1724-Pos Board B454

The Role of the Fifth Subunit on Sensitivity of α4β2-Containing Nicotinic Acetylcholine Receptors to Allosteric Modulators

Constanza Alcaino.
Oxford Brookes University, Oxford, United Kingdom.

α4β2 nicotinic acetylcholine receptors (nAChRs) belong to the family of pentameric ligand gated ion channels, which includes muscle nAChRs, 5-HT3, GABA receptors type A and C, and glycine receptors. α4β2 nAChRs are the predominant heteromeric nAChR in the brain. The α4β2 nAChR is the predominant nAChR subtype in the brain. Here, it constitutes one of the most important modulatory receptor systems influencing activities such as cognition, mood, consciousness and nociception and converging evidence indicates that this type is a key mediator of the rewarding and reinforcing effects of nicotine. α4 and β2 nAChR subunits assemble into alternate stoichiometries (α4β2)2α4 and (α4β2)2β2 that display remarkable differing sensitivity to activation by agonists and allosteric modulators (Moroni et al., 2006; Carbone et al., 2009). Positive allosteric modulators of α4β2 nAChRs have attracted considerable interest as potential tools for the treatment of cognitive impairment, chronic pain and depression, and as an aid for smoking cessation. However, despite the therapeutic potential of these types of compounds, it is not yet know the structural elements determining stoichiometry-specific sensitivity to α4β2 nAChR allosteric modulators. Here, we have used fully concatenated (α4β2)2α4, (α4β2)2β2 and (α4β2)2α5 nAChRs in combination with chimeric receptors, single point mutations and homology models of the alternate α4β2 nAChRs to determine the effects of desformylflustrabromine (dFBr), a selective positive allosteric modulator of α4β2 nAChR, on α4β2nAChRs and structural elements responsible for those effects. We show that dFBr effects are receptor stoichiometry-dependent and that the fifth subunits is a major determinant of the potentiating effects of dFBr.

1725-Pos Board B455

An Integrative Model of the Pleiotropic Effects of Genistein and Cyclosporine a in Functional Upregulation of Alpha 7 nAChRs

Joseph Farley[1], Mohammad Faridul Islam[1], Patrick Beard Schwartz[1], Kristi DeBoeuf[1], Jed Rose[2].
[1]Neuroscience, Indiana University, Bloomington, IN, USA, [2]Depart of Psychiatry and Behavioral Sci., Duke University Med School, Durham, NC, USA.

We have identified several distinct mechanisms by which inhibitors of tyrosine kinases (genistein) and calcineurin/PP2B (cyclosporine A, CsA) produce functional upregulation of α7 Rs expressed in *Xenopus* oocytes. These effects varied with the duration of drug exposure and time course of expression. Genistein's potentiation of peak currents was greater early in expression (2 vs. 5 days following α7-cRNA injection), and for prolonged drug exposures (24 hr vs 1 min). CsA's potentiation was limited to 24 hr exposure and was independent of expression time. When combined 5-days post-injection, genistein and CsA produced a facilitation greater than either alone: 5-6 fold for CsA + 1 min genistein vs. 2.6-fold for CsA and 2.3-fold for 1 min genistein; 8.5 fold for CsA + 24 hr genistein vs. 2.6 fold for CsA and 4.3 fold for 24 hr genistein. Similarly, 2-days following cRNA injections, 24 hr CsA plus 1 min genistein produced facilitation greater than either alone: 4.5 fold vs. 1.5 for CsA and 2.8 for genistein. However, the combination of CsA and genistein (24 hr) produced facilitation (5.3 fold) that was *less* than genistein alone: 1.5 for CsA, and 12 for genistein. We propose a model to explain this pattern that assumes: 1) 1 min genistein's effects are exclusively PAM, while 24 hr genistein's effects are PAM and non-PAM (all effects are positive), 2) the effects of CsA alone are net positive, but reflect two opposing processes. CsA facilitates currents by increasing surface membrane numbers of α7-Rs and/or single-channel gating. But CsA also inhibits cRNA-translation and/or chaperoning of α7-Rs. The inhibitory effect is greater early in expression, reducing the number of functional α7-Rs distal to the ER/Golgi pathway that genistein can positively interact with.

1726-Pos Board B456

Structure Function Studies at Two Different Nicotinic Acetylcholine Receptor Subtypes

Michael R. Post[1], Henry A. Lester[2], Dennis A. Dougherty[1].
[1]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, USA, [2]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA.

