negative ([^{3}H]TFD-etomidate) and positive ([^{3}H]TDBzl-etomidate) allosteric modulators.


TDBzl-Etomidate


TFD-Etomidate

680-Pos

Dynamics of Acetylcholine Receptor-Channel Gating: Pre-M1 of the Epsilon Subunit

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Neuromuscular acetylcholine receptors (AChRs) mediate fast chemical synaptic transmission. Neurotransmitters bind to two sites in the α subunit extracellular domain (ECD) and trigger an isomerization that opens/closes the pore in the transmembrane domain (TMD). The TMD/ECD interface is a complex and important domain that links 'binding' and 'gating'. We have examined one component of this interface, the pre-M1 region (linker connecting β10-strand of the ECD to M1 of the TMD) in both α and non-α subunits. We recorded single-channel currents (mouse $\alpha_2\beta\delta\epsilon$; HEK cells, −100mV, cell-attached) and estimated gating rate constants and rate-equilibrium relationships for AChRs having a mutation at the pre-M1 linker of the ϵ subunit. This region is a stretch of five amino acids (217-221) that contains three positively charged residues (ϵR217, ϵR218, ϵK219). ϵR218 is homologous to αR209 and is conserved among other AChR subunits. AChRs were activated by 20mM choline or 0.5mM acetylcholine. So far we have measured the gating rate constants of ϵ subunit mutants at positions R217, R218, and K219. R217D and N decreased the diliganded equilibrium isomerization constant (E_2) by only 1.5-fold. R218A and N decreased E_2 by 184-fold and 235-fold, respectively. K219A, D and N increased E_2 by <6-fold. So far, out of the three scanned positions, R217 and K219 show moderate energy changes (~1 kcal/mol) and R218 shows a larger energy sensitivity (~3.2 kcal/mol). In all cases, both the forward and backward rate constants changed with the mutation, with the larger effect being on the forward rate constant. The results indicate that ϵ pre-M1 changes energy ('moves') during gating isomerization. More experiments should reveal more precisely both the energy sensitivity of each position and the relative timing of the side chain motions within the AChR isomerization. Supported by NIH (NS-23513, NS-064969).

681-Pos

Potential Implications of Cholesterol and Phosphatidylinositol 4,5-Bisphosphate (PIP2) Interactions With The Cholesterol-Sensitive AC418W Acetylcholine Receptor Mutation at Lipid Rafts

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Lipid rafts, specialized membrane microdomains in the plasma membrane that are rich in cholesterol and sphingolipids, are hot-spots for a number of important cellular processes. The novel acetylcholine receptor (AChR) mutation αC418W was shown to be cholesterol-sensitive (Santiago et al., 2001) and to accumulate in microdomains rich in the membrane raft marker protein caveolin-1 (Baez-Pagan et al., 2008). The objective of this study is to gain insight into the mechanism by which lateral segregation into specialized raft membrane microdomains regulates the activatable pool of AChRs. We performed Fluorescent Recovery After Photobleaching (FRAP) experiments and whole-cell patch clamp recordings of GFP-encoding *mus musculus* AChRs transfected into HEK 293 cells to assess the role of cholesterol levels in the diffusion and functionality of the AChR (WT and αC418W). Our findings support the hypothesis that a cholesterol-sensitive AChR might reside in a specialized membrane microdomain; however, when cholesterol is depleted in vitro or in vivo, the caveolae disrupt and the cholesterol-sensitive AChRs are released to the pool of activatable receptors. Furthermore, our results suggest that phosphatidylinositol 4,5-bisphosphate (PIP_2), which is concentrated in lipid rafts, may be responsible for the increase in whole-cell currents observed upon cholesterol depletion for the αC418W AChR mutant.

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682-Pos

The N Terminal M2 Cap of Nicotinic Acetylcholine Receptors

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Nicotinic acetylcholine receptors (AChRs) isomerize ('gate') between a low affinity/non-conducting (R) and a high affinity/ion-conducting (R*) conformation. Many different residues in this large, heteropentameric membrane protein have been shown to contribute to the free energy difference between the R and R* structures. Previously we showed that at the N-terminal 'cap' of the M2, pore-lining helix (positions 18′-27′) there are large and early energy changes in the α subunit whereas in the ϵ subunit the residues are mostly iso-energetic. We measured the energy sensitivity (computed from the apparent range in diliganded equilibrium constant E_2) and relative timing (Φ values) of the isomerization movements of residues in the N-terminal 'cap' domain of the δ and β subunits. We used cell attached, single-channel analysis to quantify the energetic consequences of point mutations (mouse $\alpha_2\beta\delta\epsilon$, HEK 293 cells, +70 mV pipette potential, cell-attached, 22° C , activated by 20 mM choline or 0.5 mM ACh). The probed positions were δ: I18′, S19′, K20′, R21′, L22′, P23′, A24′, T25′ and M27′ (a total of 43 mutants) and β:A19′, K21′, V22′, P23′, E24′, S26′, and L27′ (a total of 34 mutants). Of these, only one position, δS19′, showed a >2 kcal/mol range-energy (Φ = 0.20 ± 0.07). The only residues

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)

683-Pos

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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684-Pos

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.

685-Pos

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).

686-Pos

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.

687-Pos

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