Cys-Loop Receptors

3256-Pos Board B411
Loop-C and a Cyclic Activation Scheme for Acetylcholine Receptor-Channel (AChRs)
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The ‘capping’ of loop-C at each transmitter binding site has been proposed to initiate the AChR channel-opening isomerization. We recorded single-channel currents from various loop-C constructs (adult mouse; both α-subunits, 188-198: VPFYVTITTPY) with and without ACh, to probe its role in binding vs. gating. One construct (11gly) had glycines at all positions, and others had n=9, 7, 5 or 3 glycines. The loop A mutation αA96H was added as a background to increase constitutive activity. 1) None of these constructs altered the unliganded gating activity. 2) The open-probability of the 11gly-construct was not increased by 1mM ACh. 3) Reverting only two amino acids (bold) in the 11gly-construct to tyrosines generated AChRs that were activated by ACh, again without altering unliganded gating. These results suggest that loop-C is mainly involved in agonist binding.

A sequential kinetic scheme has been used to describe brief and long unliganded openings in AChRs with background pore mutations (βM2,9-S). We compared this scheme with others using AChRs activated by low [ACh], in a construct that had only one functional binding site (ζW149F+90°S+P121R). The intervals were fitted across-concentrations using three different kinetic schemes, each having four states (C, O, A, and AO). The best fit was obtained by using the full cycle (MWC model). The estimated increase in the gating equilibrium constant with ACh (74-fold) was similar to that obtained previously by saturating the binding site (~690-fold). These results suggest that a cyclic scheme describes brief/long unliganded openings at low [ACh] and can be used to estimate the association/dissociation rate constants to the high-affinity, open transmitter binding site.

3257-Pos Board B412
Intrinsic Gating Energy of Fetal-Type Neuromuscular Acetylcholine Receptors
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Fetal-type neuromuscular AChRs are composed of (z1),β0βy subunits, whereas adult-type have the stoichiometry (z1)b,b.e. γ-AChRs are indispensable for functional synapse formation, and mutations in this receptor subtype have been associated with congenital myasthenia, multiple pterygium syndrome and premature death. Cellular responses to transmitter molecules are influenced by a receptor’s unliganded gating energy, G0. In mouse e-AChRs, G0 = 8.4 kcal/mol (~100 mV). We estimated this intrinsic gating energy in γ-AChRs (single-channels, HEK cells), from measurements of the unliganded gating equilibrium constant En (G0 = 0.59nEm). Em was extrapolated from observed E0 values in γ-AChRs having different combinations of background mutations that increased constitutive activity. First, 12 different background mutations were characterized separately (by measuring the unliganded gating equilibrium constant with choline) and were found to alter only G0 and not the energy from the agonist. The effects of the mutations in γ-AChRs were approximately the same as in e-AChRs. The extrapolation was based on two assumptions, that the change in the diliganded gating equilibrium constant (E2) is caused by only an equivalent change in G0, and that the G0 changes are independent. A log-log plot of E2 vs. E1 resulted in straight line with slope 0.94 ± 0.05, validating the two assumptions. From the extrapolation, E1 = 4.8 x 10^-3, or G0 = 9.9 kcal/mol. This value is ~+1 kcal/mol larger than e-AChR value. The inherent ‘slower’ kinetics of γ- vs. e-AChRs may, in part, be attributed to the elevated intrinsic energy barrier of the gating transition state. The results are discussed in the light of a MWC thermodynamic cycle, to derive other equilibrium and kinetic parameters for γ-AChRs. This works lays the foundation for understanding the fetal-type AChR transmitter binding site.

