Synaptic Neurotransmitter-Gated Receptors

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Since the discovery of the major excitatory and inhibitory neurotransmitters and their receptors in the brain, many have deliberated over their likely structures and how these may relate to function. This was initially satisfied by the determination of the first amino acid sequences of the Cys-loop receptors that recognized acetylcholine, serotonin, GABA, and glycine, followed later by similar determinations for the glutamate receptors, comprising non-NMDA and NMDA subtypes. The last decade has seen a rapid advance resulting in the first structures of Cys-loop receptors, related bacterial and molluscan homologs, and glutamate receptors, determined down to atomic resolution. This now provides a basis for determining not just the complete structures of these important receptor classes, but also for understanding how various domains and residues interact during agonist binding, receptor activation, and channel opening, including allosteric modulation. This article reviews our current understanding of these mechanisms for the Cys-loop and glutamate receptor families.

To understand how neurons communicate with each other requires a fundamental understanding of neurotransmitter receptor structure and function. Neurotransmitter-gated ion channels, also known as ionotropic receptors, are responsible for fast synaptic transmission. They decode chemical signals into electrical responses, thereby transmitting information from one neuron to another. Their suitability for this important task relies on their ability to respond very rapidly to the transient release of neurotransmitter to affect cell excitability.

In the central nervous system (CNS), fast synaptic transmission results in two main effects:

neuronal excitation and inhibition. For excitation, the principal neurotransmitter involved is glutamate, which interacts with ionotropic (integral ion channel) and metabotropic (second-messenger signaling) receptors. The ionotropic glutamate receptors are permeable to cations, which directly cause excitation. Acetylcholine and serotonin can also activate specific cation-selective ionotropic receptors to affect neuronal excitation. For controlling cell excitability, inhibition is important, and this is mediated by the neurotransmitters GABA and glycine, causing an increased flux of anions. GABA predominates as the major inhibitory transmitter throughout

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the CNS, whereas glycine is of greater importance in the spinal cord and brainstem. They both activate specific receptors—for GABA, there are ionotropic and metabotropic receptors, whereas for glycine, only ionotropic receptors are known to date.

Together with acetylcholine- and serotonin-gated channels, GABA and glycine ionotropic receptors form the superfamily of Cys-loop receptors, which differs in many aspects from the superfamily of ionotropic glutamate receptors. Over the last two decades, our knowledge of the structure and function of ionotropic receptors has grown rapidly. In this article, we summarize our current understanding of the molecular operation of these receptors and how we can now begin to interpret the role of receptor structure in agonist binding, channel activation, and allosteric modulation of Cys-loop and glutamate receptor families. Further details on the regulation and trafficking of neurotransmitter receptors in synaptic structure and plasticity can be found in accompanying articles.

CYS-LOOP RECEPTOR SUPERFAMILY

In the central and peripheral nervous systems, neurotransmitter-gated ion channels underpin the rapid transfer of information between neurons by permitting the passage of ions across the cell membrane. They are located at presynaptic and postsynaptic sites to affect the excitability of neurons and muscle. Within a few milliseconds following a presynaptic action potential, receptors bind neurotransmitter molecules to initiate channel opening. These channels close just as rapidly after the dissociation of neurotransmitters to terminate synaptic events. The importance of these receptors is exemplified by the diseases that are caused following their dysfunction and by the increasing number of therapeutic agents that target them. Postsynaptic receptors are not confined to synapses—their extrasynaptic counterparts are responsive to low levels of neurotransmitter mediating a persistent tonic control over cell activity. Thus, their roles are manifold, and with improved structural techniques, it is now possible to assign some function to various domains of Cys-

loop receptors, although much remains to be discovered.

Cys-Loop Receptor Gene Families

The founding members were defined as “Cys-loop receptors” because they contain a highly conserved structural signature—a loop formed by a disulfide bridge in the extracellular domain. They are nicotinic acetylcholine receptors (nAChR), type 3 5-hydroxytryptamine receptors (5HT₃R), γ -aminobutyric acid receptors (type A and C, GABA_{A/C}R), and glycine receptors (GlyR) (Barnard et al. 1987; Grenningloh et al. 1987; Betz 1990). Also included are the Zn²⁺-activated cation channel (Davies et al. 2003) and invertebrate receptors activated either by glutamate or serotonin (anionic channels) or GABA (cationic channels) (Ortells and Lunt 1995; Lester et al. 2004), but the related bacterial homologs, *Gloeobacter violaceus* (GLIC) (Bocquet et al. 2009) and *Erwinia chrysanthemi* (ELIC) (Hilf and Dutzler 2008), lack the characteristic Cys-loop signature. Consequently, Cys-loop receptors are more precisely referred to as “pentameric ligand-gated ion channels.”

Their diversity is extensive, with most subunit families encoded by multiple genes. For the major ligand-gated anion channels, such as GABA_{A/C}Rs, eight subunit families have been identified (α 1-6, β 1-3, γ 1-3, δ , ϵ , π , θ , and ρ 1-3), yielding 19 subunits. Although multiple subunit combinations are possible, prominent among these are $\alpha\beta\gamma$ (stoichiometry 2:2:1) and $\alpha\beta\delta$. In contrast, the ρ -subunits preferentially combine together forming either homomeric or heteromeric receptors that are referred to as GABA_CRs; for GlyRs, two subunit families exist, α 1-4 and β , yielding a total of five subunits. Early on during development, GlyRs form α -subunit homomers, later changing to $\alpha\beta$ heteromers (Alexander et al. 2008).

The major ligand-gated cation channels include the extensive nAChR family. Five subunit families are known (α 1-7, 9 and 10, β 1-4, δ , ϵ , and γ), providing 16 subunits in vertebrates. Subunit combinations at the neuromuscular junction are well known (embryonic: $\alpha_2\beta\gamma\delta$; and adult: $\alpha_2\beta\epsilon\delta$). For neuronal nAChRs, sub-

unit assemblies are less clear with $\alpha\beta$ combinations comprising $\alpha(2-6)$ and $\beta(2-4)$. The $\alpha 7$ and $\alpha 9$ subunits can form homomers but also combine with other subunits, such as $\alpha 10$ ($\alpha 9\alpha 10$) or $\beta 2$ ($\alpha 7\beta 2$). The less-extensive 5-HT₃R family forms cation-permeable channels from only two main subunits, 5-HT_{3A} and 5-HT_{3B}. The 5-HT_{3A} subunit either forms homomers or coassembles with 5-HT_{3B}. Three other subunits have been reported—5-HT_{3C-E}—but these primarily affect the expression levels of 5-HT_{3A} and not the receptor's physiological profile (Alexander et al. 2008).

Anchoring Cys-Loop Receptors: Receptor-Associated Molecules and Phosphorylation

The efficacy of synaptic transmission relies heavily on receptor subunit composition and the numbers that cluster opposite axon terminals. Receptor-associated molecules provide a scaffold near the surface membrane that influences receptor trafficking. Moreover, through post-translational modifications of receptors and/or scaffolds, receptor expression, function, and plasticity can be regulated (Moss and Smart 2001; Araud et al. 2010; Luscher et al. 2011).

