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

Prasad Purohit, Anthony Auerbach.
Suny at Buffalo, Buffalo, NY, USA.

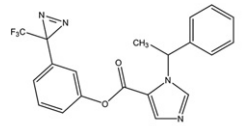
Rarely, an AChR mutation will increase the affinity of the resting receptor for ACh (reduced K_d). One such example is αG153S, a cause of slow-channel congenital myasthenic syndrome. The presence K vs. G side chain at this position in neuronal α4β2 vs. neuromuscular $(\alpha1)_2\beta\delta\epsilon$ AChRs 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.

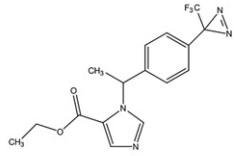
679-Pos

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

Ayman K. Hamouda[1], Deidre Stewart[2], S. Shaukat Husain[2], Jonathan B. Cohen[1].
[1]Harvard Medical School, Boston, MA, USA, [2]Massachusetts General Hospital, Boston, MA, USA.

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

Iva Bruhova, Archana Jha, Anthony Auerbach.
State University of New York at Buffalo, Buffalo, NY, USA.

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

Jessica Oyola-Cintrón[1], Daniel Caballero-Rivera[1], Leomar Ballester[1], Leonardo Martínez[2], Carlos J. Nogueras[1], Ramón Y. Ríos-Morales[1], Orestes Quesada[1], José A. Lasalde-Dominicci[1].
[1]UPR-Rio Piedras Campus, San Juan, PR, USA, [2]California State University Dominguez Hills, Carson, CA, USA.

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.

This work was supported by NIH Grants 2RO1GM56371-12 and 2U54NS43011.

682-Pos

The N Terminal M2 Cap of Nicotinic Acetylcholine Receptors

Shaweta Gupta, Snehal Jadey, Prasad Purohit, Anthony Auerbach.
University at Buffalo, Buffalo, NY, USA.

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