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The coupling interface and pore domain codetermine the single-channel activity of the α7 nicotinic receptor

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ABSTRACT

Ligand-gated ion channels play a role in mediating fast synaptic transmission for communication between neurons. However, the structural basis for the functional coupling of the binding and pore domains, resulting in channel opening, remains a topic of intense investigation. Here, a series of α7 nicotinic receptor mutants were constructed for expression in cultured mammalian cells, and their single-channel properties were examined using the patch-clamp technique combined with radio ligand binding and the fluorescence staining technique. We demonstrated that the replacement of the four pore-lining residues in the channel domain of the α7 nicotinic receptor with the hydrophilic residue serine prolongs the open-channel lifetime, although the conductance of these mutants decreases. At the coupling interface between the extracellular and transmembrane domains, when the VRW residues in the Cys-loop were substituted with the corresponding residues (i.e., IYN) in the 5-HT_{3A} receptor, the single-channel activity elicited by acetylcholine is impaired. This effect occurred despite the expression of the mutant receptors on the cell surface and despite the fact that the apparent K_d values were much lower than those of the wild-type α7 receptor. When we further lowered the channel-gating barrier of this chimera to enhance the open-channel probability, the loss of function was rescued. Overall, we explored the microscopic mechanisms underlying the interplay between the channel domains and the coupling interface that affect the channel activity, and we generated an allosteric gating model for the α7 receptor. This model shows that the gating machinery and coupling assembly codetermine the single-channel gating kinetics. These results likely apply to all channels in the Cys-loop receptor family.

1. Introduction

Ligand-gated ion channels (LGICs) play a role in mediating fast synaptic transmission for communication between neurons. A superfamily of LGICs is the Cys-loop receptor family, and its members contain a signature cysteine loop in the amino-terminal domain. This family includes the cation-selective nicotinic acetylcholine receptor (nAChR) (Thompson et al., 2010), serotonin receptor type 3 (5-HT_3R) (Ranganathan et al., 2000), the zinc-activated ion channel, the anion-selective γ-aminobutyric acid receptor ($GABAR_{A/C}$) (Beg and Jorgensen, 2003), glycine receptors (GlyRs), invertebrate glutamate-gated chloride channels (GluCls) (Hibbs and Gouaux,

Abbreviations: nAChR, nicotinic acetylcholine receptor; ACh, acetylcholine; α-BTX, alpha-bungarotoxin; LGIC, ligand-gated ion channel; 5-HT_3R, serotonin receptor type 3; GABAR, γ-aminobutyric acid receptor; GlyR, glycine receptor; GluCl, glutamate-gated chloride channel; pLGIC, pentameric ligand-gated ion channel.

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2011), the GABA-gated cation channel, the histamine-gated chloride channel, and the serotonin-gated chloride channel (Gengs et al., 2002). Cys-loop receptors are widely expressed in the nervous system and are known to be involved in learning, memory, sleep–wake cycles, and other processes.

The channels in the Cys-loop receptor family are pentameric assemblies of five subunits (Corringer et al., 2010; Hilf and Dutzler, 2009; Miller and Smart, 2010). Each subunit has a topology with a large extracellular amino-terminal domain, four transmembrane segments (M1-M4), and a large intracellular loop between M3 and M4. Electron micrograph analysis of the *Torpedo* nAChR (Miyazawa et al., 2003; Unwin, 2005) and X-ray crystal structure studies of the bacterial channels ELIC (Hilf and Dutzler, 2008) and GLIC (Bocquet et al., 2009; Hilf and Dutzler, 2009), as well as of the invertebrate glutamate-gated chloride channel GluCl (Hibbs and Gouaux, 2011), have led to the suggestion that the channel-gating residues in the middle of M2 form the constriction girdle via hydrophobic forces. Within the coupling interface of each subunit, six segments from different parts of the linear sequence merge: three loops from the extracellular domain (loop 2, loop 7 and the β8-β9 loop); the covalent links between the extracellular and pore domains, the β10 strand and the pre-M1 region; and the linker joining the M2 and M3 helices (the M2-M3 linker) (Bouzat et al., 2008). The coupling interface functions as a structural transition zone in which β-sheets from the binding domain merge with α-helices from the pore (Fig. 1A). The large intracellular loop, including a curved MA stretch (MAS), can interact with intracellular proteins, presumably for receptor targeting or clustering.