3258-Pos Board B413
α-Conotoxin Regia: Targeting Nicotinic Acetylcholine Receptors: Mutagenesis Studies Improving Selectivity and Potency
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Nicotinic acetylcholine receptors (nAChR) play important roles in various physiological functions including pain, anxiety, fatigue, memory and learning. The α3β4 nAChR subtype has been implicated in various conditions, including lung cancer and nicotine addiction. Selective nAChR antagonists are invaluable for evaluating the functional roles of nAChR subtypes in the nervous system. α-Conotoxins that act as nAChR antagonists have been identified from the venom of predatory marine cone snails. We previously reported the discovery of a new α47-conotoxin, RegIA, isolated from Conus regius. RegIA inhibits acetylcholine (ACh)-evoked currents mediated by α3β2, α3β4 and α7 nAChR subtypes. RegIA is the most potent α3β4 nAChR antagonist known, to date, with an IC50 of 48 nM (Franco, A., et al. 2012, Biochem. Pharmacol. 83:419-426). This study aims to understand and improve RegIA’s selectivity profile at the α3β4 nAChR subtype. It uses alanine scan mutants of non-cysteine residues within loop 2 of RegIA, which were synthesised using solid-phase peptide synthesis. These mutants were functionally tested on nAChRs expressed in Xenopus oocytes using two-electrode voltage clamp recording. Of these mutants, [N11A] and [N12A] RegIA exhibited a three-fold increase in selectivity for α3β4, compared with the α3β2 nAChR subtype. Molecular dynamics simulations of RegIA bound to the ACh binding pocket of the α3β4 nAChR subtype revealed crucial molecular interactions, including residues of the “esthetic cage” at the α3(+) principal face (W174, Y215, Y222) as well as the αβ(-) complementary face (W79, K81, R135). This study extends our understanding of RegIA interactions with various nAChR subtypes and elucidated the key residues involved on both the peptide and the receptor binding site. Furthermore, we have obtained valuable information about the future design and development of α3β4-selective drugs that could target lung cancer and nicotine addiction.

3259-Pos Board B414
Energetic Changes in the β Subunit during Gating of the Muscle Nicotinic Receptor
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The transmitter-binding sites in the adult muscle-type nicotinic receptor are located at the interfaces between the α subunit and the δ or ε subunits, while the β subunit does not contribute to binding. The β subunit is termed a "structural" subunit as a result. We made mutations to 27 residues in the mouse muscle β subunit (2 to 4 mutations at each location) to measure the consequences on the channel opening and closing rates. We analyzed the results in terms of the effect on the ratio of the opening to closing rates (θ) and, when sufficiently large changes occurred, on the slope of the logarithmic relation between the opening rate and θ (φ). φ can be interpreted in terms of the timing during channel activation when a given residue makes an energetic contribution to gating. We examined 12 positions in the extracellular domain (ECD) of the β subunit, chosen because mutations to homologous residues in other subunits had large effects on θ. In contrast, mutations at the locations in β produced small changes in gating. We examined 15 positions in the second transmembrane (TM2) and TM2-TM3 linker regions. The majority of locations showed larger changes in θ (typically > 1 kcal/mole). Estimates of φ were consistent with the idea that the energetic contributions from residues in the β subunit occurred later than for homologous residues in the α subunit but before residues in δ. Overall, the data suggest that the ECD and the interface between the ECD and TM domains of β make little contribution to the energetics of gating. However, channel-lining residues do contribute, although the data suggest that the timing is later than for the α subunit. (NS22356 & NS72770)

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Effect of Membrane Hydrophobic Thickness on the Uncoupling of Binding/Gating in the Nicotinic Acetylcholine Receptor
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The effects of allosteric modulators on the nicotinic acetylcholine receptor (nAChR) are usually interpreted in terms of a model involving pre-existing resting, open, and desensitized conformations. The nAChR also adopts a lipid-dependent non-activatable conformation, referred to as the “uncoupled” state. Neither the global secondary structure nor the thermal denaturation of the nAChR is greatly affected by uncoupling, although uncoupling leads to an increase in peptide hydrogen exchange suggesting that previously buried peptide hydrogens become exposed to aqueous solvent. We propose that uncoupling results from a weakening of the physical interactions at the interface between the agonist binding and transmembrane pore domains because of lipid-dependent alterations of M4/Cys-loop interactions. As a preliminary test of this model, we reconstituted the nAChR into phosphatidyethanolamine...