A key component for anchoring GABA_A/C Rs and GlyRs at inhibitory synapses is gephyrin, which forms a lattice connecting receptor subunits to the cytoskeleton (Feng et al. 1998; Kneussel and Betz 2000; Sassoe-Pognetto and Fritschy 2000; Luscher and Keller 2004; Jacob et al. 2008; Tyagarajan and Fritschy et al. 2008; Fritschy 2010). There are numerous other proteins, including GABA_AR-associated protein, Plic-1, Huntingtin-associated protein 1, which affect the surface stability and trafficking of GABA_ARs (Luscher and Keller 2004; Luscher et al. 2011). Although not subject to the same research intensity, extrasynaptic receptors can associate with other proteins, for example, $\alpha 5$ -subunit-containing GABA_ARs and radixin, which slows diffusion of the receptor in the surface membrane (Loebrich et al. 2006). Anchoring is reversible because neurotransmitter-gated receptors are laterally mobile in the surface membrane, which is a major pathway for replen-

ishing receptors at synapses (Thomas et al. 2005; Triller and Choquet 2005; Bogdanov et al. 2006).

Many of these interactions are regulated by phosphorylation of receptor subunits and their associated molecules, affecting receptor trafficking, assembly, and stability in the surface membrane. Consensus sites for serine/threonine and tyrosine kinase phosphorylation of GABA_A/C R and GlyR subunits are located in the intracellular domains between M3 and M4 of GABA_AR $\alpha 4$, β -, and γ -subunits, and GlyR α -subunits (Moss and Smart 1996). Gephyrin can also be phosphorylated, which is important for inhibitory synaptic plasticity (Tyagarajan and Fritschy 2010).

With regard to clustering nAChRs in skeletal muscle, rapsyn plays a major role with the receptor tyrosine kinase, MuSK, linking via β -catenin and β -dystroglycan to form a scaffold between nAChRs and the cytoskeleton (Bruneau et al. 2009). For neuronal nAChRs, although receptor-associated molecules are less well defined, PSD-93, PSD-95, SAP102, and neuregulin-1 affect receptor clustering and surface expression (Neff et al. 2009; Araud et al. 2010; Fernandes et al. 2010). Similar to GABA_ARs and GlyRs, nAChRs can also be phosphorylated in their major intracellular domains to affect expression levels and cell surface clustering (Wallace et al. 1991; Charpentier et al. 2005; Lee et al. 2008).

Irrespective of their location, the main function of neurotransmitter-gated ion channels is to rapidly transmit the binding “signal” to the ion channel, opening the gate and permitting ion flux (Lester et al. 2004; Bartos et al. 2009; Cederholm et al. 2009; Chang et al. 2009; Miller and Smart 2010). Our understanding as to how the receptor's underlying molecular machinery is structurally and functionally configured to achieve this rapid response is constantly evolving.

Pentameric Receptor Structure

Although structure–function studies have been hampered by a lack of crystal structures for Cys-loop receptors, the density of nAChRs in *Torpedo marmorata* (Unwin 2005) has provided high-resolution images down to 4 Å. These

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reveal a modular design for nAChRs comprising pseudosymmetrical rings of five subunits, each contributing an extracellular domain (ECD) of anti-parallel sets of inner and outer β -sheets, a four- α -helical transmembrane domain (TMD), and an intracellular domain (ICD) of largely unknown structure (Fig. 1). The ECDs are positioned over the TMDs, providing a central aqueous pathway that stretches from the external vestibule formed by the ECDs, on through the TMDs and channel gate, before dissipating via the ICDs (Fig. 1). The signature Cys-loop structure is formed by a disulfide bond and contains 13 amino acids situated at the base of the ECDs. It is an important conduit for communication between the neurotransmitter-binding sites and the ion channel. This overall structure of the receptor agrees reasonably well with the first crystal structure of a Cys-loop receptor, the glutamate-gated Cl^- channel (GluCl) from *Caenorhabditis elegans* (Fig. 1) (Hibbs and Gouaux 2011). This recep-

tor also contains a large ECD, four TMDs, and a Cys-loop, in addition to another Cys bridge in loop C, a feature also present in GlyR α -subunits, with which GluCl shares 43% primary sequence identity.

The Primary Step: Ligand Binding

The initial step in the activation pathway involves the binding of a neurotransmitter between neighboring subunit ECDs, coordinated by six binding loops, A–F. Binding loops A–C from one subunit form the principal (P or +) face of the binding site, and loops D–F are contributed by the complementary (C or –) face of the next subunit (Fig. 2) (Corringer et al. 2000). For a homomeric receptor (e.g., GlyR α 1 or GABA C R ρ 1), up to five binding sites are presented, although occupancy of each one is unnecessary for full receptor activation (Amin and Weiss 1996; Beato et al. 2004). For GABA A -Rs comprising α 1 β 2 γ 2 subunits, two identical

Figure 1. Architecture of Cys-loop receptors. Structure of (left) *Torpedo* nAChR solved from electron microscopic images at the 4 Å level (pdb 2BG9) (Unwin 2005) and (right) the *C. elegans* glutamate-gated anion channel at 3.3 Å (pdb 3RIA) (Hibbs and Gouaux 2011). The pentameric subunit assembly and secondary structure are shown for the extracellular domain (ECD) and transmembrane domain (TMD). The ECDs are composed of inner and outer β -sheets with an α -helix, and each subunit's TMD is formed by four α -helices (M1–M4). Note that for nAChR, the intracellular (MA) helices preceding M4 are omitted, whereas the M3–MA stretch is disordered and is thus not included in the structure. For the GluCl structure, the M3–M4 domain is replaced by a tripeptide, A-G-T (Hibbs and Gouaux 2011).

Figure 2. Synaptic view of a Cys-loop receptor. Looking across from the presynaptic terminal over to the post-synaptic membrane, an image of the structure for a typical Cys-loop receptor is shown. This is generated from the atomic resolution structure for AChBP (pdb 2BYQ) (Hansen et al. 2005) for the ECD, linked to the trans-membrane domains taken from images of GLIC (Bocquet et al. 2009). (A) The five subunits form a pseudosymmetrical ring with interfacial binding sites between principal (P, +) and complementary (C, –) binding faces. Note the central aqueous pathway for ion conduction. (B) A cut-away slab from A depicts the loop C structures on each subunit and the relative stoichiometry for a muscle nAChR and neuronal GABA_AR. The identity of the subunits and neurotransmitter-binding sites are illustrated. (C) Further cut-away to reveal the tops of the TMDs showing M2 lining the ion channel and the support formed by M1, M3, and M4.

β – α interfaces are able to bind neurotransmitter, whereas in muscle nAChRs ($\alpha\beta\gamma\delta$), these sites are different, being located at γ – α and δ – α interfaces (Karlin and Akabas 1995). Although the agonist binding loops are readily identifiable across the Cys-loop receptor family, the aromatic and hydrophobic residues coordinating agonist binding vary (Ashton and Young 2003; Bohme et al. 2004; Celie et al. 2005a; Hansen et al. 2005; Gay and Yakel 2007; Young et al. 2007).

Understanding how agonist molecules bind has been facilitated by X-ray crystallography of acetylcholine-binding proteins (AChBPs), a group of molluscan proteins (Brejc et al. 2001; Smit et al. 2001; Celie et al. 2005b; Geitmann et al. 2010). Although AChBPs lack TMDs and thus an ion channel, they *do* assemble into pentamers with a topology resembling the ECDs of nAChRs. As a consequence, they are used exten-

sively as templates for structural homology modeling of Cys-loop receptors (Geitmann et al. 2010; Miller and Smart 2010). Such studies reveal that cation– π interactions between an agonist's terminal amine and hydrophobic residues in the receptor are critical determinants of binding (Zhong et al. 1998; Beene et al. 2002; Mu et al. 2003; Celie et al. 2004; Padgett et al. 2007; Pless et al. 2008). Modeling studies will now be considerably enhanced by using the GluCl structure at 3.3 Å resolution (Hibbs and Gouaux 2011).

