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Review

Nicotinic acetylcholine receptor–lipid interactions: Mechanistic insight and biological function☆

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ABSTRACT

Membrane lipids are potent modulators of the nicotinic acetylcholine receptor (nAChR) from *Torpedo*. Lipids influence nAChR function by both conformational selection and kinetic mechanisms, stabilizing varying proportions of activatable versus non-activatable conformations, as well as influencing the transitions between these conformational states. Of note, some membranes stabilize an electrically silent uncoupled conformation that binds agonist but does not undergo agonist-induced conformational transitions. The uncoupled nAChR, however, does transition to activatable conformations in relatively thick lipid bilayers, such as those found in lipid rafts. In this review, we discuss current understanding of lipid–nAChR interactions in the context of increasingly available high resolution structural and functional data. These data highlight different sites of lipid action, including the lipid-exposed M4 transmembrane α-helix. Current evidence suggests that lipids alter nAChR function by modulating interactions between M4 and the adjacent transmembrane α-helices, M1 and M3. These interactions have also been implicated in both the folding and trafficking of nAChRs to the cell surface. We review current mechanistic understanding of lipid–nAChR interactions, and highlight potential biological roles for lipid–nAChR interactions in modulating the synaptic response. This article is part of a Special Issue entitled: Lipid–protein interactions.

Contents

1. Introduction

The nicotinic acetylcholine receptor (nAChR) from *Torpedo* is the prototypic member of a broad family of pentameric ligand-gated ion channels (pLGICs) that are found in pre-, post-, and non-synaptic

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membranes of the central and peripheral nervous systems. These neurotransmitter receptors perform important roles in both synaptic communication and information processing, have been implicated in a variety of neurological processes and diseases, and are targets of numerous pharmaceuticals [1–5].

Early attempts to isolate and reconstitute the *Torpedo* nAChR in model membranes first highlighted the functional sensitivity of the nAChR to lipids. To retain agonist-induced channel flux, the nAChR must be solubilized and purified in the presence of lipid, and then placed in a membrane with an appropriate lipid composition [6–9]. The exquisite lipid sensitivity of the *Torpedo* nAChR is of interest because even subtle changes in human nAChR activity have profound effects on human biology [10,11]. In addition, the lipid environment of the human nAChR changes as the nAChR traffics from intracellular membranes to its pre-, post-, or non-synaptic locations in the plasma membrane, as well as during both aging and neurodegenerative disease [12–14]. These changes in the nAChR lipid micro-environment during both normal and abnormal brain functions likely influence cholinergic biology to alter synaptic communication.

The relative abundance of the nAChR in the electric fish *Torpedo* has made it the ideal model for studies of ligand-gated ion channel structure/function relationships. Biophysical and biochemical studies over the past three decades have led to an extensive literature on lipid–nAChR interactions, which has been summarized in several comprehensive reviews [15–19]. In the past decade, a 4 Å resolution cryo-electron microscopy model of the *Torpedo* nAChR (Fig. 1) [20], as well as X-ray crystal structures of homologous pLGICs [21–29] and water-soluble homologs of the nAChR extracellular agonist-binding domain [30,31], and NMR structures of nAChR transmembrane domains [32,33] have provided an increasingly detailed picture of both nAChR structure and the nature of ligand-induced conformational change. With this structural data in hand, we are now in an unprecedented position to probe the mechanisms underlying lipid–nAChR interactions at a structural/mechanistic level.

This review focuses on our current understanding of lipid–nAChR interactions in the context of these recently solved pLGIC structures. We review the structural properties of the *Torpedo* nAChR, the *Torpedo* nAChR's lipid requirements, and current models of lipid–nAChR interactions. We also highlight potential roles for lipids in the folding, cell-surface trafficking, and domain localization of the nAChRs in mammalian tissues.

2. nAChR structure

There are seventeen homologous nAChR subunits in mammals (α1–α10, β1–β4, γ, ε, and δ) that combine to form a variety of either homo-pentameric or hetero-pentameric structures [34]. The *Torpedo* nAChR is most similar to the muscle-type nAChR found at the neuromuscular junction, being formed from four distinct subunit types organized in an $(\alpha1)_2\beta1\gamma\delta$ pentamer. In the adult muscle, the fetal γ-subunit of the $(\alpha1)_2\beta1\gamma\delta$ pentamer is replaced by the ε-subunit. nAChRs are also an important part of the central nervous system, with both heteromeric α4β2 and homomeric α7 nAChRs abundant throughout the human brain, and less abundant combinations, such as α3β4, α3β2, and α6β2β3 nAChRs, targeted to specific brain regions [1,2].

