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Review

Structural insights into Cys-loop receptor function and ligand recognition

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ABSTRACT

This review outlines recent insights into ligand recognition, channel gating and ion permeation for the family of pentameric ligand-gated ion channels (pLGICs). These receptors are involved in the fast inhibitory and excitatory neurotransmission. Prototypical anion-selective members are the γ-amino butyric acid type A ($GABA_A$), γ-amino butyric acid type C ($GABA_C$) and glycine receptor. The cation-selective members are the 5-HT_3 serotonin and nicotinic acetylcholine (nACh) receptors. They are the target for a wide variety of drugs and dysfunction in these receptors is associated with several diseases.

We summarize recent structural knowledge in combination with electrophysiological data and molecular dynamic simulations, thereby describing key features of ligand binding, channel gating and ion permeation. A conserved cation-π interaction between ligand and aromatic residues of the ligand binding site critically contributes to ligand recognition, as revealed by X-ray crystal structures of acetylcholine binding proteins, as well as the integral pLGICs, ELIC and GluCl. In addition, we summarize the possible downstream effects on gating of structural rearrangements in the extracellular ligand-binding domain, which mainly occur in loop C and loop F. These data are discussed in the context of different conformational states of the pore-forming domain observed in crystal structures of GLIC and GluCl, which likely represent the open pore conformation, and ELIC, which likely corresponds to a closed pore conformation. We conclude with a current structural view on the determinants of ion selection and permeation.

Contents

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1. Introduction

Our expanding structural knowledge of membrane proteins, including ion channels, G-protein coupled receptors, transporters and pumps, progressively shifts the drug design process for these important drug targets from high-throughput screening toward a structure-based discovery approach. The treatment of neurological disorders could greatly benefit from rational drug development since many of these disorders could be better treated with more potent or efficacious drugs with fewer side effects. However, structure-based drug discovery approaches require a thorough insight into the structural features of target receptors, including the family of pentameric ligand-gated ion channels (pLGICs). This family consists of allosterically regulated membrane proteins located at synapses in the nervous system. They convert binding of a specific neurotransmitter released in the synaptic cleft into an ion flux over the postsynaptic membrane, which subsequently triggers excitatory or inhibitory postsynaptic potentials. Known vertebrate anion-selective pLGICs are the γ-amino butyric acid type A ($GABA_A$), γ-amino butyric acid type C ($GABA_C$) and glycine receptor, whereas the cation-selective pLGICs are the 5-HT_3 serotonin and nicotinic acetylcholine (nACh) receptors. pLGICs serve as targets for a wide variety of frequently prescribed drugs, including smoking cessation aids, anxiolytics, anticonvulsants, muscle relaxants, hypnotics and anti-emetics. Dysfunction of pLGICs also plays an important role in several disorders of the central nervous system, including hyperekplexia [1], myasthenia gravis [2], epilepsy [3], irritable bowel syndrome (IBS) [4], Alzheimer's disease [5], schizophrenia [6] and Parkinson's disease [7].

The current understandings concerning structure, gating mechanism and ligand recognition of pLGICs (Fig. 1) are derived from electron microscopic imaging of the nACh receptor from the *Torpedo marmorata* (*Tm*) electric organ [8] and X-ray crystallographic studies of the extracellular domain (ECD) from the muscle α1 nACh receptor [9] and the water-soluble acetylcholine binding proteins (AChBP) [10]. Until 2005 it was assumed that this class of membrane proteins was uniquely expressed in multicellular eukaryotic organisms, but Tasneem et al. also identified several homologues in unicellular prokaryotic organisms (Fig. 2) [11]. In 2008 the first X-ray crystal structure of an integral prokaryotic pLGIC, derived from *Erwinia chrysanthemi* (ELIC) was determined [12]. This structure likely corresponds to a non-conducting conformation of the ion channel. About one year later a second crystal structure was determined for another prokaryotic homologue, derived from the cyanobacterium *Gloeobacter violaceus* (GLIC) [13,14], which likely displays an open ion-conducting conformation. Both ELIC and GLIC form cation-selective ion channels, similar to the nACh receptor (nAChR) and the 5-HT_3 receptor (5-HT_3R). ELIC can be activated by primary amines such as GABA [15,16], GLIC on the other hand responds to an increase in extracellular proton concentration [17]. In 2011 the first 3-dimensional structure of a eukaryotic, anion-selective pLGIC was determined. This glutamate-gated chloride (GluCl) channel is derived from *Caenorhabditis elegans* and reveals an open pore conformation, similar to GLIC [18].