Agonist Binding Loops A–F

After agonist binding, structural rearrangements around the binding site are transmitted through the ECD to the TMDs to initiate ion channel opening. Agonist-induced conformational changes to

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loops A, D, and E (Fig. 3) are not readily apparent from AChBP structures, but subtle (~ 0.5 Å) movements are detected in residues of loops C and D in high-resolution images of GluCl crystals containing the coagonists glutamate and ivermectin (Hibbs and Gouaux 2011). Mutating specific residues in loops A, D, and E causes Cys-loop channels to open spontaneously in the absence of agonist, indicating their importance for receptor activation (Boileau et al. 2002; Torres and Weiss 2002; Sedelnikova et al. 2005; Miller et al. 2008). In particular, loop A and parts of the ECD's hydrophobic core are vital for linking allosteric modulator sites to neurotransmitter binding sites (Miller et al. 2008) and for stabilizing the closed state of the ion channel (Chakrapani et al. 2003; Miller et al. 2008).

For loop F in GABA_A/C_{Rs}, conformational changes do not appear to be associated with receptor activation (Khatri et al. 2009; Wang et al. 2010) with minor movement thought to result from loop F's connection to loop C, possibly signaling the presence of bound ligand (Khatri and Weiss 2010). Nevertheless, movement of loop F during activation of muscle nAChRs has been proposed (Gleitsman et al. 2008).

In comparison with these minimal movements, structural changes can occur in loop B, which sits behind loop C and connects the inner and outer β -sheets near the top of the ECD. Loop B may interact with loops A and C on the principal binding face (Grutter et al. 2003; Lee and Sine 2004; Cashin et al. 2007). After agonist binding, loop B appears to rotate and envelop the agonist molecule in the binding site, and substituting residues in this loop initiates spontaneous channel opening in GABA_A-Rs (Newell et al. 2004).

The Role of Loop C

The most pronounced movement involves loop C. Cocrystallizing AChBP with an agonist indicates that loop C moves toward the receptor by ~ 7 Å, "capping" the agonist-binding site (Celie et al. 2004; Hansen et al. 2005). In contrast, antagonist binding to the same site either keeps loop C uncapped or displaces it further away from the receptor (Bourne et al. 2005; Celie et al. 2005a; Hansen et al. 2005). Evidence of loop C repositioning itself closer to the agonist molecule is also evident in GluCl (Hibbs and Gouaux 2011). The precise role of capping is

Figure 3. Structure of the extracellular domain. Side view of two adjacent subunits of the AChBP. The positions of the binding loops and other loops from the amino terminus to the carboxyl terminus that adjoins the pre-M1 domain in Cys-loop receptors are shown. β -strands that form the inner and outer β -sheets are also indicated with labeling according to Brejc et al. (2001).

undefined. It could trap agonist molecules at the binding site and/or prime the channel for activation by moving two β -strands ($\beta 9$ and $\beta 10$, which support loop C) to initiate movement in the pre-M1 domain. This would transfer the conformational wave from the ECD to the TMD for channel activation (Fig. 3) (Unwin 2005). Loop C capping *does* occur in spontaneously opening mutant nAChRs in the absence of agonist, suggesting that it is important for channel activation (Mukhtasimova et al. 2009).

A preactivation or “flip” state, which might relate to the loop C capped state, has been proposed from studies of full and partial agonists at nAChRs and GlyRs (Lape et al. 2008). After binding full agonists, the receptor attains the preactivated state far easier than with partial agonists; once preactivated, channel gating proceeds with equal efficiency for either type of agonist. Full agonists are characterized by their higher affinity for preactivated states (Lape et al. 2008). Whether preactivation represents a discrete structural state (e.g., loop C capped state) remains uncertain. If these states are equivalent, then whether an agonist is full or partial will depend on its ability to promote loop C capping. Presently, it is likely that preactivation is one of several structural intermediates that are passed through during the process of receptor activation.

The view that loop C capping is an important early step in preactivation is supported by studies analyzing the effect of mutations on rate equilibrium free-energy relationships. This relies on a simple kinetic scheme and a statistical value Φ , indicating whether a group of residues is similar to an open ($\Phi = 1$) or shut channel state ($\Phi = 0$) (Miller and Smart 2010). Agonist binding induces conformational changes to residues near the binding site early on during activation (Φ near to 1) (Grosman et al. 2000). Next the Cys-loop and loop 2 change at near identical times, and finally the M2–M3 linker, which adjoins the ion channel, follows slightly later (lower values of Φ) (Purohit and Auerbach 2007). This is plausibly modeled by discrete groups of residues contributing to a conformational wave from the ECD to the TMD. The near-identical changes that occur after different ago-

nists bind to the receptor indicate that the conformational wave is independent of the nature of the agonist (Han et al. 2004). Interestingly, non-binding interfaces also undergo conformational rearrangement when agonist is bound (Wang et al. 2010), which is indicative of a propagated conformational wave.

It is notable that the binding sites for agonists and many allosteric modulators are located at subunit interfaces where movements of the binding loops (Chang and Weiss 2002) and interactions across the interface are important for stabilizing the resting receptor in a shut state (Harvey et al. 1999; Nevin et al. 2003; Miller et al. 2005; Mukhtasimova and Sine 2007; Gleitsman et al. 2008). It is therefore not surprising that destabilizing the interface by mutation can lead to spontaneous channel activation (Miller et al. 2008; Todorovic et al. 2010).

Coupling the Extracellular Domain to the Ion Channel

Several structures are important for transferring the conformational wave from the ECD to the TMD (Fig. 3), specifically loop 2 ($\beta 1$ – $\beta 2$, inner β -sheet), loop 7 (Cys loop, connects inner and outer β -sheets) and loop 9 (loop F) at the base of the ECD, together with the pre-M1 domain (linked to $\beta 10$ and thus loop C) and the M2–M3 linker (Bouzat et al. 2004).

Loops 2 and 7 arch over the M2–M3 linker, and their displacement after agonist binding may trigger channel opening (Unwin 2005), affecting the accessibility of residues in the M2–M3 linker (Bera et al. 2002). An alternative view of channel opening comes from comparing open and shut conformations of GLIC and ELIC. This suggests that loop 2 moves down toward the ion channel (Bocquet et al. 2009), outwardly displacing the Cys-loop and the M2–M3 linker. This allows the M2 helices to retract toward M1, M3, and M4, opening the channel (Fig. 2).

Loop 9 and the pre-M1 domain in the ECD could also interact with the TMD (Lyford et al. 2003; Newell and Czajkowski 2003; Bouzat et al. 2004). Although the pre-M1 domain is structurally rigid during receptor activation (Unwin 2005; Hilf and Dutzler 2008, 2009; Bocquet

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et al. 2009), it may interact with the Cys-loop and the M2–M3 linker to link agonist binding to channel activation (Castaldo et al. 2004; Lee and Sine 2005; Mercado and Czajkowski 2006; Lee et al. 2009) acting as a pivot around which other domains move (Hu et al. 2003; Lee and Sine 2005).

The nature of the contacts between the ECD and TMD relies on hydrophobic and electrostatic interactions (Absalom et al. 2003; Kash et al. 2003; Lee and Sine 2005; Unwin 2005; Xiu et al. 2005; Price et al. 2007). The hydrophobic contacts are well conserved across the Cys-loop family, whereas the electrostatic interactions vary. Overall, the electrostatic environment is more important than the location of individual charged residues (Absalom et al. 2003; Kash et al. 2003; Xiu et al. 2005).

