

1502-Pos Board B412**Energy Changes at the Acetylcholine Receptor Gate Region**

Tapan K. Nayak, Anthony Auerbach.

Abstract

The neuromuscular acetylcholine receptor (AChR) alternatively switches between an inactive (closed) and active (open) conformation. during the gating isomerization ($R \leftrightarrow R^*$) process. The 'gate' region is formed by the equatorial residues ($9'-16'$). We and others have speculated that channel gating involves a wetting/de-wetting of this region of the pore, based on measurements of gating equilibrium changes caused by mutations, phi value analysis and molecular dynamics simulations. We are studying the coupling energies between $9'-13'$ residues in different subunits, by estimating the effect of mutations, separately vs. in combination, on the unliganded gating equilibrium constant E_0 . The end states and conformational pathway for AChR gating is fundamentally the same with and without agonists, and E_0 can be estimated without obfuscation by channel block. We characterized two background mutants ($\alpha A96N/W149S$ and $\alpha A96H/Y96H$) that greatly increase spontaneous gating and exhibit simple two-state kinetics ($E_0 = 0.076$ and 0.031). These E_0 values predict an E_0^{wt} value that is similar to previous estimates ($\sim 6.5 \times 10^{-7}$). With these spontaneously-opening backgrounds, the individual free energy changes for $\beta V13A$ and $\delta V13'L$ (-4.1 kcal/mol and -1.9 kcal/mol) are similar to previous estimates obtained by using diliganded AChRs. Initial results suggest that these $13'$ mutants are only weakly coupled (~ 1.2 kcal/mol). We expect these studies will help unravel the structural changes in protein and water that occur at the AChR gate region.

1503-Pos Board B413**Characterization of Nicotinic Acetylcholine Receptor from Torpedo Californica Using Lipid Analog Detergents**

Luis F. Padilla-Morales, Claudio L. Morales-Pérez, Pamela C. De La Cruz-Rivera, Luis M. Lopez-Cruz, Guillermo A. Asmar-Rovira, Raymond C. Stevens, Orestes Quesada, Jose A. Lasalde-Dominicci.

In the present study, we characterized the effect of detergent solubilization and affinity column purification on *Torpedo californica* nicotinic acetylcholine receptor (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* (18:1, 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-16), while cholesterol analogs were represented by cholate, taurocholate and CHAPS. Each detergent was used to 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 stability and function of the solubilized and purified receptor is preserved in lipid-analog detergents that have acyl chains similar to the most abundant lipid (18:1, 18:0, 16:1, and 16:0) in the endogenous *Torpedo* lipid environment, providing a suitable set of detergents for future structural studies. We also performed lipidic cubic phase (LCP) fluorescence recovery after photo bleaching (FRAP) experiments using fluorescently-tagged-*Torpedo* nAChR solubilized and affinity purified in FC-12, -14 and lyso-FC-16 detergents to estimate the mobile fraction and diffusion coefficient with the goal of using these data to perform LCP crystallization trials within a narrower crystallization space. These detergents display a linear increase in mobile fraction and diffusion coefficient (LFC-16 > FC-12 > FC-14) which is inversely proportional to the percentage of dimmer in these detergents (FC-14 > FC-12 > LFC-16). These experiments will serve to establish correlations to define suitable conditions for nAChR crystallization in LCP.

1504-Pos Board B414**Identification and Functional Expression of Nicotinic Acetylcholine Receptors in Mcn Terminals**

Jeremy Florman, Coral Capó, Edward Custer, Jose R. Lemos, Jose A. Lasalde, Sonia Ortiz-Miranda.

Chronic and acute nicotine exposure causes pregnancy and fertility problems as well as increased blood pressure and heart rate, vasoconstriction and cardiovascular disease. These physiological processes are regulated by the neuropeptides arginine vasopressin and oxytocin, which are synthesized and released from magnocellular neurons (MCNs) in the hypothalamic-neurohypophysial system (HNS).

