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Ali Jazayeri & Fiona Marshall

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Implications of metabotropic glutamate receptor structures for drug discovery in neurotherapeutics

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Ali Jazayeri

Heptares Therapeutics Ltd, BioPark Broadwater Road, Welwyn Garden City, Hertfordshire, AL7 3AX, UK

Fiona Marshall

Author for correspondence: Heptares Therapeutics Ltd, BioPark Broadwater Road, Welwyn Garden City, Hertfordshire, AL7 3AX, UK
fiona.marshall@heptares.com

Recent technological advances in the field of membrane protein structural biology have led to significantly improved success rates in the structure resolution of G protein-coupled receptors. Apart from gaining insight into the mechanics of receptor biology, these technical advances facilitate the application of structure-based drug discovery to G protein-coupled receptors. Structure-based drug discovery has the potential to significantly increase the efficiency and success rate of drug discovery campaigns against this important family of drug targets. Recently, structures of mGlu1 and mGlu5 transmembrane domains were reported in complex with negative allosteric modulators. Analysis of these structures reveals a fascinating insight into the historical difficulties associated with the drug discovery efforts for these receptors and provides an important novel template for structure-based drug discovery approaches to identify more diverse and better quality chemotypes.

Glutamate, the major excitatory neurotransmitter of the brain, mediates its activities via ion channels and through a family of eight metabotropic receptors (mGlu) that are part of the G protein-coupled receptor (GPCR) superfamily. Modulation of glutamate transmission has the potential in the treatment of a wide range of neurological disorders; however, currently, there are no approved drugs directed at mGlu receptors. The mGlu5 receptor is highly expressed in the basal ganglia, lateral septum, cortex and hippocampus and has a key role regulating the activity of NMDA receptors. To gain selectivity over other mGlu receptors drug discovery efforts have focused on allosteric modulators that target the transmembrane regions of the receptor. Negative allosteric modulators (NAM) of mGlu5 have been of particular interest in the development of treatments for fragile X disorder, depression and anxiety, levodopa-induced dyskinesia and migraine [1–4]. Although many compounds have progressed into clinical development, there have been many failures. In the absence of structural information, designing allosteric modulators for the mGlu receptors has proved challenging. For example, there is a lack of diverse chemotypes, issues with active metabolites and pharmacological mode switching, poor compound solubility and poor pharmacokinetic properties [5]. Recently, x-ray structures of mGlu5 and the related mGlu1 receptor have been solved and this opens the way to a new era in the design of drugs for this class of receptors.

It is widely recognized that structure-based drug design (SBDD) is a powerful and efficient approach for drug discovery. Structural information has increased the efficiency of the drug discovery process from the early stages of hit identification to the later stages of lead optimization. Compared with the traditional trial and error approaches, SBDD has the potential to significantly reduce time, cost and labor associated with the search for new drugs. More importantly, an analysis of success rates in discovery programs has shown that structural enablement significantly reduces attrition rate at different stages of the drug discovery pipeline [6].

Keywords: allosteric modulators • G protein-coupled receptor • mavoglurant • metabotropic glutamate receptor • mGlu5 • structure based drug design

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The power of SBDD has been demonstrated in success stories of drug discovery against historically challenging targets with prime examples being the HIV and HCV protease inhibitors as well as the identification of highly selective kinase inhibitors [7–9]. The main limitation of SBDD is the challenges associated with generating routine, reliable and high quality crystal structures. For this reason, despite the established importance of GPCRs as drug targets, this class of proteins have until recently been refractory to SBDD approaches [10]. The process of protein crystallization requires high quantities of purified and homogenous preparations. Achieving such preparations for GPCRs is particularly challenging due to their hydrophobic nature, instability in detergent micelles as well as inherent flexibility that is intrinsic to their biological function as these receptors exist in equilibrium between active and inactive conformations [11]. These factors mean that upon detergent solubilization and purification, GPCRs tend to yield protein preparations with high levels of heterogeneity and aggregation that are refractory to formation of well-ordered crystals. Over the past few years, a number of technical advances have addressed these issues as evidenced by the increase in success rate of GPCR structure determination efforts. These include the advent of novel detergents and crystallization techniques that are more suitable for membrane proteins [12,13]. In addition, protein engineering solutions have been developed to further facilitate GPCR structure resolution. These approaches include the addition of a fusion protein or an antibody to increase the hydrophilic surface that in turn aids the process of crystallization [14,15]. An alternative protein engineering solution is conformational stabilization, which involves identification of point mutations that concomitantly increase receptor thermal stability outside membrane environment and reduce conformational heterogeneity. Successful application of this approach yields a stabilized receptor that not only maintains its structural integrity for long periods of time in detergent but also primarily occupies a single conformation. Such combination of attributes significantly reduces receptor aggregation and heterogeneity thus facilitating crystallization [16]. This methodology has led to structure determination of a number of different receptors in both agonist and antagonist conformations.