The critical importance of the M2–M3 linker for Cys-loop receptor activation is shown by the dysfunction that is caused by naturally occurring pathogenic mutations within this domain, which include congenital myasthenic syndrome (nAChR), epilepsy (GABA_AR), and hyperekplexia (GlyR) (Breitinger and Becker 2002; Engel et al. 2003; MacDonald et al. 2004; Chung et al. 2010).

Ion Channel Domain

Electron microscopy of nAChR channels shows that each subunit contributes its M2 domain to a ring of five α -helices (Miyazawa et al. 2003). A tapering of the helices causes the ion channel pore to widen at the extracellular end with a “kink” in the middle of each α -helix (Fig. 4). The ion channel is stabilized by contacts between neighboring M2 helices and by the M1 and M3 helices behind forming a supporting ring for the channel (Miyazawa et al. 2003).

Structurally, the pore lining of the channel is highly conserved and composed of concentric rings of similar residues. Some of these rings are hydrophobic (9'-leucine and 16'-leucine in nAChRs). Highly conserved, positively charged residues at the cytoplasmic end of M2 are defined as 0', increasing to another ring of negatively charged residues at the extracellular end defined as 20'. Others are polar (2'-threonine,

6'-serine). At both ends of M2, rings of residues with charged side chains can be found (–1'-glutamate, 20'-glutamate for nAChRs). These homologous rings are also broadly conserved in ELIC, GLIC (Bocquet et al. 2009), and GluCl (Fig. 4) (Hibbs and Gouaux 2011).

Opening the Ion Channel

How the ion channel opens is still not completely understood. For nAChRs, a rotation of M2 α -helices to remove the kinked region from the conduction pathway has been proposed (Unwin 2005). It is around the M2 kink that the α -helices converge to form the principal channel gate with hydrophobic rings (9'-leucines and 13'-valines), constricting the channel to ~ 6 Å in diameter (White and Cohen 1992; Banks et al. 2000; Miyazawa et al. 2003; Arevalo et al. 2005; Unwin 2005). The two α -subunit M2 helices are the first to move during receptor activation (Purohit et al. 2007), presumably destabilizing the channel gate to allow other α -helices to retract and complete channel opening. When the channel opens, the pore diameter increases following retraction of M2 toward M1 and M3 (Miyazawa et al. 2003; Unwin 2005), which is permitted by an aqueous cavity (Wick et al. 1998; Bera et al. 2002; Bera and Akabas 2005; Brannigan et al. 2008). An outward tilting motion in M2 could also explain channel opening (Cymes et al. 2005; Paas et al. 2005) following a comparison of ELIC and GLIC channels. This involves an outward movement of the M2–M3 linker, pulling and tilting M2 to open the channel (Fig. 4) (Hilf and Dutzler 2008; Bocquet et al. 2009).

Ion Selectivity Filter

At either end of the ion channel, there are charged rings of residues that regulate channel conductance (Fig. 4) (Imoto et al. 1988; Langosch et al. 1994; Wang et al. 1999; Moorhouse et al. 2002). For nAChRs and 5HT₃Rs, the rings are negatively charged, selecting for cations, whereas for GABA_{A/C}Rs and GlyRs, the rings are net positive and thus anion selective (Keramidas et al. 2004).

Figure 4. Cys-loop receptor ion channel domain. The M2 domains of several Cys-loop receptors are aligned; those residues that are exposed and lining the channel lumen are boxed. The structural model shows the M2 domains of open GLIC and closed ELIC. For GLIC, the β 1-2 loop (green) moves downward and outwardly displaces the M2–M3 linker (blue) hauling the M2 domains (blue) upward and tilting outward to open the channel compared with ELIC (pink and red).

Reducing charge density at the extracellular 20'-ring promotes outward rectification (Imoto et al. 1988; Moorhouse et al. 2002), in accord with an effect on the concentration of permeant ions in the channel vestibule (Keramidas et al. 2004). In comparison, mutating the cytoplasmic ring ($-1'$) symmetrically affected membrane current. This ring is located at the narrowest point of the open channel and contributes to the main ion selectivity filter. Indeed, substituting residues in nAChR α 7 and 5HT₃R α s to replicate those in GlyRs switched the ionic permeability from cationic to anionic (Galzi et al. 1992; Corringer et al. 1999; Gunthorpe and Lummis 2001). Switching GlyR and GABA_CR permeability from anionic to cationic can be achieved by the reverse substitutions (Kerami-

das et al. 2000, 2002; Miller and Smart 2010). Analysis of anion binding in GluCl crystals suggests that the $-2'$ -proline forms part of an electropositive ion binding "pocket," increasing bulk ion concentration at the cytoplasmic end of the channel. As such, the $-2'$ -position is thought to be part of the pore lining for anion- and cation-selective Cys-loop receptors (Fig. 4) (Hibbs and Gouaux 2011). Interestingly, for heteromeric $\alpha\beta\gamma$ GABA_AR α s, switching ion permeability only requires substitution of residues in the β 3 subunit (Jensen et al. 2002), indicating its greater contribution toward ion selection.

Outside the ion channel, there are key residues that have a significant impact on channel conductance. For 5-HT_{3A}R α s, by replacing a group of arginine residues in the M3–M4

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intracellular domain with alanines or by coexpressing with the 5-HT_{3B} subunit, single-channel conductance was markedly increased (Davies et al. 1999; Kelley et al. 2003). Positively charged arginines were thought to line the portals formed by the internal subunit interfaces (Miyazawa et al. 2003; Unwin 2005) hampering cation flux by electrostatic interaction (Miyazawa et al. 2003; Peters et al. 2005; Unwin 2005).

With respect to the extracellular domain, ions entering the channel must first pass through an outer vestibule formed from the ECDs of each subunit. This vestibule narrows to ~20 Å in diameter (Brejc et al. 2001; Unwin 2005) and contains either a ring of negatively charged residues in nAChRs or positively charged residues in anion-selective channels (Hansen et al. 2008). Increasing positive charge density in the vestibule lowered the nAChR channel conductance (Hansen et al. 2008). These rings of residues may be important for bulk concentration of permeant ions in the vestibule just outside the ion channel; equally plausible is a role as an outer selectivity filter along the ion permeation pathway.

Our understanding of the main components involved in Cys-loop receptor activation continues to evolve. Nevertheless, we need greater clarity regarding the role of agonist-binding loops and interactions across the subunit interfaces during receptor activation, and how the conformational wave spreads toward the key interface between the ECD and the TMD. We are less clear about the mechanisms involved in channel opening—exactly what role does loop C capping actually play? No doubt the structures for AChBP, GluCl, GLIC, and ELIC, in tandem with the high-resolution images of nAChRs, will provide insight.

Finally, one area that has lagged significantly behind concerns the structural determination of a Cys-loop receptor's large intracellular domain and how it is modified in response to signaling and other receptor-associated molecules and scaffold proteins. An understanding of the fundamental processes is important for establishing how drugs modulate the activities of Cys-loop receptors and, importantly, how dysfunction to their signaling is a pivotal cause of debilitating disease.