The five subunits of the *Torpedo* nAChR pentamer are arranged pseudo-symmetrically around a central axis that functions as an ion channel (Fig. 1A) [20]. Each subunit contributes three distinct domains, a roughly 200-residue long N-terminal extracellular domain (ECD) responsible for agonist binding, a roughly 150-residue long transmembrane domain (TMD) responsible for ion channel conductance, and a cytoplasmic domain of variable length that links to the cytoskeleton. The ECD of each subunit consists of 10 β-strands (β1–β10) forming two β-sheets that fold into a classic β-sandwich. The α-subunit contributes the principal face of each agonist site [35], with the complementary face formed by the adjacent γ- and δ-subunits [36,37]. The TMD of each subunit contributes four transmembrane α-helices (M1–M4) organized in a four-helix bundle. The five M2 α-helices line the channel pore, while M1 and M3 from each subunit form a ring of α-helices that shield M2 from the membrane [20,38,39]. The M4 α-helices are located on the periphery of each subunit where they are exposed to the lipid bilayer. The cytoplasmic domain of each subunit contributes an α-helix that extends away from the membrane surface. The cytoplasmic domain is located in a long loop positioned between transmembrane α-helices M3 and M4.

Communication between the ECD and TMD in each subunit is mediated primarily by the covalent link between the C-terminus of β10 in the ECD and the N-terminus of M1 in the TMD, as well as by non-covalent connections between the β1/β2 and β6/β7 loops (the latter is referred to as the Cys-loop in eukaryotic pLGICs) of the ECD and the M2–M3 linker of the TMD (Fig. 2) [40,41]. Although the detailed structural changes that occur when agonist binding couples to channel gating remain controversial, it is thought that concerted movements of the two β-sheets in the ECD lead to changes in structure of both the

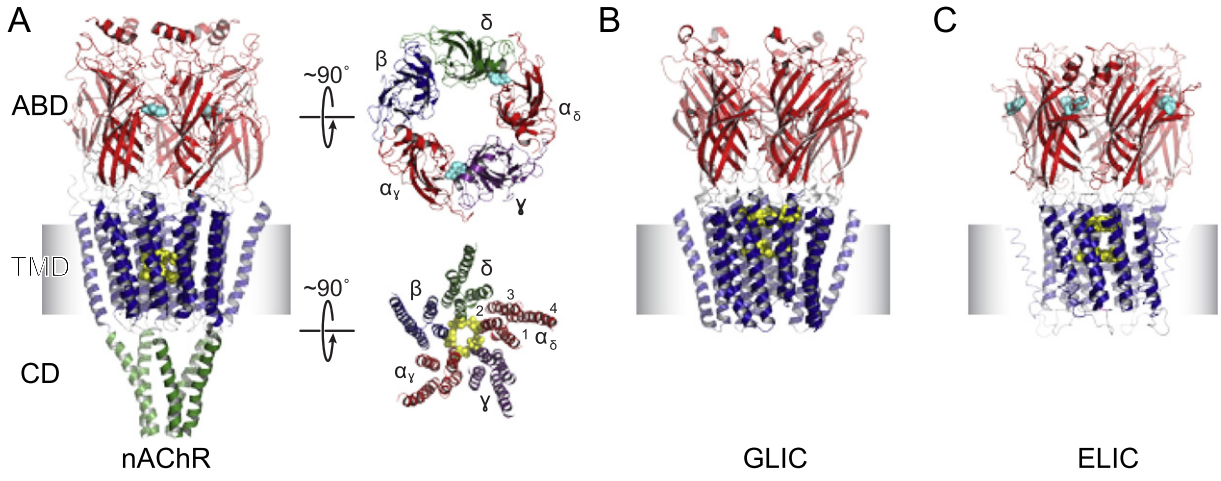

Fig. 1. Structure of the A) nAChR, B) GLIC, and C) ELIC. A) A side view of the *Torpedo* nAChR structure (PDB ID: 2BG9) is shown on the left with the agonist-binding domain (ABD) in red, the transmembrane domain (TMD) in blue, and the cytoplasmic domain (CD) in green. Residues contributing to the proposed channel gate (αLeu251, αVal255 and homologous residues in the β, γ, and δ subunits) are shown as yellow spheres. The agonist binding site αTrp149 is shown as cyan spheres. Top down views of the ECD and TMD are shown on the right. B) Side view of the GLIC structure (PDB ID: 3EAM) with coloring as in A). Residues contributing to the proposed channel gate (I233, I240, L241) are shown as yellow spheres. C) Side view of the ELIC structure (PDB ID: 2VL0) with coloring as in A). Residues contributing to the proposed channel gate (L239 and F246) are shown as yellow spheres. The agonist binding site F187 is shown as cyan spheres.