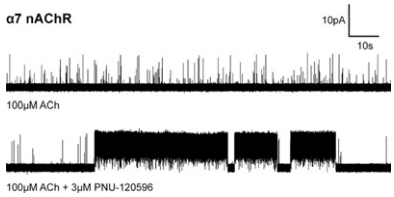
1880-Plat
Quantitation and Dynamics of Dc-Sign Microdomains on the Cell Surface
Ping Liu, Michelle S. Itano, Xiang Wang, Aaron K. Neumann, Nancy L. Thompson, Ken Jacobson.
University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
DC-SIGN, a dendritic cell (DC)-specific C-type lectin that binds many different pathogens, is a receptor for HIV-1 and promotes subsequent infection of T cells. Our previous study demonstrated that DC-SIGN forms sub-micron scale domains on the surface of immature DCs as well as cell lines that ectopically express DC-SIGN. In this study, we investigated the occupancy and dynamics of DC-SIGN surface domains. First, we developed a single molecule approach, based on total internal reflection fluorescence (TIRF) microscopy, to examine the number of DC-SIGN molecules in a single domain. By comparing the brightness of a single fluorophore to the total brightness of a fluorescently-labeled DC-SIGN domain, we show that the number of DC-SIGN proteins in a domain ranges from a few to over hundred molecules. The size of each domain, as measured from the full-width half-maximum (FWHM) of a Gaussian fit of the emission profile of a domain, varies from the diffraction limit to micron scale. Second, scanning fluorescence correlation spectroscopy (sFCS) and fluorescence recovery after photobleaching (FRAP) were carried out to investigate the mobility of DC-SIGN in a domain, both of which showed that DC-SIGN is highly immobile. This was corroborated by single particle tracking of quantum dots attached to DC-SIGN molecules within a domain. By contrast, photobleaching of a lipid modified-fluorescent protein (mRFP) in a DC-SIGN domain area showed full recovery similar to that outside of the domain, indicating that lipids inside the DC-SIGN domain are highly mobile and and can freely exchange with the surrounding membrane. Finally, a deletion mutation study of DC-SIGN was carried out to further investigate which moiety of DC-SIGN facilitates surface domain formation. Supported by NIH GM41402.

Platform AI: Acetylcholine Receptors & Channel Regulation

1881-Plat
Spiroimine Toxins in Complex with Nicotinic Acetylcholine Receptors: Structure and Dynamics
Rómulo Aráoz[1], Laurent Chabaud[2], Catherine Guillou[2], Jordi Molgó[1], **Bogdan I. Iorga**[2].
[1]Institut de Neurobiologie Alfred Fessard, Laboratoire de Neurobiologie et Developpement, Gif sur Yvette, France, [2]Institut de Chimie des Substances Naturelles, Gif sur Yvette, France.
Macrocyclic spiroimine phycotoxins belong to an emerging class of chemical agents associated with marine algal blooms and shellfish toxicity. [1] Representative members of this family are gymnodimines, spirolides, pinnatoxins, pteriatoxins, and spiro-prorocentrimine. Gymnodimine A and 13-desmethyl spirolide C have been shown to be potent antagonists of nicotinic acetylcholine receptor (nAChR), and have prompted the development of new tools to detect these spiroimine toxins in shellfish and for the design of novel drugs. [2,3] Very recently, crystal structures of Acetylcholine Binding Protein complexed with gymnodimine A and 13-desmethyl spirolide C were solved, thus providing insight into the orientation and the conformation adopted by these ligands in the binding pocket and their interactions with neighbouring amino-acid residues. [3]
In this work we have explored the interaction of several members of spiroimine toxins family with different nAChR subtypes. Given the lack of general methods for flexible docking of macrocyclic ligands, we have developed a two-step, general approach to circumvent this problem, and applied it specifically to the docking of macrocyclic spiroimines on nAChRs. Subsequent molecular dynamics studies of the resulting receptor-ligand complexes allowed us to identify the key interactions responsible for the high affinity and target selectivity experimentally determined for some of these phycotoxins.
[1] J. Molgó, E. Girard, E. Benoit, "The cyclic imines: an insight into this emerging group of bioactive marine toxins" in: Phycotoxins: Chemistry and Biochemistry (Eds.: Y. H. Hui, L. M. Botana), Blackwell Publishing, 2007, pp. 319-355.
[2] R. Kharrat, D. Servent, E. Girard, G. Ouanounou, M. Amar, R. Marrouchi, E. Benoit, J. Molgó, J. Neurochem., 2008 107, 952-963.
[3] Y. Bourne, Z. Radic, R. Araoz, T. T. Talley, E. Benoit, D. Servent, P. Taylor, J. Molgó, P. Marchot, Proc. Natl. Acad. Sci. USA, 2010, 107, 6076-6081.

