Acetylcholine Receptors

668-Pos

The Human Neuronal Nicotinic α4β2 Receptor has a High Maximal Probability of Being Open

Joe Henry Steinbach, Ping Li.
Washington University, Saint Louis MO, MO, USA.

Studies of the functional properties of the nicotinic α4β2 receptor have been hampered by the facts that the receptors desensitize rapidly and extensively even to low concentrations of agonist, and the receptors ''run down'' rapidly upon patch excision. We have analyzed macroscopic (whole cell) responses of HEK cells stably transfected with human α4 and β2 subunits using nonstationary noise analysis. The variance does not increase linearly with the mean current, and even decreases for large responses evoked by acetylcholine or nicotine. The maximal response to ACh or nicotine has an estimated open probability of about 0.7. The estimated average single channel chord conductances (−60 mV) are: ACh 18 ± 6 pS (mean ± SD) and nicotine 17 ± 5 pS. The selective agonist 5-Iodo A85380 has a similar single channel conductance (17 ± 2 pS), whereas the agonist cytisine has a larger average estimated conductance (24 ± 4 pS). The estimated numbers of channels activated by ACh or nicotine are about 1000 - 2000 per cell, which is lower than the estimated numbers of surface receptors per cell (about 15,000; Zhang and Steinbach, Brain Res. 959:98 2003). These results demonstrate that the full agonists ACh and nicotine are efficacious agonists at this receptor. Supported by NIH NS 22356.

669-Pos

Length and Composition of the 5HT3A Receptor M3M4 Loop Effects Channel Expression and Desensitization

Nicole McKinnon, Moez Bali, Myles H. Akabas.
Albert Einstein College of Medicine, Bronx, NY, USA.

$5HT_{3A}$ receptors are members of the Cys-loop receptor family of neurotransmitter-gated ion channels. These proteins share a similar structure; each subunit contains an amino-terminal extracellular domain, four transmembrane (TM) domains and a large intracellular loop (~100 residues) between TM3 and TM4. Prokaryotic Cys-loop homologues lack a large intracellular loop, having instead a loop of less than 15 residues. To date, very few studies have looked at the effects of the M3M4 loop on channel function, kinetics and desensitization.

To investigate the role of the M3M4 loop in receptor function we replaced the 5-HT_{3A} M3M4 loop with chains of alanine repeats (5-HT_{3A}-$A_{n=1-7}$) and studied the electrophysiological and biochemical properties of the resulting homomeric channels. All mutants were functional with 5-HT EC_{50}'s similar to wild type receptors; however desensitization times differed greatly amongst the mutants. 5-HT_{3A}-A_2, 5-HT_{3A}-A_4, 5-HT_{3A}-A_6, or 5-HT_{3A}-A_7 demonstrated channel desensitization rates similar to wild type 5-HT_{3A} channels; however 5-HT_{3A}-A_1, 5-HT_{3A}-A_3, or5-HT_{3A}-A_5 had desensitization rates an order of magnitude faster than wildtype. Additionally, the 5-HT_{3A}-A_1 construct enters a non-binding, non-functional state after initial opening, from which it cannot recover. These results suggest that the large M3M4 loop of eukaryotic Cys-loop channels is not required for receptor assembly or function; however the length of the loop and its amino acid composition can have significant effects on channel kinetics and desensitization rate. We infer that the cytoplasmic ends of the M3 and M4 segments may undergo conformational changes during channel gating and desensitization and/or the loop may influence the position and mobility of these segments in the channel protein as it undergoes gating-induced conformational changes. Thus, while the M3M4 loop is not essential for channel assembly and function it can exert significant effects on the channel kinetics.

670-Pos

A Deep Non-Conducting State in the Nicotinic Acetylcholine Receptor

Giovanni Gonzalez-Gutierrez, Claudio Grosman.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.