Nicotinic acetylcholine receptors (nAChRs) are ligand gated ion channels which bind nicotine as well as the endogenous neurotransmitter acetylcholine and are widely distributed throughout the central nervous system. However, little is currently known about the distribution and function of nAChRs in the HNS. Using RT-PCR in conjunction with immunolabeling, Ca imaging and release studies, we provide a comprehensive description of the types, localization

and role of nAChRs in the HNS. RT-PCR identified the presence of alpha7, alpha4, alpha2, beta4 and beta2 subunits in tissue obtained from the Supraoptic and Paraventricular nuclei; with alpha7 and beta2 being predominately expressed. Immunolabeling supports this finding and was used to quantify subunit expression and determine the anatomical location of subunits within these neurons. Immunolabeling identified alpha7, alpha4, alpha2, beta4 and beta 2 subunits in MCN terminals. The presence of alpha7 and alpha4 nAChRs was also confirmed using pharmacological and electrophysiological measurements from isolated terminals. Furthermore, neuropeptide release in the presence of nicotine and/or anatoxin was enhanced in isolated terminals and whole glands. Finally, we confirmed the presence of the vesicular acetylcholine transporter in MCN terminals indicating a possible cholinergic autocrine modulation of peptide release in the HNS. (Supported by UMass Grant P60037094900000 to SOM)

1505-Pos Board B415**Modulation of the Nicotinic Acetylcholine Receptor Function by Cholesterol**

Natalie del Hoyo-Rivera, Carlos A. Báez-Pagán, Orestes Quesada, José A. Lasalde-Dominicci.

The nicotinic acetylcholine receptor (nAChR), located in the cell membranes of neurons and muscle cells, mediates the transmission of nerve impulses across cholinergic synapses. The nAChR is also found in the electric organs of electric rays (e.g. *Torpedo californica*). Cholesterol is a key lipid for maintaining the correct functionality of membrane proteins and has been found to alter the nAChR function. We were thus interested to probe the changes in the functionality of different nAChRs when expressed in cell membranes with modified cholesterol to phospholipid ratios (C/P). In this study, we examined. The effect of increasing the C/P of *Xenopus laevis* oocytes expressing the muscle-type, *Torpedo californica*, neuronal $\alpha 7$ or $\alpha 4\beta 2$ nAChRs in the function of the nAChR was studied. Using the two-electrode voltage clamp technique it was found that the neuronal $\alpha 7$ and *Torpedo* nAChRs are significantly more sensitive to small increases in C/P than the muscle-type nAChR and the neuronal $\alpha 4\beta 2$ nAChR, which is more sensitive than the muscle-type nAChR, but less sensitive than $\alpha 7$ and *Torpedo* nAChRs. This study clearly illustrates that a physiologically relevant increase in membrane cholesterol concentration alters the neuronal $\alpha 7$ and *Torpedo* nAChRs functionality whereas the muscle-type nAChR tends to resist this inhibition in function.

1506-Pos Board B416**Glycine Hinges with Opposing Roles at the Acetylcholine Receptor-Channel Transmitter Binding Site**

Prasad G. Purohit, Anthony Auerbach.

The nicotinic acetylcholine receptor-channel (AChR) is an allosteric protein and an important model for understanding how transmitter molecules activate synaptic receptors. The extent to which agonists activate synaptic receptor-channels depends on the intrinsic tendency of the unliganded receptor to open and the amount of agonist binding energy realized in the channel-opening process. We examined mutations of the nicotinic acetylcholine receptor transmitter binding site (alpha subunit loop B) with regard to both of these parameters. alphaG147 is an 'activation' hinge where backbone flexibility maintains high values for intrinsic gating, the equilibrium dissociation constant ratio of the resting conformation for agonists and net ligand binding energy. alphaG153 is a 'deactivation' hinge that maintains low values for these parameters. alphaW149 (between these two glycines) mainly serves to provide ligand binding energy for gating. A concerted motion of the two glycine hinges (plus other structural elements at the binding site) positions alphaW149 so that it provides physiologically-optimal binding and gating function at the nerve-muscle synapse.

1507-Pos Board B417**Effects of N-Terminal-Helix and M3-M4-Loop Deletions on Channel Activity in a Chimeric Acetylcholine-Glutamate Cys-Loop Receptor**

Dekel D. Bar-Lev, Nurit Degani-Katzav, Alexander Perelman, Asia Bak, Yoav Paas.

Eukaryotic Cys-loop receptors are pentameric ligand-gated ion channels (pLGICs) that respond to neurotransmitters such as acetylcholine, serotonin, γ -aminobutyric acid, glycine, glutamate or histamine by opening (and closing) of an intrinsic transmembrane channel. Each subunit has a long extracellular N-terminal segment, four transmembrane helices (M1 to M4) and a long intracellular M3-M4 connecting segment ("M3-M4 loop"). Recently, the X-ray crystal structure of a prokaryotic pLGIC from the cyanobacterium *Gloeobacter violaceus* (termed "GLIC") was determined at atomic resolution. This prokaryotic receptor does not respond to any of the aforementioned neurotransmitters, but it is activated by protons. In addition, compared to eukaryotic Cys-loop receptors, GLIC lacks a short N-terminal helical segment in its ligand-binding domain,