1882-Plat
Potentiation of the Human α7 Acetylcholine Receptor: A Single-Channel Study
Corrie J.B. daCosta, Chris R. Free, Steven M. Sine.
Mayo Clinic College of Medicine, Rochester, MN, USA.
Allosteric modulation of neuronal nicotinic acetylcholine receptors by exogenous ligands is a promising strategy for the treatment of several neurological disorders including Alzheimer's disease and Schizophrenia. To gain insight into the molecular mechanisms underlying allosteric modulation, we have examined with single-channel recordings, the potentiation of human α7 nicotinic acetylcholine receptors by both a Type I, and a Type II positive allosteric modulator (PAM). Dwell time analysis reveals that both NS-1738 (Type I PAM) and PNU-120596 (PNU; Type II PAM) lead to prolonged openings in the presence of agonist. While co-application of NS-1738 with agonist results in the appearance of a single new component in open time histograms, co-application of PNU leads to two additional components, the longest of which coalesce into clusters of openings whose frequency and duration depend on PNU concentration. At saturating concentrations of PNU these clusters last as long as several minutes, representing a ~10,000-100,000 fold prolongation of the notoriously brief α7 openings. The differences between Type I and Type II potentiation evident at the microscopic/single-channel level will be discussed in the context of the variable phenotypes previously characterized at the macroscopic/whole cell level.



1883-Plat
Why is Choline Such a Low-Efficacy Agonist of the Neuromuscular Acetylcholine Receptor-Channel?
Iva Bruhova[1], Timothy Gregg[2], Anthony Auerbach[1].
[1]State University of New York at Buffalo, Buffalo, NY, USA.
[2]Canisius College, Buffalo, NY, USA.
Acetylcholine receptor-channels (AChRs) mediate fast chemical transmission at the synapse. Agonist molecules, such as acetylcholine and nicotine, bind at two transmitter binding sites and trigger the channel to switch from a closed to an open conformation. At the synaptic cleft, acetylcholine is hydrolyzed by acetylcholinesterase to acetate and choline. Replacing an ester acetyl group by a hydroxyl group (from acetylcholine to choline) decreases the resting affinity by ~50-fold and efficacy by ~600-fold. To understand the action of these ligands and to design novel drugs, it is essential to measure both affinity and efficacy of agonist derivatives using wild type and binding-site mutated AChRs. A technical limitation is that agonists block the channel. Thus, experiments at high agonist concentrations, required due to a low affinity agonist or mutant, are difficult at −100 mV membrane potential. Using a background mutation and depolarization, we have developed an approach to measure AChR single-channel currents activated by various agonists without channel block even at concentrations >140 mM. Using this method, we have directly measured the efficacy of several derivatives of choline on the mouse neuromuscular AChR. Replacing the hydroxyl group of choline with different substituents, such as hydrogen, chloride, methyl, or amine, increases the relative efficacy by 2-9 fold. Extending the ethyl hydroxide tail of choline to propyl and butyl hydroxide also produces more efficacious agonists, by 4.8 and 25-fold, respectively. Perhaps the neuromuscular AChR has specifically evolved to be poorly activated by choline. Our findings reveal the amount of the energy available for AChR conformational change from different agonists. We are searching for the site(s) in the AChR that make choline such a low-efficacy agonist. Supported by NIH (NS-23513, NS-064969).