We studied the effect of systematically introducing aromatic residues in the pore domain of the muscle nicotinic acetylcholine receptor (AChR). Specifically, we mutated positions 9′, 13′, 16′, and 20′ of the second transmembrane segments (M2), four positions that face the channel's lumen in the open conformation. Mutations were engineered one position at a time in individual subunits or as combinations of mutations in multiple subunits. We used single-channel and ensemble-type current recordings to monitor the single-channel conductance and the kinetics of gating-desensitization, and used equilibrium 125I alpha-bungarotoxin binding to estimate cell-surface expression. Kinetic analysis revealed that most of these mutations prolong the mean duration of diliganded bursts of openings (and, as expected, slow the deactivation time course of macroscopic currents) whether present in one or all five subunits, consistent with a 'gain-of-function' effect that is typical of mutations in M2. The effect on the kinetics of entry into desensitization varied among mutants, but was generally mild. The most striking effect of these mutations was observed at positions 16′ and 20′, and consists of a marked decrease in the magnitude of the peak currents in response to single ACh-concentration jumps (from zero to 100 uM) to an extent that cannot be accounted for by the number of mutated AChRs on the cell surface, their single-channel conductance, or their open probability at 100-uM ACh. We conclude that these mutants can enter some sort of refractory state even in the absence of bound ACh, in such a way that only a small fraction of the receptors in the membrane remain activatable. Analysis of the effects of single and multiple mutations at these positions indicate that this desensitized-like state results directly from the interaction among same-ring aromatic side chains (very likely through pi-pi interactions).

671-Pos

Single-Channel Recording of Muscle and Neuronal Nicotinic Acetylcholine Receptors: Implications for Allosteric Transitions

Jai A.P. Shanata, Henry A. Lester, Dennis A. Dougherty.
California Inst Tech, Pasadena, CA, USA.

The muscle and neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric ligand gated ion channels. In most cases these receptors open when two molecules of an agonist, endogenously acetylcholine, bind to the receptor at binding sites located in the extracellular domain. Agonist binding causes a conformational change in the channel pore that produces channel isomerization from the nonconducting to the conducting form. Whole-cell studies revealed two distinct subunit combinations of the α4β2 neuronal receptor: 3 α subunits and 2 β subunits (A3B2) and 2 α subunits and 3 β subunits (A2B3). The A2B3 stoichiometry has higher sensitivity for nicotine than the A3B2 receptor. Based on recent whole-cell work that distinguishes the two stoichiometries by their rectification behaviors, we are developing a single-channel recording-based voltage ramp method to distinguish different stoichiometries of the α4β2 receptors. We also seek to quantify the different types of openings and conductances of the 2 stoichiometries.

In our studies of the muscle nAChR, our manipulations include mutations in the pore region that facilitate gating. We are evaluating a recently proposed model that invokes multiple open states of the nAChR, even in the absence of agonist.

672-Pos

Calcineurin Suppression of Alpha$_7$ nAChR Currents in *Xenopus* Oocytes

Joseph Farley[1], Brent Hallahan[1], Jordan Raynor[1], Krystina Neuman[1], Joseph Rumers[1], Minesh Rajkotia[1], Jed Rose[2].
[1]Indiana University, Bloomington, IN, USA, [2]Department of Psychiatry and Behavioral Sciences, Duke University Medical School, Durham, NC, USA.

As part of our study of mechanisms underlying upregulation of neuronal nicotinic acetylcholine receptors (nAChRs) in brain, due to chronic nicotine exposure, we are characterizing signaling pathways that influence the magnitude of α_7 nAChR currents in *Xenopus* oocytes. In previous studies of Kv1.1 channels in oocytes, we found that calcineurin (CaN) and protein tyrosine kinases (PTKs) mediated calcium-dependent suppression of Kv1.1 currents, through both endocytosis of channels as well as effects on channel gating. Thus, we hypothesized that these molecules would similarly regulate nAChR currents. We first determined that 100 uM bath-applied genistein (an inhibitor of many PTKs) enhanced murine peak α_7 nAChR currents in oocytes, by a factor of 2-3, as reported by others (Cho et al., 2005, J. Neuroscience). We also measured an increase in membrane capacitance (C_m), as expected if genistein's potentiation of peak currents was due to increased addition of channels to the plasma membrane via exocytosis. Conversely, injection of vanadate ion (VO_4^{3-}) into oocytes expressing α_7 nAChRs produced a dramatic reduction (~90%) in peak currents, but no clear decrease in C_m. Exposure of oocytes expressing α_7 nAChRs to 10 uM cyclosporine A (CsA), a potent and specific inhibitor of CaN, increased the magnitude of α_7 currents by a factor of ~2, and also increased C_m. The facilitatory effect of CsA upon α_7 current was blocked by 100 uM H-7, a non-specific inhibitor of serine/threonine PKs. The combination of genistein and CsA produced a greater enhancement of the α_7 current than either agent alone; their combined effect was approximately additive. Our results suggest that CaN activity may provide a readout of the intracellular calcium concentration, due to recent activity of α_7 nAChRs, and thereby negatively regulate the number of α_7 nAChRs expressed on the plasma membrane.