The process of receptor stabilization relies on measuring the thermal stability of detergent solubilized receptors using a ligand binding assay. The use of ligand-binding assay provides a sensitive and quantitative read-out of receptor structural integrity. More importantly, it allows identification of mutations that bias the receptor equilibrium toward the state that is preferentially stabilized by the ligand [17]. As an indirect consequence, stabilized receptors become independent of stabilization that is conferred by the ligand thus facilitating structure resolution with weak ligands. This is critical for successful SBDD campaigns as early hits tend to be weak ligands that are unlikely to work with non-stabilized receptors that tend to be dependent on stabilization derived from high-affinity ligands for successful crystallography.

Using these technologies, structures of mGlu1 and mGlu5 transmembrane domains have been reported recently [18,19]. In both cases, the structures have been solved with NAM revealing details of the allosteric binding sites in these receptors. The overall structural arrangements of the transmembrane helices are in good agreement with each other; however, compared with inactive state structures of receptors from Family A and B, the extracellular half of mGlu receptors appears more compact. This closed configuration is primarily achieved by shifting of TM2, 5 and 7 closer to the central axis of the receptor. In addition to this helical arrangement, the extracellular loop 2 further closes the structure such that no open entrance to the transmembrane domains for a putative ligand binding can be observed. This is consistent with the fact that glutamate (the orthosteric ligand) binding site is not in the transmembrane domains.

These structures reveal the atomic details of the NAM-binding sites. In mGlu1, the NAM (FITM) bins on an analogous region to orthosteric ligand-binding sites in class A receptors. Part of the mGlu5 NAM (mavoglurant) binding site overlaps with this region, the rest of the molecule extends down, with the ligand alkyene linker traversing a narrow channel formed by TM3 and 7. This extension of the ligand binding in mGlu5 explains the subtype selectivity of mavoglurant as a number of non-conserved residues in mGlu1 effectively close this lower region of the binding site. In general, the mGlu5 allosteric binding site is restricted in size and further analysis shows that it lacks favorable druggable feature [20]. Taken together, these features explain the narrow structure–activity relationships that have been observed in mGlu5 allosteric drug discovery.

An interesting feature of the mGlu5 structure is the presence of a crystallographic water molecule in close proximity to the lower part of the allosteric binding site. This water molecule participates in a hydrogen bonding network that also includes the side chains of Tyr 659, Thr 781 and the main-chain carbonyl of Ser 809. In addition to this observed water molecule, further water molecules can be computationally modeled in this region, providing evidence for the presence of a water network in this region of the receptor. We postulate that these water molecules can facilitate different patterns of hydrogen bonding networks resulting in stabilization of different receptor conformations. This proposition is supported by the observation that subtle changes to the 3-methyl substituent that sits in close proximity to this network can result in dramatic switching of highly related ligands. Modification of this substituent from methoxy to chloro to fluoro changes ligand pharmacology from NAM to neutral binder to positive allosteric modulator (PAM), respectively [21], indicating that perhaps the perturbations of the water network and ensuing hydrogen bonding pattern is the explanation for this observation in mGlu5 ligands. It is possible that different hydrogen bonding networks selectively lower the energy required to achieve either active or inactive conformations. Thus, a ligand that preferentially stabilizes an 'active water network' will engender PAM activity and vice versa. The exact mechanics of how this is achieved requires further investigation, but the ultimate answer is likely to provide fascinating additional insight into the

mechanisms of mGlu5 receptor activation that might be applicable to other receptor systems.

The availability of x-ray structures for class C receptors will enable a different approach to drug discovery. In the past, cell-based high-throughput screening was the usual starting point for drug discovery programs. With structures in hand, computational approaches such as virtual screening and fragment-based design can now be used. Such methods can discover novel chemical templates that can be optimized to achieve improved selectivity, solubility and pharmacokinetic properties. It is clear that targeting such an important class of receptors needs a careful strategy for compound selection such that a balance of activation or inactivation is achieved depending on the disease state. This is crucial to avoid side effects that may be associated with excessive activation of blockade of the receptor. Multi-parametric optimization of multiple properties, including degree of allosteric modulation, potency, brain penetration and pharmacokinetic properties is required. This challenge will be made easier with the advent of x-ray structures, which will hopefully increase the probability of drugs directed at mGlu receptors reaching the market place.

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