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New insights into the structural bases of activation of Cys-loop receptors

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ABSTRACT

Neurotransmitter receptors of the Cys-loop superfamily mediate rapid synaptic transmission throughout the nervous system, and include receptors activated by ACh, GABA, glycine and serotonin. They are involved in physiological processes, including learning and memory, and in neurological disorders, and they are targets for clinically relevant drugs. Cys-loop receptors assemble either from five copies of one type of subunit, giving rise to homomeric receptors, or from several types of subunits, giving rise to heteromeric receptors. Homomeric receptors are invaluable models for probing fundamental relationships between structure and function. Receptors contain a large extracellular domain that carries the binding sites and a transmembrane region that forms the ion pore. How the structural changes elicited by agonist binding are propagated through a distance of 50 Å to the ion channel gate is central to understanding receptor function. Depending on the receptor subtype, occupancy of either two, as in the prototype muscle nicotinic receptor, or three binding sites, as in homomeric receptors, is required for full activation. The conformational changes initiated at the binding sites are propagated to the gate through the interface between the extracellular and transmembrane domains. This region forms a network that relays structural changes from the binding site towards the pore, and also contributes to open channel lifetime and rate of desensitization. Thus, this coupling region controls the beginning and duration of a synaptic response. Here we review recent advances in the molecular mechanism by which Cys-loop receptors are activated with particular emphasis on homomeric receptors.

1. Introduction

The human brain is a vast and complicated network, where billions of nerve cells use signals to communicate with each other. Chemical synaptic transmission is the main process by which nerve cells signal one another. It offers the advantages of signal amplification, reversal of polarity and great potential for modulation, all important properties for higher brain function. At chemical synapses, the neurotransmitter is released into a narrow synaptic gap after depolarization of the presynaptic terminal and binds to a postsynaptic receptor. Neurotransmitter-gated ion channels are a family of synaptic receptors that convert the chemical signal into an electrical one by rapidly opening a channel that allows the flux of ions through the membrane. Just as important, the channel closes within a few milliseconds as the transmitter dissociates to terminate the synaptic event. Thus, moment-to-moment communication relies on rapid on and off responses of synaptic receptors.

Neurotransmitter-gated ion channels of the Cys-loop superfamily play key roles in chemical synapses throughout the nervous system, and include receptors activated by acetylcholine (ACh), γ-aminobutyric acid (GABA), glycine, and serotonin (5-HT) (Le and Changeux, 2001; Lester et al., 2004; Sine and Engel, 2006; Bartos et al., 2009a). They are known as Cys-loop receptors because all family subunits contain a pair of disulfide-bonded cysteines separated by 13 residues which form a loop located at the interface between extracellular and transmembrane domains. Their vital role in converting chemical recognition into an electrical impulse makes these receptors prime loci for learning, memory and disease processes, as well as targets for clinically relevant drugs. Cys-loop receptors are targets of widely prescribed drugs, such as neuromuscular blockers, barbiturates and benzodiazepines. In the last years, an ever increasing number of human and animal diseases has been found to be caused by defective function of Cys-loop receptors, such as Alzheimer's and Parkinson's disease, schizophrenia, hereditary epilepsies, attention-deficit, hyperactivity disorder, autoimmune autonomic neuropathy, autism, myasthenia gravis, and congenital myasthenic syndromes (Kalamida et al., 2007).

In vertebrates, Cys-loop receptors can be cation-selective, as nicotinic ACh (nAChRs) and 5-hydroxytryptamine type 3 (5-HT_3) receptors, or anion-selective channels, as $GABA_A$, $GABA_C$ and glycine receptors. The selectivity of the channels for cations or

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anions governs the sign of the current, and in most of the cases, the type of response: inhibitory, for anionic channels because they hyperpolarize the cell, and excitatory, for cationic channels because they induce membrane depolarization by allowing a net influx of Na^+ ions into the cell.

Nicotinic receptors have been object of attention since Claude Bernard investigated the action of the Central American arrow poison, curare. The muscle nAChR was the first to be identified and purified, and the first to be characterized biochemically and electrophysiologically. The nAChR is widely distributed throughout the animal kingdom, from nematodes to human (Le and Changeux, 1995). It is expressed in many regions of the central and peripheral nervous system and plays a major role in neuromuscular transmission. This receptor is the target of competitive blockers, such as curare, and other muscle relaxants used in surgery and it is modulated by a great variety of compounds (Arias et al., 2006). nAChRs are also present in various non-neuronal tissues, such as glia, blood cells (De Rosa et al., 2005), keratinocytes (Maus et al., 1998), endothelial cells (Macklin et al., 1998), multiple cell types of the digestive system and lung cells (Wessler et al., 2003) .

5-HT_3 receptors are found in the central and peripheral nervous system. They are involved in sensory processing, nociception, emesis, cardiovascular regulation, and gut function (Thompson and Lummis, 2007). Selective antagonists are used as antiemetic agents during antineoplasic therapy. To date, five different subunits are known in human, and all subunits show splice variants (A–E) (Niesler et al., 2003, 2007).