IONOTROPIC GLUTAMATE RECEPTORS

The vast majority of excitation in the central nervous system is mediated by glutamate, a ubiquitous amino acid (Watkins and Jane 2006). In neurons, glutamate is packed within synaptic vesicles by dedicated vesicular transporters (the vGluTs) and following presynaptic activity is released in the synaptic cleft. It then diffuses rapidly across the synaptic cleft and activates ionotropic glutamate receptors (iGluRs) localized primarily on the postsynaptic membrane. Because iGluRs form ion channels that flux cations, and not anions, activation of iGluRs results in membrane depolarization. Besides mediating the basic excitatory synaptic responses, iGluRs are also central in the induction and maintenance of synaptic plasticity, long-term changes in synaptic efficacy that underlie the remarkable capacity of the brain to store and retrieve information over extended periods of time.

Multigenic Families and Subunit Composition Diversity

Mammalian iGluRs are encoded by a total of 18 genes that assemble into four major families: the AMPA, kainate, NMDA, and delta receptors (AMPA, kainate, NMDA, and delta receptors (AMPA, kainate, NMDA, and delta receptors, respectively) (Fig. 5) (Hollmann et al. 1989; Seeburg 1993; Nakanishi and Masu 1994; Dingledine et al. 1999). The excitatory postsynaptic current is typically mediated by members of the AMPA and NMDA families. KainateRs are found both on presynaptic and postsynaptic compartments and have a role more modulatory than direct at synapses (Lerma 2006; Contractor et al. 2011). DeltaRs are the least understood iGluRs largely because, in contrast to other iGluRs, their apparent incapacity to gate an ion channel following ligand binding makes them electrically “silent” (Kohda et al. 2000; Schmid et al. 2009). There are four AMPAR genes (GluA1-4, also known as GluR1-4); five kainateR genes (GluK1-5, also known as GluR5-7, KA1-2); seven NMDAR genes (GluN1; GluN2A-D; GluN3A-B, also known as NR1; NR2A-D; NR3A-B), and two δ -subunits (GluD1-2, also known as $\delta 1$ and $\delta 2$).

Ionotropic glutamate receptors (iGluRs)			
AMPA	Kainate		Delta
GluA1	GluK1	GluK4	GluD1
GluA2	GluK2	GluK5	GluD2*
GluA3	GluK3	GluN1* GluN2A GluN3A* GluN2B GluN3B* GluN2C GluN2D	
GluA4			

* These subunits bind glycine or D-serine.

Figure 5. The four families of iGluR subunits. Each subunit is encoded by a distinct gene. There is no known mixing of subunits between families.

Functional iGluRs are formed exclusively by subunits within the same family. AMPAR subunits can form both homo- and heteromers, most native AMPARs being of the latter type (Jonas and Burnashev 1995; Rossmann et al 2011). In kainateR, only GluK1–GluK3 subunits can function as homomers; GluK4 and GluK5 form functional receptors only when coexpressed with GluK1–GluK3 (Contractor et al. 2011). NMDARs are obligatory heteromers associating two GluN1 subunits with either two GluN2 subunits (which can be of different type) or a combination of GluN2 and GluN3 subunits (Cull-Candy and Leszkiewicz 2004; Ulbrich and Isacoff 2007; Paoletti 2011). Functional GluN1/GluN3 complexes, activated by glycine but not glutamate, have also been described in heterologous systems but not in native situations (except on myelin) (Henson et al. 2010; Piña-Crespo et al. 2010). Finally, the δ -subunits mainly form homomeric receptors. Overall, it appears that within each family (the deltaR excepted), differential coassembly of subunits generates a large number of receptor subtypes. This diversity is further enhanced by posttranscriptional modifications of several iGluR genes. The GluN1 subunit, all four AMPAR subunits, and kainateR subunits GluK1–GluK3 are subjected to alternative splicing (Monyer et al. 1991; Dingledine et al. 1999). AMPA and kainate receptor genes can also undergo RNA editing, which leads to single-amino-acid exchanges with important functional consequences (Seeburg and Hartner 2003). The iGluR subunits also experience posttranslational modifications including phosphorylation (see below),

palmitoylation, and SUMOylation (Traynelis et al. 2010).

The iGluRs are thus diverse in their subunit composition, and each receptor subtype displays unique functional properties. In vivo, the extent of iGluR diversity ultimately depends on which genes are expressed in a given neuron. In the hippocampus, for instance, interneurons and principal cells express different iGluR subunits (Isaac et al. 2007). Even within an individual neuron, iGluR subtypes can differ from one synapse to another, in an input-specific manner, highlighting the critical importance of subunit composition in defining the receptor signaling properties (Köhr 2006). The synaptic content in iGluR subtypes varies according to developmental stages, activity, and disease. During certain forms of synaptic plasticity, subunits can rapidly (within minutes) be replaced one for another, thus affecting the synaptic phenotype (Liu and Cull-Candy 2000; Bellone and Nicoll 2007; Mameli et al. 2007).

Interactions with Intracellular Proteins

Similarly to Cys-loop receptors, iGluRs are the targets of a variety of intracellular kinases. Serine/threonine phosphorylation by cAMP-dependent protein kinase A (PKA), protein kinase C (PKC), and calcium/calmodulin-dependent protein kinase II (CAMKII), as well as tyrosine phosphorylation by Src and Fyn kinases have been described. Phosphorylation sites are usually found in the carboxy-terminal tails and regulate receptor trafficking, localization, binding to other intracellular proteins, and, in

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some cases, channel activity (Salter and Kalia 2004; Chen and Roche 2007; Shepherd and Huganir 2007; Traynelis et al. 2010).

The intracellular tails of iGluR subunits, which in the case of GluN2 NMDAR subunits are particularly long (>400 residues), are also the locus for direct interactions with numerous intracellular proteins other than kinases. Many of these proteins are scaffolding or cytoskeletal proteins that are core components of the post-synaptic density (PSD). This is the case of the actin-binding proteins α -actinin and spectrin and of several members of the large family of modular PDZ-containing proteins (also known as membrane-associated guanylate kinases, or MAGUKs), including GRIP and PICK1 for AMPARs and PSD-95, SAP102, SAP97, and PSD-93 (also known as chapsyn-110) for NMDARs (Wenthold et al. 2003; Kim and Sheng 2004; Montgomery et al. 2004; Shepherd and Huganir 2007). By controlling the transport and clustering of glutamate receptors as well as the recruitment of multiple signaling proteins, MAGUKs play key roles in the structural and functional changes that excitatory synapses experience during development, plasticity, and disease (Lau and Zukin 2007; Sheng and Hoogenraad 2007; Gardoni et al. 2009).

Modular Architecture

Unlike Cys-loop receptors, which assemble as pentamers, iGluRs form tetramers (Rosenmund et al. 1998; Sobolevsky et al. 2009). Each iGluR subunit has a characteristic modular organization consisting of four discrete domains (Wo and Oswald 1995; Mayer 2006; Traynelis et al. 2010): (1) a large extracellular amino-terminal domain (ATD or NTD; ~380 amino acids) that participates in subunit-specific receptor assembly and modulation; (2) an agonist-binding domain (ABD, also known as the S1S2 domain; ~300 amino acids), binding glutamate or glycine (or D-serine) in GluN1, GluN3, and GluD2 subunits; (3) a transmembrane domain (TMD) composed of three membrane-spanning segments (M1, M3, and M4) plus a short reentrant loop (M2) and that forms the ion channel; and (4) a cytoplasmic carboxy-

terminal domain (CTD), highly variable in length and involved in receptor trafficking, subcellular localization, and coupling to intracellular signaling complexes.

