EDITORIAL

Advances and hold-ups in the study of structure, function and regulation of Cys-loop ligand-gated ion channels and receptors

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The Cys-loop receptor channels, including the nicotinic acetylcholine (nAChR), serotonin 5-HT_3, $GABA_A$, $GABA_C$ and glycine receptor channels, are widely expressed in the nervous system (both central and peripheral) where they regulate excitability and neurotransmitter release, as well as at the endplate where they induce neuromuscular contraction. Furthermore dysfunctions in these receptors are thought to be involved in a variety of diseases and disorders, including (but not limited to) Alzheimer's disease, Parkinson's disease, epilepsy, schizophrenia, myasthenic syndromes, depression and substance abuse. Presently the Cys-loop receptors serve as molecular targets for a variety of clinically important drugs, such as muscle relaxants, tranquillizers, anti-convulsants and anti-emetics. Thus, these receptors are presently critical targets for the development of therapeutics to treat a variety of neurological diseases and disorders.

The Cys-loop receptor channels are pentameric assemblies, with each subunit arranged around a central ion-conducting pore. The binding of ligand to the extracellular interface between two subunits induces channel opening. With the discovery and crystallization of a molluscan ACh-binding protein, a soluble pentameric protein that is analogous to the extracellular ligand-binding domain of the Cys-loop receptors, along with the 4 Å resolution of the *Torpedo* nAChR, much has been learned about the structure of the ligand binding domain and the channel pore, as well as major structural rearrangements that may confer channel opening and function.

The Journal of Physiology held a satellite symposium, 'Advances and hold-ups in the study of structure, function and regulation of Cys-loop ligand-gated ion channels and receptors', at the 2009 Society for Neuroscience annual meeting in Chicago, IL, USA. It was a half-day event that brought together leading experts in the field to discuss the advances and challenges in studying the structure and function of the Cys-loop receptor channel superfamily.

August Smit (Vrije Universiteit Amsterdam, the Netherlands) began by talking about the lessons learned from 8 years of AChBP structure and function research, taking us through his discovery of this unique molluscan protein, how it was isolated and crystallized, what we have learned thus far, how it relates to studies on understanding the ligand-binding domain of the nicotinic ACh receptors (nAChRs) as well as other members of the superfamily, and how they are using this to screen for compounds that might be useful in the treatment of various neurological diseases and disorders.

However, since AChBPs do not function as ion channels, this protein my lack the structural features necessary for ion channel function and be of limited use if we want to understand channel gating, and how this is regulated by ligand binding. Lin Chen (Keck School of Medicine, University of Southern California, Los Angeles) discussed his work on the first high-resolution crystal structure of the extracellular domain of the α1 subunit of the nAChR, and the similarities and differences between the AChBP structure and those of other members of the Cys-loop receptor families. In addition, he discussed the current search for the structure of the neuronal nAChRs (Chen, 2010). These high resolution structures are critical for mechanistic studies of nAChRs and other members of the Cys-loop family, as well as in drug development. For example, close examination of the structure of the α1 nAChR extracellular domain (ECD) revealed four surface pockets that bind clusters of ordered solvent molecules, and these pockets may help in the search for small molecules that might act on the nAChRs, and guide the optimization of leading therapeutic compounds.

To date, there is no crystal structure of the entire protein of any member of the Cys-loop ligand-gated ion channel (LGIC) family. However, over 20 homologues of the Cys-loop LGICs have been found in bacteria, indicating the prokaryotic origin of this family of receptors. Pierre-Jean Corringer (Pasteur Institute, Paris) discussed his work on the cloning and crystallization of one of these prokaryotic proton-gated ion channels in an apparently open-pore conformation, GLIC (from *Gloeobacter violaceus*), the first transmembrane Cys-loop channel to be crystallized. This structure was compared to the crystal structure of another prokaryotic ion channel in an apparently closed conformation, ELIC (from *Erwinia chrysanthemi*). He compared and contrasted the structures of these bacterial homologues in the putative open and closed configuration, with the aim of trying to understand from a structural perspective the movements that occur during channel gating; these movements appear to involve both a quaternary twist and a tertiary deformation (Corringer *et al.* 2010). Furthermore, these structures tell us a lot about the structure of the pore, and interactions between the protein and the lipids.

The next set of speakers focused on the physiological and chemical aspects of how ligands that interact with these receptor channels alter the equilibrium constant of the global conformational change that leads to channel gating. Anthony Auerbach (State University of New York, Buffalo) reviewed work from his lab (and others) on the years of work involved in understanding how the energies of ligand binding and conformational change are coupled. In particular he measures the distribution of energy propagating through the system, and relates this dynamic energy map to specific chemical forces and atomic structures (Auerbach, 2010).

