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Invited review

The nicotinic acetylcholine receptor and its prokaryotic homologues: Structure, conformational transitions & allosteric modulation

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ABSTRACT

Pentameric ligand-gated ion channels (pLGICs) play a central role in intercellular communications in the nervous system by converting the binding of a chemical messenger – a neurotransmitter – into an ion flux through the postsynaptic membrane. Here, we present an overview of the most recent advances on the signal transduction mechanism boosted by X-ray crystallography of both prokaryotic and eukaryotic homologues of the nicotinic acetylcholine receptor (nAChR) in conjunction with time-resolved analyses based on single-channel electrophysiology and Molecular Dynamics simulations. The available data consistently point to a global mechanism of gating that involves a large reorganization of the receptor mediated by two distinct quaternary transitions: a global twisting and a radial expansion/contraction of the extracellular domain. These transitions profoundly modify the organization of the interface between subunits, which host several sites for orthosteric and allosteric modulatory ligands. The same mechanism may thus mediate both positive and negative allosteric modulations of pLGICs ligand binding at topographically distinct sites. The emerging picture of signal transduction is expected to pave the way to new pharmacological strategies for the development of allosteric modulators of nAChR and pLGICs in general.

This article is part of the Special Issue entitled 'The Nicotinic Acetylcholine Receptor: From Molecular Biology to Cognition'.

1. Introduction

A critical event in the history of biological chemistry was, 44 years ago, the chemical identification of the first neurotransmitter receptor, the nicotinic acetylcholine receptor (nAChR) from fish electric organ (Changeux et al., 1970; Miledi et al., 1971; Corringer et al., 2000; Karlin, 2002; Changeux and Edelstein, 2005) (rev (Changeux, 2012)). The success was due to the convergence of disciplines as diverse as electrophysiology, pharmacology, and biochemistry with the common goal of successfully identifying the molecular switch that converts a chemical input into an electrical output at neuronal synapses. Since then, the cationic nicotinic receptor has become the titular head of a broad family of pentameric ligand-gated ion channels (pLGICs), paving the way to the identification of the homologous inhibitory $GABA_A$ (rev (Barnard, 1995; Olsen and Sieghart, 2009; Miller and Aricescu, 2014)) and glycine (rev (Dutertre et al., 2012)) receptors, along with the excitatory 5-hydroxytryptamine receptor (rev (Yan et al., 1999)) and, in invertebrates, the glutamate-gated chloride channel (GluCl) (rev (Hibbs and Gouaux, 2011)). The recent discovery of cationic orthologs in prokaryotes (Tasneem et al., 2005; Bocquet et al., 2007) has extended the superfamily, plunging its evolutionary origins back 3 billion years and leading to the first crystallization and full atomistic structure of a pentameric ligand-gated ion channel (Hilf and Dutzler, 2008, 2009; Bocquet et al., 2009). These oligomeric membrane proteins are allosterically regulated by the binding of a neurotransmitter —the agonist— to an orthosteric site that is topographically distinct from the transmembrane ion channel (rev (Corringer et al., 2012; Taly et al., 2014)). At rest, the ion channel is closed and binding of the agonist to the extracellular domain triggers a rapid conformational change that results in the opening of the transmembrane pore, a process referred to as *gating* (Changeux and Edelstein, 1998). Recently, the extension of computational approaches based on Molecular Dynamics (rev (Karplus and McCammon, 2002)) to pentameric receptors (Taly

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et al., 2005; Cheng et al., 2006; Nury et al., 2010; Calimet et al., 2013) has introduced a new temporal dimension in the understanding of the signal transduction mechanism. Also, an important outcome of the most recent structural and functional studies is that many of these regulatory proteins carry a variety of allosteric modulatory sites, – in addition to the main categories of orthosteric regulatory and biologically active sites – which have become important new targets for drug design (Mulle et al., 1992; Vernino et al., 1992; Krause et al., 1998; Bertrand and Gopalakrishnan, 2007) (rev (Changeux, 2013a)). In this review, we briefly present the most recent advances in the structure, conformational transitions and allosteric modulation of nAChR and its prokaryotic homologues. We do not intend to exhaustively review electrophysiological studies of nAChR (see e.g. Auerbach and others in this volume) nor early EM analyses (Brisson and Unwin, 1985; Unwin, 2005), but place special emphasis on the recent X-ray structures and Molecular Dynamic simulations.

2. Molecular architecture of nAChRs and homologues

nAChRs and homologues are integral allosteric membrane proteins with a molecular mass of ~290 kDa, comprising five identical or homologous subunits symmetrically arranged around a central ionic channel with a fivefold axis perpendicular to the membrane plane. In mammals, there are multiple types of nAChR oligomers, which differ in their subunit compositions throughout the body (Wang et al., 2015; Zoli et al., 2015). Of these, nine β-subunits and three α-subunits are expressed in the brain. They assemble into various homo- and hetero-pentameric combinations, which differ in their pharmacological, physiological and kinetic properties along with their localization in the brain (Zoli et al., 2015).