$GABA_A$ and glycine receptors are mainly involved in inhibition in the central nervous system, with the $GABA_A$ receptor distributed throughout the central nervous system and the glycine receptor found predominantly in the brainstem and spinal cord. The activity of GABA receptors is allosterically enhanced by benzodiazepines, barbiturates, intravenous anesthetics, alcohols, steroids and volatile anesthetics; and it is blocked by picrotoxin (Steinbach and Akk, 2001; Akk et al., 2007; Hanson et al., 2008; Olsen and Sieghart, 2009). Glycine receptors are targets of the plant alkaloid strychnine which acts as a competitive antagonist, leading to agitation, muscle spasms, and convulsions. The development of therapeutic agents against these receptors may therefore have significant utility as muscle relaxants and analgesic agents (Connolly and Wafford, 2004).

The essential function of Cys-loop receptors is to couple the binding of the agonist to the opening of the ion channel. Given that this process governs synaptic transmission, elucidation of its mechanism and the structures involved has been a long-standing challenge.

2. Overall structure

Cys-loop receptors are composed of five identical (homopentamers) or different (heteropentamers) polypeptide chains arranged around an axis perpendicular to the membrane (Fig. 1a). A wide number of subunits have been cloned for all members of the superfamily (Ligand-gated ion channel database, http://www.ebi.ac.uk/compneur-srv/LGICdb/cys-loop.php). In nAChRs, subunits are classified in two types, α and non-α, with the α-type subunits containing a disulfide bridge in the binding site. Present day homomeric Cys-loop receptors likely descended from a homomeric bacterial counterpart (Tasneem et al., 2005). They are the simplest structural class of receptors of the superfamily, and they therefore represent a model system to examine structural and mechanistic aspects of channel activation. Homomeric receptors include the neuronal α7 nAChR that is involved in a range of neurological and psychiatric disorders, including Alzheimer's disease, attention deficit hyperactivity disorder and schizophrenia (Kalamida et al., 2007).

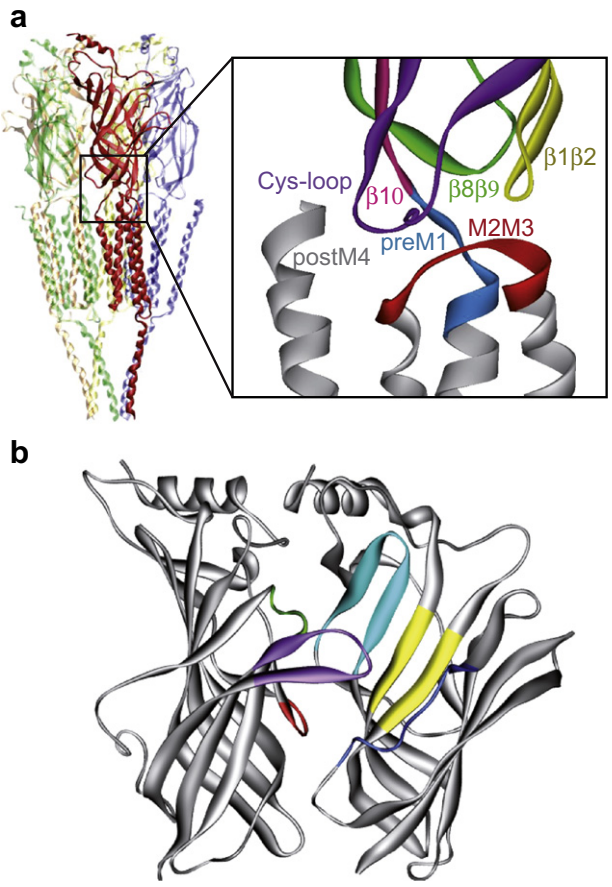

Fig. 1. Structure of the nAChR. (a) Cartoon diagram for the *Torpedo* nAChR (PDB 2BG9) and view of the structures at the interface between extracellular and transmembrane domains: β1β2 in yellow, Cys-loop in purple, β8β9 in green, β10 in pink, pre-M1 in light blue, and M2–M3 linker in red. (b) Extracellular domain showing the loops at the principal (Loop A in red, Loop B in green, and Loop C in pink) and complementary faces of the binding site (Loop D in yellow, Loop E in light blue, and Loop F in blue) (AChBP, PDB: 1UW6).

In serotonin-activated receptors, only 5-HT_3A subunits are able to form functional homomeric channels in heterologous expression systems and probably in native cells (Hussy et al., 1994; Holbrook et al., 2009).

All subunits share a basic scaffold composed of: (1) a large N-terminal extracellular domain of ~200 amino acids; (2) three transmembrane domains (TM) separated by short loops; (3) a cytoplasmic loop of variable size and amino acid sequence; and (4) a fourth transmembrane domain with a relatively short and variable extracellular COOH-terminal sequence (Bartos et al., 2009a).

Recent structural studies have provided an insight into the three dimensional structure of nAChRs and all members of the superfamily. In particular, a high resolution structural model (4 Å) of the nAChR from the marine ray *Torpedo* (Unwin, 2005) has been invaluable in the interpretation of functional and pharmacological data (PDB code 2BG9, Fig. 1a). This refined 4 Å resolution electron microscopy structure shows that the N-terminal extracellular portion is built around a β-sandwich core consisting mainly of ten β-strands from each subunit, resulting in a whole domain that contains two binding sites for ACh. The membrane-spanning portion, composed of the four α-helical segments from each subunit, is joined covalently to the extracellular domain at the N-terminal end of M1 (Fig. 1a). Thus, Cys-loop receptors are built on a modular basis, with the extracellular domain containing the agonist binding sites, and the transmembrane domain containing the pore and the channel gate (Fig. 1a).