Previous studies have shown that the channel domain, the binding domain, the coupling interface and even the intracellular loop can contribute to the kinetics of receptor gating and desensitization. Revah et al. (Revah et al., 1991) first suggested that the well-conserved L9′ residue of the M2 domain is an important determinant of receptor activation and desensitization. Replacing the 9′ leucine with serine or threonine in $GABA_AR$, nAChR, or 5-HT_3R apparently stabilizes open states whether agonists are bound or not. These mutant receptors exhibit decreased agonist EC50 values, prolonged channel openings, and spontaneous openings (Chang et al., 2009; Revah et al., 1991; Zhang et al., 2011). For the binding domain, it has been indicated that the desensitization of the β4-containing receptor can be accelerated by multiple elements of the N-terminal domain of the β2 subunit. Conversely, in the β2-containing receptor, desensitization can be decreased only by substitution with the β4 N-terminal domain (residues 1–212) (Bohler et al., 2001). For the coupling interface, the Cys-loop (Rayes et al., 2009), pre-M1 region (Yakel, 2010a), the N-terminus of M1 (Bianchi et al., 2001; Engblom et al., 2002; Lu and Huang, 1998; McCormack et al., 2010), and the M2-M3 linker (Yakel, 2010b) all contribute to channel gating and desensitization. Moreover, the intracellular loop, especially the MAS, also plays a role in the rate of the Cys-loop receptor desensitization. For example, in the 5-HT_3 receptor, the extent of receptor desensitization was positively correlated with the polarity of the amino acid residue at the 427′ position in the MAS (Hu et al., 2006). Even more recently, some chemicals, such as PNU-120596, were found to be closely associated with the channel-gating kinetics (Kalappa et al., 2013; Pesti et al., 2014; Szabo et al., 2014). Despite previous intense investigation, the structural basis for the functional coupling of agonist binding to channel gating, which results in channel opening and desensitization, is still unclear (Giniatullin et al., 2005; Katz and Thesleff, 1957).

In this study, we attempted to gain insight into the microscopic origin of Cys-loop receptor coupling and gating. Our previous results from two-electrode voltage clamp measurements in an oocyte expression system demonstrated that some key residues in the α7 nAChR play a role in the channel gating and desensitization (Zhang et al., 2011). However, because of technical limitations of the macroscopic oocyte recording, the detailed single-channel profiles and structural underpinnings behind it are still not clear. Here, we employed patch-clamp, site-directed mutagenesis, radio ligand binding and fluorescence staining techniques to study the structural basis of the receptor coupling and gating at the microscopic level. We found that the coupling interface and the pore domain codetermine the channel open probability and the channel lifetimes, resulting in the differing functional properties of the channel. Based on these results, we generated an allosteric gating model of the α7 nicotinic receptor.

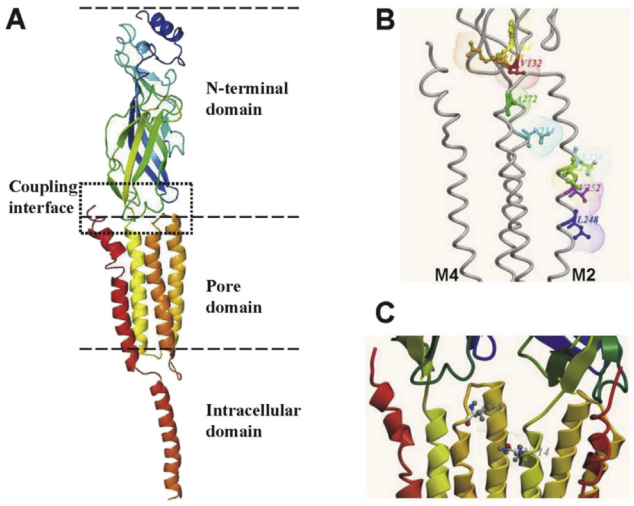

Fig. 1. The homology model of the α7 nAChR subunit based on the structural template of the α1 subunit of the *Torpedo* nACh receptor (2BG9). (A) The locations of the N-terminal domain, channel domain, and intracellular domain are indicated. The boxed region is the coupling interface, which divides the extracellular and channel domains. (B) Residues from the channel domain and three regions of the coupling interface (Cys-loop, pre-M1 region and the M2-M3 linker) are shown in stick representation overlaid with colored van der Waals surfaces. These residues were investigated in this study. (C) Insight into the interaction between the N214 residue in the pre-M1 region and the A272 residue in the M2-M3 linker.

2. Materials and methods

2.1. Molecular biology

The cDNA encoding the wild-type human α7 nAChR subunit was cloned into the pCDNA3.1 vector. The residues in the second transmembrane domain, the Cys-loop of the N-terminal domain, the pre-M1 region and the M2-M3 linker (see Fig. 1A and B) were mutated for single and multiple mutations. This mutation was performed using the PCR-based QuikChange method of site-directed mutagenesis with *PfuUltra* DNA polymerase (Agilent Technologies, Hercules, CA). The mutations were confirmed by sequencing the entire cording region. For single-channel patch recording, to promote receptor expression on the cell surface, human Ric-3 cDNA was subcloned into pIRES2-EGFP, and this construct was cotransfected with a human plasmid encoding the human α7 receptor or its mutants. The parent plasmid pIRES2-EGFP also encodes the green fluorescent protein, allowing for the identification of transfected cells under fluorescence optics.

2.2. Single-channel patch-clamp recording

HEK 293T cells were transfected by calcium phosphate precipitation with the receptor subunit cDNAs together with Ric-3 cDNA for cell surface expression of the receptor (Andersen et al., 2013). Single-channel recordings were obtained in the cell-attached patch configuration. The pipette solution contained 150 mM KCl, 2.5 mM $MgCl_2$, 10 mM HEPES, and 0.2 mM EGTA at pH = 7.4, and the bath solution contained 150 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES and 10 mM glucose at pH = 7.4. The recording was conducted with an HEKA EPC-10 patch-clamp amplifier with the associated software (PULSE and PatchMaster, HEKA Electronic Inc., Germany). The currents were sampled at 50 kHz and filtered at