Cynthia Czajkowski (University of Wisconsin, Madison) then talked about her work linking structure to function in $GABA_A$ receptors. Her lab uses a combination of approaches including mutagenesis, disulfide cross-linking, voltage-clamp fluorimetry, and homology modelling to identify conformational movements in the $GABA_A$ receptor ($GABA_AR$) triggered by agonist binding/channel gating. Neurotransmitter

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binding is believed to initiate an inward capping movement of the loop C region of the ligand-binding site, which ultimately triggers channel gating. They have identified a critical intra-subunit salt-bridge between conserved charged residues (βE153, βK196) in the $GABA_AR$ that is involved in regulating loop C position. By monitoring disulfide bond formation between cysteines substituted at these positions (E153C–K196C), the mobility of loop C in resting and ligand-bound states was probed. Disulfide bond formation was significantly reduced in the presence of activating concentrations of GABA and pentobarbital, suggesting that activation of the $GABA_AR$ proceeds via restricting loop C mobility. She also discussed her work where they demonstrated that the $\beta4$–$\beta5$ linker (β2K102–G108) in the $GABA_AR$ β-subunit links GABA binding site movements in loop A to the inner β-sheet, indicating that the length and flexibility of this linker region in the β-subunit is a key structural determinant of $GABA_AR$ function.

Jeremy Lambert (University of Dundee, Scotland, UK) then talked about the work from his lab on the structural basis of ion selectivity and conductance in the cation-selective members of this family, with a particular emphasis on the 5-HT_3 receptor. It has been thought for decades that the lining of the channel of the Cys-loop receptors is formed by the α-helical second transmembrane (TM2) domain of each of the five subunits present within the receptor, and that residues within and adjacent to the TM2 domain influence single channel conductance, ion selectivity, gating and desensitization. However they found that the intracellular domain of LGICs, in particular a region of the loop linking TM3 and TM4 termed the membrane associated (MA) stretch, exerts a strong influence upon ion channel biophysics (Peters *et al.* 2010). In addition, he also discussed recent work implicating the extracellular domain as an additional important determinant of ion conduction. This issue of *The Journal* also contains a paper by Wu *et al.* (2010) where the authors show that there is a conserved cysteine residue (position 312) in the TM3 domain of the serotonin 5-HT_3 receptor channel. When this residue in the 5-HT_3A subunit was mutated to various other residues, the authors showed that this residue was essential for homomeric (but not heteromeric) receptor gating.

Finally, I discussed two aspects of the work from my lab: our search for (1) novel AChBPs from outside of the phylum Mollusca, and (2) residues in the extracellular domain responsible for regulating desensitization (Yakel, 2010). We identified a homologue of the molluscan acetylcholine-binding protein (AChBP) in the marine polychaete *Capitella teleta*, from the annelid phylum. The *Capitella teleta* AChBP (ct-AChBP) has 21–30% amino acid identity with known molluscan AChBPs. Sequence alignments indicate that ct-AChBP has a shortened Cys-loop compared to other Cys-loop receptors, and a variation on a conserved C-loop triad, which is associated with ligand binding in other AChBPs and nAChR α subunits. In addition nAChRs can undergo desensitization, the process whereby the channel closes in the continued presence of agonist. Although the mechanism of desensitization is not completely understood, it is thought to be an important factor in controlling cholinergic signalling, and perhaps in certain nAChR-related disease. We have found two residues in the extracellular domain of the α7 nAChR that when mutated dramatically slowed the kinetics of onset of desensitization. We are trying to understand how structural elements in the extracellular domain link to the function of these receptors.

In addition for this issue, Meyer Jackson (University of Wisconsin, Madison, Wisconsin, USA) has written a Classical Perspectives article (Jackson, 2010) on the paper by Neher & Steinbach (1978). This paper came soon after the first observations of single channel currents in cells, and the issue of the day was what could this information tell us? As detailed in this Classical Perspective, Neher and Steinbach turned to address the question of mechanism of action of local anaesthetics. In this study, they established the idea of open channel block as an important mechanism of action, while also demonstrating the power of single channel recordings.

How the structure of the Cys-loop LGICs affects binding, gating and desensitization is still unknown, but a general hypothesis has emerged of how ligands that interact with these receptor channels alter the equilibrium constant of the global conformational change that leads to channel gating and desensitization. With the continued use of a variety of experimental, structural and modelling techniques, major advances await in the near future. These studies are likely to continue to elucidate the various steps linking ligand binding to gating and desensitization, as well as how we might be able to affect function through external ligands that might be useful in treating various neurological disorders and diseases.

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