Based upon this structural information, the overall architecture of pLGICs appears to be conserved and consists of five homologous or identical membrane-spanning subunits. Each of these subunits is composed of a N-terminal extracellular ligand binding domain, four transmembrane domains, an intracellular loop between the 3rd and 4th transmembrane domain and an extracellular C-terminus. They are also referred to as the family of Cys-loop receptors (CLRs) due to a highly conserved disulfide bond in one of the loops forming the interface between the extracellular ligand binding domain and the pore-forming transmembrane channel domain. This disulfide bond is not present in the prokaryotic homologues, but the architectural fold of this loop is similar to their eukaryotic homologues.

This review will outline the current structural knowledge and understandings concerning the molecular recognition of ligands, channel gating and ion permeation for this class of ion channels.

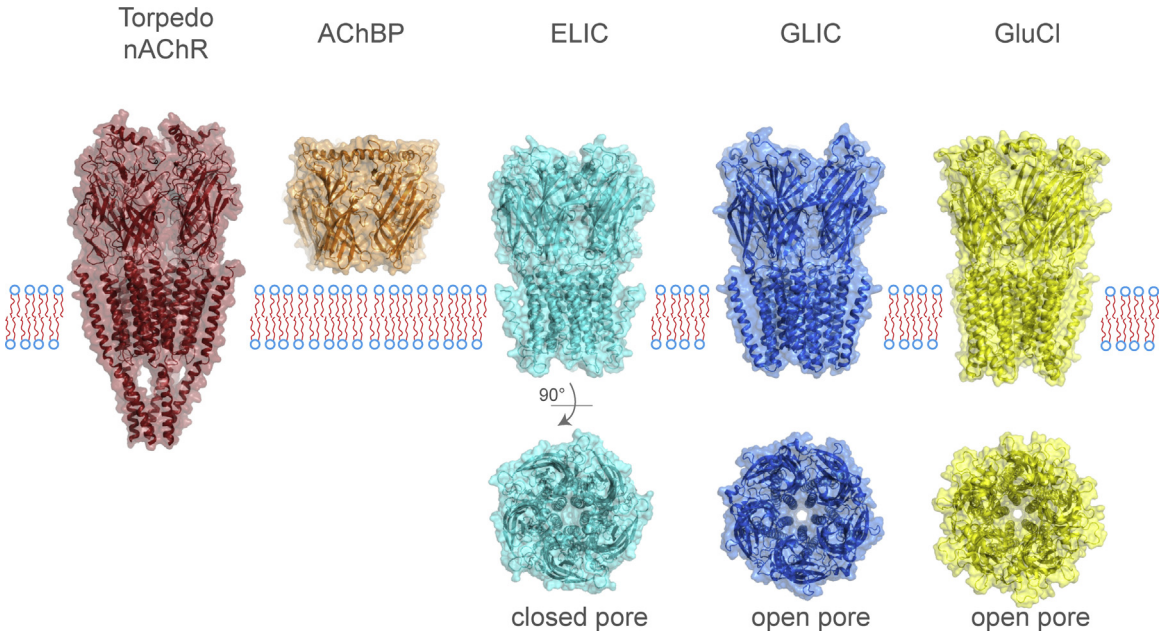

Fig. 1. Overview of the current structural knowledge concerning pentameric ligand-gated ion channels. From left to right: The electron-microscopic structure of the *Torpedo marmorata* nAChR [8], followed by X-ray structures of the water-soluble AChBP [10], ELIC [12], GLIC [13,14] and GluCl [18]. Torpedo nAChR, *Torpedo marmorata* nicotinic acetylcholine receptor; AChBP, Acetylcholine binding protein; ELIC, *Erwinia chrysanthemi* ligand-gated ion channel; GLIC, *Gloeobacter violaceus* ligand-gated ion channel; GluCl, Glutamate-gated chloride channel derived from *Caenorhabditis elegans*.