The 3D-structure of nAChR and homologues is well-characterized (Karlin, 2002; Taly et al., 2014, 2009; Thompson et al., 2010). The primary structure of each subunit consists of a large hydrophilic amino-terminal extracellular (EC) domain, a transmembrane (TM) domain comprising four hydrophobic segments (M1–M4), and a variable hydrophilic cytoplasmic or intracellular (IC) domain, which is absent in prokaryotic pLGICs; see Fig. 1. There are 2–5 ACh-binding sites within the EC domain, which are distant (ca. 60 Å) but functionally linked to a unique cationic ion channel, located on the axis of symmetry of the TM domain. The atomic structure of the EC domain was first solved for the ACh-binding protein (AChBP) — a soluble pentameric homologue of the EC domain of nAChR — which was initially cloned from invertebrate snails (Smit et al., 2001; Brejc et al., 2001). Complete structures of pLGICs at atomic resolution were then obtained with two prokaryotic homologues of nAChR from *Gloeobacter violaceus* (GLIC) (Hilf and Dutzler, 2009; Bocquet et al., 2009) and *Erwinia chrysanthemi* (ELIC) (Hilf and Dutzler, 2008) and only recently with three eukaryotic receptors: GluCl from *Caenorhabditis elegans* (Hibbs and Gouaux, 2011), the $5HT_3$ receptor from *mouse* (Hassaine et al., 2014), and the $GABA_A$ receptor from *human* (Miller and Aricescu, 2014). At this stage, no full X-ray structure of any nAChR has ever been obtained. Only low resolution images of the electric organ/muscle nAChR have been published (see (Unwin, 2013), however see also (Taly et al., 2014)).

In agreement with structural studies of the AChBP (Brejc et al., 2001), the EC domain of all prokaryotic and eukaryotic pLGICs is folded into a highly conserved immunoglobulin-like β-sandwich stabilized by inner hydrophobic residues. However, the connecting loops, as well as the N-terminal α-helix present in most eukaryotic pLGICs but not in the prokaryotic ones, are variable in length and structure. To date, the role of this N-terminal α-helix, which is duplicated in the $GABA_A$ β3 receptor (Miller and Aricescu, 2014), remains unknown (see (Corringer et al., 2012)). Consistent with early EM data of *Torpedo* nAChR at low (maximum 4 Å) resolution (Unwin, 2005), the four transmembrane segments fold into α-helices and are organized as a well conserved bundle strikingly different from the K^+ channel pore (Zhou et al., 2001). The second segment M2 lines the channel walls (Giraudat et al., 1986, 1987; Hucho et al., 1986; Imoto et al., 1988, 1986) and is surrounded by a ring of α helices made of M1 and M3. M4 lies at the side of the bundle. In all X-ray structures investigated to date, the TM domain makes extensive interactions with the lipid bilayer, which is also thought to play a role in modulating ion permeation. Interestingly, the structure of GLIC reveals three lipid molecules per subunit that are bound in the crevices between M4 and either M1 or M3 (Bocquet et al., 2009; Nury et al., 2011). Higher resolution structures (2.4 Å) of the prokaryotic channel GLIC in its open conformation revealed the presence of ordered pentagons of water molecules within the ion pore at the level of two rings of hydroxylated residues (named Ser6′ and Thr2′), which are thought to contribute to the ion selectivity filter (Sauguet et al., 2013a). Finally, the very recent structure of the eukaryotic $5HT_3$ receptor, which first visualized the conformation of the intracellular domain (IC) in pLGICs, suggests the IC domain would also contribute to ion permeation (Hassaine et al., 2014).

The major loop contributing to the EC/TM domains interface, namely the Cys loop carrying the canonical FPFD motif (Rendon et al., 2011), is not ordered in the structure of the isolated EC domain of GLIC (Nury et al., 2010) but adopts a well-defined conformation in the full-length receptor structure through extensive interactions with the four α-helical bundle of the TM domain (Bocquet et al., 2009). The superposition of GLIC and GluCl reveals striking similarities, but also differences that are found at the subunit–subunit interfaces including the β8–β9 loop (Loop F, see below), which is located on the outside of the EC domain, and an insertion loop in strand β5 that faces the inner part of the vestibule (Miller and Aricescu, 2014; Hibbs and Gouaux, 2011). The former region is important for ligand binding (Bourne et al., 2005), the latter is thought to influence assembly specificity in GluCl and the $GABA_A$ receptor (Miller and Aricescu, 2014) and be involved in the Zinc modulation of Gly receptors (Miller et al., 2008).

Overall, the available X-ray structures of prokaryotic and eukaryotic pLGICs reveal a striking similarity in the 3D structure of the nAChR with its homologues (Corringer et al., 2012; Taly et al., 2014); see Fig. 1. Yet, the absence of high-resolution structures of *heteromeric* pseudo-symmetrical pLGICs makes it difficult to extend all conclusions drawn on the prokaryotic channels to the eukaryotic members of the superfamily. From a physiological perspective, the particularly well-conserved architecture is suggestive of conservation of function despite significant differences in the primary structure do exist. Indeed, chimeric constructs produced by merging the EC and TM domains from different pentameric receptors were shown to fully preserve function. For instance, functional chimeras of eukaryotic α7, α4, and β2 nAChR subunits with the $5HT_3$ receptor (Eisele et al., 1993; Cooper et al., 1999) and α7 nAChR with α1 GlyR (Grutter et al., 2005) have been produced. Even more striking, functional chimeras have been constructed using the bacterial GLIC and the eukaryotic α1 GlyR (Duret et al., 2011), demonstrating a remarkable conservation of the structural organization from bacteria to humans.

3. Functional interpretation of structures

Signal transduction by nAChR was proposed since the 60's to be mediated by a global isomerization of the receptor coupling the neurotransmitter binding site in the EC domain and the transmembrane ion channel, which was referred to as an *allosteric transition* (Changeux, 1964, 1966; Changeux et al., 1967; Changeux