The typical kit design of iGluRs likely arose during evolution through the fusion of separate genes that in bacteria encode individual proteins (Mayer 2006). Thus, whereas the TMD has sequence homology with bacterial potassium channels, the NTD and ABD are each related to clamshell-shaped bacterial periplasmic-binding proteins. Domains behave in a semi-autonomous fashion. They can be transplanted between subunits or expressed in isolation while retaining functionality (such as ligand binding). Interestingly, prokaryotes appear to express “simplified” versions of iGluRs, lacking both the NTDs and CTDs but with a functional gating core machinery (ABDs + TMD) (Chen et al. 1999; Janovjak et al. 2011).

The first views of a full-length iGluR were obtained using electron microscopy (EM) imaging of single particles from recombinant and native AMPARs (Tichelaar et al. 2004; Nakagawa et al. 2005). Despite their low resolution (~30 Å), EM images revealed interesting features such as twofold, rather than fourfold, rotational symmetry, and in the membrane region, electron dense material tightly associated with the TMD and assigned to auxiliary subunits (see below). A major breakthrough in the field was achieved with the crystal structure of a full-length AMPAR (rat GluA2 homotetramer), thus providing the first atomic map of an iGluR (Fig. 6) (Sobolevsky et al. 2009). The structure revealed a massive Y-shaped complex in which the three major domains are arranged in layers: at the “bottom,” the TMD that forms the ion channel; at the “top,” the NTD layer with the four individual NTDs; and sandwiched in between these two layers, the ABDs. There are surprisingly few contacts between the various layers. Connections between layers are made by short (<20 amino acids) polypeptide stretches, or linkers, that adopt an extended conformation. In agreement with their location, these linkers play critical roles in transducing conformational changes in the extracellular domains into modifications of channel activity (Yelshansky et al.

Synaptic Neurotransmitter-Gated Receptors

Figure 6. The tetrameric structure of the AMPA GluA2 receptor. (*Left*) X-ray crystal structure of the AMPA GluA2 homotetrameric receptor (Sobolevsky et al. 2009). Each subunit is in a different color. The tetramer shows a typical layer organization with at the “top” the amino-terminal domains (ATDs or NTDs), at the “bottom” the transmembrane domain (TMD) where the ion channel sits, and sandwiched between the two the agonist-binding domains (ABDs or S1S2 domains) binding glutamate (or glycine/D-serine). (*Right*) Subunit non-equivalence. α -Carbon traces of subunit A and subunit B with the ABDs similarly oriented. Note the striking difference in overall domain orientation between the two subunits.

2004; Schmid et al. 2007; Gielen et al. 2009; Talukder et al. 2010). In the NTD layer, the NTDs arrange as two dimers. Similarly, each ABD partners with the neighboring ABD, forming two pairs of local dimers. Contrasting with this twofold symmetrical dimer-of-dimers organization, the pore region exhibits the typical fourfold symmetry seen in potassium channels. Accompanying this symmetry mismatch, another key feature revealed by the GluA2 structure is “domain swapping.” Thus, if at the NTD level, subunit A pairs with subunit B (A/B dimer) and subunit C with subunit D (C/D dimer), at the ABD level, the two pairs engage different subunits (A/D and B/C dimers). Crossover of subunit pairing likely helps to stabilize the large and loosely packed extracellular region. As a consequence of symmetry mismatch and domain swapping, the GluA2 receptor presents two pairs of conformationally distinct subunits (despite identical sequences). This non-equivalence in subunit structure is without precedent for an ionotropic receptor. It suggests differential effects on the gating machinery depending on which pair of subunits is considered. The significance of domain swapping

and structural non-equivalence is likely to be of paramount importance in the function of iGluRs composed of distinct subunits (heteromers), which in native tissues are the majority.

The Ion Channel and Permeation Properties

Resembling an inverted potassium channel, the pore of iGluRs has a cone shape structure, with a cytoplasmic base and an extracellular apex (Sobolevsky et al. 2009). The ion conduction pathway resembles an hourglass, with a region of narrow constriction separating two cavities. The M2 segment (or P-loop) lines the inner cavity, whereas the four M3 helices form the wall of the central cavity. Ion replacement experiments have estimated the dimension of the narrow constriction to be $\sim 0.55\text{--}0.75$ nm in diameter (Villarroel et al. 1995; Zarei and Dani 1995; Burnashev et al. 1996). The M1 and M4 helices reside on the exterior of the ion channel domain. In the GluA2 structure, the M4 segments have a striking location, with each M4 helix resting on the ion channel core (M1–M3) of the neighboring subunit. The extensive subunit–subunit

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interactions mediated by the M4s likely contribute to the stabilization of the tetrameric assembly.

Although Cys-loop receptors can either flow cations or anions, iGluRs display strong cation selectivity. The flush of Na^+ ion entry following synaptic iGluR activation is what depolarizes the neuronal membrane. The mechanisms that exclude anions from permeating are ill defined but likely involve both steric and electrostatic effects (Kuner et al. 2001). The various iGluR subtypes differ in their unitary conductance, selectivity for divalent cations, and channel block (Traynelis et al. 2010).

Two key permeation properties that have major impact on synaptic function are the high calcium permeability and magnesium block of NMDAR channels. Extracellular Mg^{2+} ions inhibit NMDARs by “plugging” the receptor ion channel (Mayer et al. 1984; Nowak et al. 1984). The strong voltage dependence of Mg^{2+} block endows NMDARs with a unique capacity of coincidence detection between presynaptic and postsynaptic activities, which is central to associative learning (Bliss and Collingridge 1993). The high calcium permeability is another prominent feature of NMDARs that is essential for synaptic plasticity by triggering intracellular cascade activation (Lau and Zukin 2007; Bingol and Sheng 2011). The calcium permeability of NMDAR channels is higher than that of any other ligand-gated ion channel (Rogers and Dani 1995). Thus, at typical GluN1/GluN2A or GluN1/GluN2B receptors, >15% of the total inward current is carried by Ca^{2+} ions (vs. ~4% for the most Ca^{2+} -permeable AMPARs) (Burnashev et al. 1995; Schneggenburger 1996). The detailed molecular mechanisms that allow Ca^{2+} ions, but not the smaller Mg^{2+} ions, to permeate NMDAR channels are still enigmatic.

A key determinant of Ca^{2+} permeation and Mg^{2+} block in NMDARs, but also of the permeation properties of all iGluRs, is the residue occupying the tip of the M2 loop. This site, coined the “QRN site,” is located halfway in the membrane and forms the region of narrowest constriction in the pore. It harbors either a glutamine (Q) or arginine (R) in AMPA and kainate receptors and an asparagine (N) in (GluN3-lacking) NMDARs (Hume et al. 1991;

Verdoorn et al. 1991; Burnashev et al. 1992; Mori et al. 1992; Sakurada et al. 1993). Not surprisingly given its strategic position in the ion conduction pathway, the QRN site influences every aspect of iGluR permeation properties (Traynelis et al. 2010). It is also a site subjected to RNA editing. In the primary transcripts of GluA2, GluK1, and GluK2, the codon encoding a glutamine can be edited to an arginine (Sommer et al. 1991; Seeburg and Hartner 2003). The presence of the positively charged arginine in edited versions of AMPA and kainate receptors causes low single-channel conductance and strongly reduces calcium permeability and block by intracellular polyamines (Traynelis et al. 2010). In the adult brain, the GluA2 subunit is widespread and exists almost exclusively in its edited version (Ca^{2+} impermeable). During development, however, many neurons express low levels of GluA2 subunits rendering AMPARs Ca^{2+} permeable, a property exploited for synapse maturation (see Traynelis et al. 2010). The paramount importance of the QRN site in iGluR function is to be found in the clinic: A defect in GluA2 RNA QRN site editing has been detected in spinal motor neurons of certain patients with amyotrophic lateral sclerosis (Kawahara et al. 2004), and a single substitution (N to K [lysine]) at the QRN site of the GluN2A NMDAR subunit causes severe mental retardation in children (Endele et al. 2010).