The nicotinic acetylcholine receptor (nAChR) is involved with regulating the release of neurotransmitter in the synapse. This research focuses on two subtypes of neuronal nAChR, the α6β2 and α4β2 receptors. Subtypes containing the α6 and β2 subunits are found mainly in dopaminergic neurons. The acetylcholine binding site in these subtypes, which sits at the interface of the two subunits, is an important therapeutic target. Expression and activation of a pure population of α6β2 receptors in *X. laevis* oocytes has previously never been reported. This study used two mutations in the β2 M3-M4 loop and a reporter mutation in the M2 helix of each subunit to successfully express α6β2 receptors. Expression has been optimized, and a method to control subunit stoichiometry has been developed. We then used unnatural amino acid mutagenesis to develop a binding model for acetylcholine and nicotine at the α6-β2 subunit interface.

The α4β2 subtype is the most prevalent nAChR subtype found in the brain and is implicated in many neurological disorders. Previous work has established a hydrogen bond interaction between the pyrrolidine nitrogen of nicotine and the carbonyl backbone adjacent to Trp149 in the α4 subunit. N'-methylnicotinium, which has a quaternary amine at the pyrrolidine nitrogen and cannot donate a hydrogen bond, was synthesized in order to probe this hydrogen bond donating interaction. Through a double-mutant cycle analysis utilizing an α-hydroxy acid mutation to perturb the carbonyl backbone hydrogen bond acceptor, we have determined that the hydrogen bonding interaction has a coupling energy of ~2 kcal/mol.

1727-Pos Board B457

Identifying Motifs Essential for Selective Activation of Nicotinic Acetylcholine Receptors

Christopher Marotta[1], Henry A. Lester[2], Dennis A. Dougherty[1].
[1]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, USA, [2]Department of Biology, California Institute of Technology, Pasadena, CA, USA.

Neuronal Nicotinic acetylcholine receptors (nAChRs) are found throughout the brain and have vital roles in memory and learning. In addition, these ligand gated ion channel are major targets of drug research for neurological disorders. Here, the two stoichiometries of α4β2 nAChRs expressed in *Xenopus* oocytes were probed using two agonists that display stoichiometry selectivity: Sazetidine A and NS9283. The results from these studies led to the following conclusions that may be generalized to the nAChR family: 1) an agonist must be bound to each α subunit in the receptor in order to fully activate the receptor and 2) three key residues located on the complementary side of the subunit dictate agonist selectivity. Through better understanding of agonist binding, more potent and selective responses with minimized off target responses can be obtained.

1728-Pos Board B458

Insights into the Distinctly Different Sensitivities of α7 and α7β2 nAChRs to the Volatile Anesthetic Isoflurane

David D. Mowrey[1], Qiang Liu[2], Vasyl Bondarenko[3], Qiang Chen[3], Edom Seyoum[3], Yan Xu[3], Jie Wu[2], Pei Tang[3].
[1]Computational Biology, University of Pittsburgh, Pittsburgh, PA, USA, [2]Division of Neurology, Barrow Neurological Institute, St Joseph's Hospital and Medical Center, Phoenix, AZ, USA, [3]Anesthesiology, University of Pittsburgh, Pittsburgh, PA, USA.

Nicotinic acetylcholine receptors (nAChRs) mediate the fast synaptic transmission in the central and peripheral nervous systems. They are also targets of general anesthetics. Among the nAChR subtypes, α7 and α4β2 nAChRs are the most abundant subtypes found in the brain. Despite their high sequence homology, the α4β2 nAChR is hypersensitive but the α7 nAChR is insensitive to volatile general anesthetics. The underlying cause for the functional discrepancy between these homologous receptors presents an intriguing enigma. In this study, we have found that the α7β2 nAChR is hypersensitive to the volatile anesthetic isoflurane and that the β2 subunit is primarily responsible for conferring anesthetic sensitivity. Using high-resolution NMR, we identified intrasubunit isoflurane binding sites at the extracellular and intracellular ends of the β2 and α7 transmembrane domains, respectively. Cavity analyses revealed that β2, but not α7, has a pocket large enough to accommodate isoflurane binding to the extracellular end of the transmembrane domain. Variations in pocket size result from subtle structural differences between β2 and α7, where the angle between the TM2 and TM4 helices differs by ~7° between β2 and α7. While isoflurane binding perturbed dynamics for both β2 and α7, only the binding to the extracellular end of the β2 transmembrane domain produced significant dynamics changes for the pore-lining residues. The study suggests that a subtle structural difference could affect drug binding. Furthermore, only the drug binding that affects the dynamics of the channel gate is able to generate the functional effect. Supported by NIH (R01GM66358, R01GM56257, and R37GM049202).