Agonist Recognition

The agonist-binding domains (ABDs) are the best characterized parts of iGluRs. Numerous high-resolution crystal structures of ABDs in complex with a wide variety of ligands, including agonists and antagonists, have been solved (Armstrong et al. 1998; Armstrong and Gouaux 2000; Mayer 2006; Pohlsgaard et al. 2011). All ABDs display a common clamshell-like fold made of two lobes separated by a large central cleft. Unlike the Cys-loop receptors, where agonist binds at an interface between subunits, in GluRs, each subunit carries its own agonist-binding site. Thus, a typical AMPA or kainate receptor exhibits four distinct glutamate-binding sites, whereas “classical” (GluN3-lacking)

NMDARs harbor two glutamate-binding sites (on GluN2 subunits) and two glycine (or D-serine)-binding sites (on GluN1 subunits) (Furukawa et al. 2005). Agonists are nestled within the interlobe cleft, interacting with both lobes (Mayer 2006). Lobe 1 residues interact with the α -amino and α -carboxy groups of the agonist molecule and are highly conserved among the four iGluR families. Subunit selectivity arises mostly from amino acid differences in lobe 2 and steric occlusion effects (Mayer 2005, 2006; Pohlsgaard et al. 2011). Another striking feature is the small size of the agonist-binding pockets of GluN1 and GluN3 subunits, which bind glycine or D-serine but not glutamate. The replacement of a tyrosine by either a methionine in GluN3A and GluN3B or a tryptophan in GluN1 greatly reduces the interlobe cavity size, thus preventing glutamate binding (Furukawa et al. 2003; Yao et al. 2008).

Channel Activation, Partial Agonism, and Antagonism

A fundamental aspect of ionotropic receptors is their ability to rapidly convert (millisecond time scale) chemical energy, that is, agonist-binding interactions, into mechanical work allowing channel gate opening. The basic principles of channel activation are shared by all iGluR subtypes and involve the following events (Fig. 7) (Madden 2002; Mayer 2006; Hansen et al. 2007): (1) Agonists bind the ABD central cleft and promote its closure. (2) Because the ABDs pack as pairs through apposition of their upper lobes whereas the lower lobes are more flexible, ABD closure increases the distance between ABD lower lobes. (3) This movement, in turn, produces tension on the ABD-TMD linkers, eventually leading to channel gate opening. In this process, subunit dimerization is essential because it allows agonist-triggered cleft closure in an ABD dimer to be transduced into separation of the ABD lower lobes. This separation is the mechanical force that drives the channel open.

Crystal structures of iGluR ligand-binding cores in complex with different ligands offer a straightforward explanation for partial agonism and competitive antagonism. Competitive an-

tagonists, such as NBQX at AMPARs or DCKA at NMDARs, prevent channel activation simply because they lock the ABD in an open-cleft conformation by a foot-in-the-door mechanism (Armstrong and Gouaux 2000; Furukawa et al. 2003; Mayer 2006). Partial agonists, on the other hand, display a submaximal efficacy because they induce less domain closure than full agonists, thus reducing the coupling efficiency to channel opening (Armstrong and Gouaux 2000; Jin et al. 2003). At AMPA and kainate receptors, there is thus a correlation between the degree of cleft closure and the level of receptor activation (Mayer and Armstrong 2004). Although appealing, this mechanism seems not to apply to NMDARs. At these receptors, partial agonists induce similar extent of ABD closure as full agonists (Inanobe et al. 2005; Yao et al. 2008). What would differ then is the stability of the closed-cleft conformation, with partial agonists inducing lower stability (certain agonists may behave likewise at AMPARs) (Birdsey-Benson et al. 2010). Another important variation between NMDARs and non-NMDA (AMPA and kainate) receptors is the stoichiometry of activation. Although a non-NMDAR can open (although submaximally) with a mixture of empty and ligand-bound ABDs (Clements et al. 1998; Rosenmund et al. 1998; Smith and Howe 2000), activation of an NMDAR requires obligate occupancy of its four agonist-binding sites (2 glutamate + 2 glycine/D-serine molecules) (Benveniste and Mayer 1991; Clements and Westbrook 1991; Schorge and Colquhoun 2005).

The channel gate, the structure that occludes ion passage in the closed state and that dilates following receptor activation, resides at the extracellular entrance of the pore. It is formed by the second half of the transmembrane M3 segment, a region that contains the most highly conserved motif (SYTANLAAF) among all iGluR subunits (Wollmuth and Sobolevsky 2004; Chang and Kuo 2008). In the GluA2 structure (captured in a closed state) (Sobolevsky et al. 2009), the four M3 helices cross at the level of this conserved motif, thus forming a tight steric closure of the ion conduction pathway. Upon receptor activation, the

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Figure 7. Structural mechanism of iGluR activation and desensitization. A single dimer is represented; a full receptor is a tetramer made of two such dimers. (*Below*) The crystal structures of the GluA2 ABD dimer in conformations that correspond to the resting state (no ligand bound; pdb code 1FT0), the active state (glutamate-bound; pdb code 1FTJ), and the desensitized state (pdb code 2I3V). The distances between the two protomers, at the top of the upper lobes (green spheres; dimer interface) and at the bottom of the lower lobes (black spheres; connections to the transmembrane segments), are indicated (Armstrong et al. 2006).

swinging of the ABD lower lobes (see above) is thought to splay apart the M3 bundle through rotating movements of the individual M3 helices. In the *Lurcher* mutant mice, neurodegeneration occurs because of a point mutation in the SYTANLA~~AF~~ motif of the GluR2 subunit producing constitutively active (open) channels (Zuo et al. 1997).

Receptor Desensitization

A hallmark of ionotropic receptors is desensitization, a process by which an activated receptor enters an inactivated state with its ion channel closed even though the neurotransmitter is still bound to the receptor (Jones and Westbrook 1996). Receptor desensitization limits ion flow following agonist binding. The extent and kinetics of desensitization vary widely between iGluR subfamilies. It is particularly fast and pronounced at AMPA and kainate receptors, occurring within a few milliseconds and producing >90% decrease in current amplitude at steady state (see Fig. 8B). Desensitization of AMPARs

affects how these receptors encode information, because the degree of receptor desensitization can influence the shape of the postsynaptic response (Jones and Westbrook 1996). The critical *in vivo* importance of AMPAR desensitization was recently revealed by the dramatic phenotype—early postnatal lethality—of mice harboring a single-point mutation eliminating GluA2 AMPAR desensitization (Christie et al. 2010).

At the molecular level, desensitization of AMPA and kainate receptors results from the disruption of the ABD dimer assembly. The repositioning of the ABD lower lobes closer one to another relaxes the tension exerted on the ABD–TMD linkers, thus allowing channel gate closure (Fig. 7) (Sun et al. 2002; Armstrong et al. 2006; Mayer 2006; Hansen et al. 2007). The stability of the ABD dimer interface is the key parameter that governs the transition to the desensitized state. Accordingly, factors that affect ABD dimerization affinity, such as subunit composition, alternative splicing, or RNA editing, influence desensitization properties (Stern-Bach et al. 1998; Traynelis et al. 2010).

Allosteric Modulation

The iGluRs, like many other membrane signaling receptors, possess binding sites distinct from the ion channel and agonist-binding sites that allow for modulation of receptor activity. These allosteric sites usually recognize ions or small molecule ligands that can either be endogenously present in the CNS, or exogenous acting like drug compounds. There is currently great interest in exploiting the allosteric potential of iGluRs for pharmacological and therapeutic purposes (Mony et al. 2009; Traynelis et al. 2010). Notably, allosteric modulation offers the possibility of targeting a specific subunit, and thus a single receptor subtype, something usually difficult to achieve at more conserved agonist or ion channel binding sites. Among iGluRs, NMDARs stand out by their numerous allosteric sites conferring to these re-

ceptors an exquisite sensitivity to their micro-environment (Dingledine et al. 1999; Paoletti 2011). Extracellular protons and zinc ions, these latter being concentrated at many glutamatergic synapses (Paoletti et al. 2009), provide potent tonic inhibition of NMDARs under physiological conditions (Peters et al. 1987; Westbrook and Mayer 1987; Traynelis and Cull-Candy 1990). In particular, receptors composed of the GluN2A subunit exhibit very high zinc sensitivity, being inhibited by nanomolar zinc concentrations (Fig. 8) (Paoletti et al. 1997). Proton and zinc inhibitions may protect against the deleterious effects of NMDAR overactivation. The amino-terminal region of NMDARs is a major locus for subunit-specific allosteric modulation. This region, the most distal to the membrane, contains clamshell-like domains (the NTDs) that undergo conformational changes that can be sensed by the downstream gating

Figure 8. Allosteric modulation of iGluRs. (A) Negative allosteric modulation of NMDARs by extracellular zinc. The GluN2A and GluN2B NTDs form subunit-specific inhibitory zinc-binding sites. (Right) Inhibition by nanomolar zinc concentrations of GluN1/GluN2A responses (adapted from Paoletti et al. 2000). (B) Positive allosteric modulation of AMPARs by cyclothiazide (CTZ). CTZ binds and stabilizes the ABD dimer interface. (Right) CTZ blocks desensitization of GluA2 receptors (Sun et al. 2002).

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machinery (Fig. 8) (Gielen et al. 2008, 2009). In addition to forming inhibitory zinc-binding sites, NMDAR NTDs harbor a site for extracellular polyamines that act as positive allosteric modulators of GluN2B-containing NMDARs (Mony et al. 2011).

At AMPA, kainate, and δ receptors, a major site of action for allosteric modulators is the dimer interface between two neighboring agonist-binding domains. Compounds like cyclothiazide at AMPARs (Sun et al. 2002), Na^+ and Cl^- ions at kainateRs (Bowie 2002; Plested et al. 2008), or Ca^{2+} ions at deltaRs (Hansen et al. 2009) all act as positive allosteric modulators by reducing receptor desensitization through stabilization of the ABD–dimer interface (Fig. 8). Positive allosteric modulators of AMPARs are currently evaluated for their ability to restore cognitive dysfunction associated with neurodegenerative conditions (Ward et al. 2010).

Auxiliary Subunits

Native iGluRs were long believed to be stand-alone receptors composed of just four pore-forming subunits. Recent evidence, however, has established that, in vivo, many membrane proteins interact specifically with iGluRs and influence their function. Notably, single-particle EM analysis of purified native AMPARs from rat brain revealed the existence of a substantial electron density in the transmembrane region not attributable to the receptor ion channel per se but, rather, to tightly associated TARP proteins (for transmembrane AMPAR regulatory proteins) (Nakagawa et al. 2005). TARPs, or stargazing-like proteins, form a large family (six members, γ -2, -3, -4, -5, -7, and -8) of four membrane-spanning segments acting as auxiliary (or accessory) subunits of AMPARs (Jackson and Nicoll, 2011).

Virtually every aspect of AMPAR function is affected by association with TARPs including receptor trafficking and localization, channel conductance and open probability, activation and deactivation kinetics, and sensitivity to allosteric modulators (Priel et al. 2005; Kato et al. 2010; Jackson and Nicoll 2011). The direct interaction of the extracellular loop that con-

nects transmembrane segments 1 and 2 of TARPs with extracellular regions of the receptor, including the ABDs, has a primary role in mediating TARP effects on receptor gating and pharmacology (Tomita et al. 2005a). Additional interactions within the membrane or cytoplasm likely play a role in TARP effects on permeation properties. Through their PDZ-binding motif, TARPs are also essential in controlling the number of AMPARs expressed at the synapse (Tomita et al. 2005b; Opazo et al. 2010). The interaction of TARPs with AMPARs is highly dynamic, being regulated in an activity-dependent manner. Thus, receptor desensitization rapidly promotes dissociation of TARP–AMPA complexes, and this dissociation mechanism contributes to synaptic short-term modulation (Morimoto-Tomita et al. 2009).

TARPs are not the only auxiliary proteins of iGluRs. Proteomic approaches identified cornichon (CNIH2 and 3) (Schwenk et al. 2009) and CKAMP44 (von Engelhardt et al. 2010) as novel auxiliary subunits of AMPARs, and NETO1 (Ng et al. 2009) and NETO2 (Zhang et al. 2009) as accessory subunits of NMDA and kainate receptors, respectively. The discovery of these families of auxiliary iGluR subunits helps solve the longstanding puzzle of why the functional properties of native iGluRs often do not faithfully match those of iGluRs expressed in heterologous cells. It also raises the enticing possibility that the structural plasticity of iGluR–auxiliary subunit complexes is a major determinant of experience-dependent changes in synaptic efficacy.

CONCLUSION

The mammalian brain has evolved to rely on two major classes of neurotransmitter receptors for the provision of rapid synaptic excitation and inhibition. It is intriguing that these two classes, which perform an equivalent task—coupling agonist binding to fast opening of a transmembrane pore—have adopted strikingly different structural designs. The most obvious divergence is their subunit stoichiometry with Cys-loop receptors being pentamers, whereas iGluRs are tetramers. Other major differences are transmembrane topology and neurotransmitter

mode of action, with Cys-loop receptors having external amino and carboxyl termini and relying on neurotransmitter binding at subunit interfaces, whereas iGluRs have an intracellular carboxyl terminus and use individual bilobate domains to capture glutamate. The need for two agonists, glutamate and the coagonist glycine or D-serine, to activate NMDARs is another striking distinction that renders these receptors unique among neurotransmitter receptors. Despite this, similarities between the two receptor classes are also apparent: the reliance on large extracellular domains distal from the membrane for housing neurotransmitter binding sites, the use of helical segments for constructing ion channels, and the presence of specialized intracellular domains acting as the pivots for scaffold proteins and regulatory ports for signaling pathways. The past years have seen an explosion in our knowledge of the structure and function of neurotransmitter-gated receptors, in particular, through crystallographic efforts. We now have realistic structural and mechanistic schemes to explain how Cys-loop and iGluRs receptors have been optimized by evolution so that their thermodynamic and kinetic properties match the strict requirements of fast and reliable synaptic function. Nevertheless, neurotransmitter-gated receptors are particularly large and complex multimeric molecular machines, and several aspects of their operation, conformational dynamics, interaction with partner proteins, and regulation remain ill defined. The use of a variety of experimental approaches, including structural, biophysical, and electrophysiological techniques, appear more necessary than ever to decipher the intricate functioning of these key signaling molecules.

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