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Review

Allosteric modulation of metabotropic glutamate receptors: Structural insights and therapeutic potential

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ABSTRACT

Allosteric modulation of G protein-coupled receptors (GPCRs) represents a novel approach to the development of probes and therapeutics that is expected to enable subtype-specific regulation of central nervous system target receptors. The metabotropic glutamate receptors (mGlus) are class C GPCRs that play important neuromodulatory roles throughout the brain, as such they are attractive targets for therapeutic intervention for a number of psychiatric and neurological disorders including anxiety, depression, Fragile X Syndrome, Parkinson's disease and schizophrenia. Over the last fifteen years, selective allosteric modulators have been identified for many members of the mGlu family. The vast majority of these allosteric modulators are thought to bind within the transmembrane-spanning domains of the receptors to enhance or inhibit functional responses. A combination of mutagenesis-based studies and pharmacological approaches are beginning to provide a better understanding of mGlu allosteric sites. Collectively, when mapped onto a homology model of the different mGlu subtypes based on the β_2-adrenergic receptor, the previous mutagenesis studies suggest commonalities in the location of allosteric sites across different members of the mGlu family. In addition, there is evidence for multiple allosteric binding pockets within the transmembrane region that can interact to modulate one another. In the absence of a class C GPCR crystal structure, this approach has shown promise with respect to the interpretation of mutagenesis data and understanding structure-activity relationships of allosteric modulator pharmacophores.

Abbreviations: ADX47273, *S*-(4-fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]oxadiazol-5-yl]-piperidin-1-yl}-methanone; AMN082, N,N'-Bis(diphenylmethyl)-1,2-ethanediamine; ATCM, allosteric ternary complex model; BINA, Biphenyl-indanone A; Br-5MPEPy, 2-(2-(5-bromopyridin-3-yl)ethynyl)-5-methylpyridine; CaSR, Calcium-sensing receptor; CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide; CFMMC, 3-cyclohexyl-5-fluoro-6-methyl-7-(2-morpholin-4-ylethoxy)-4*H*-chromen-4-one; CPCCOEt, 7-(Hydroxyimino)cyclopropa[b]chromen-1a-carboxylate ethyl ester; CPPHA, *N*-{4-chloro-2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide; DFB, [(3-Fluorophenyl)methylene]hydrazone-3-fluorobenzaldehyde; EM-TBPC, 1-ethyl-2-methyl-6-oxo-4-(1,2,4,5-tetrahydro-benzo[d]azepin-3-yl)-1,6-dihydro-pyrimidine-5-carbonitrile; ERK1/2, extracellular signal-regulated kinases 1 and 2; FMRP, fragile X mental retardation protein; FTIDC, 4-[1-(2-fluoropyridin-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-N-isopropyl-N-methyl-3,6-dihydropyridine-1(2*H*)-carboxamide; FXS, Fragile X Syndrome; GABA, γ-aminobutyric acid; GPCR, G protein-coupled receptor; mGlu, metabotropic glutamate receptor; LY404039, (-)-(1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid; LY456066, (2-[4-(indan-2-ylamino)-5,6,7,8-tetrahydro-quinazolin-2-ylsulfanyl]-ethanol hydrochloride); LY487379, 2,2,2-Trifluoro-N-[4-(2-methoxyphenoxy) phenyl]-N-(3-pyridinylmethyl)ethanesulfonamide; M-5MPEP, 2-(2-(3-methoxyphenyl)ethynyl)-5-methylpyridine; M-MPEP, 2-methyl-6-(3-methoxyphenyl)ethynyl-pyridine; MMPIP, 6-(4-Methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo [4,5-c] pyridine-4(5*H*)-one hydrochloride; MPEP, 2-Methyl-6-(phenylethynyl)pyridine; MTEP, 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl] pyridine; NAM, negative allosteric modulator; NMDA, N-methyl-D-aspartate; PAM, positive allosteric modulator; PCP, Phencyclidine; PD, Parkinson's Disease; PET, positron emission tomography; PHCCC, N-Phenyl-7-(hydroxyimino)cyclopropa[b] chromen-1a-carboxamide; R214127, 1-(3,4-dihydro-2H-pyrano[2,3-b]quinolin-7-yl)-2-phenyl-1-ethanone; Ro 67-7476, (S)-2-(4-fluorophenyl)-1-(toluene-4-sulfonyl)pyrrolidine; S-4C3H-PG, (S)-4-carboxy-3-hydroxyphenylglycine; SAR, structure-activity relationship; SIB-1757, 6-Methyl-2-(phenylazo)-3-pyridinol; SIB-1893, 2-Methyl-6-(2-phenylethenyl)pyridine; TM, transmembrane; VFD, Venus-Flytrap domain; VU0155041, cis-2-{[(3,5-Dichlorophenyl)amino]carbonyl} cyclohexanecarboxylic acid; VU29, 4-nitro-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide; VU48, 4-nitro-*N*-(1-(2-bromophenyl)-3-phenyl-1*H*-pyrazol-5-yl)benzamide; VU71, 4-nitro-*N*-(1,4-diphenyl-1*H*-pyazol-5-yl)benzamide; YM298198, 6-amino-N-cyclohexyl-N,3-dimethylthiazolo[3,2-a]benzimidazole-2-carboxamide.

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1. Introduction

In addition to eliciting fast excitatory synaptic responses, the neurotransmitter glutamate can modulate neuronal excitability, synaptic transmission, and other cell functions by activation of metabotropic glutamate receptors (mGlus). Due to the ubiquitous distribution of glutamatergic synapses and the broad range of functions of the mGlus, members of this receptor family participate in many different processes in the central nervous system (CNS). As such, mGlus are an attractive target for therapeutic intervention for a range of neurological and psychiatric disorders. mGlus are members of the G protein-coupled receptor (GPCR) superfamily, the largest class of cell-surface receptors. Despite their tractability as drug targets, the majority of GPCR-based drug discovery programs have failed to yield highly selective compounds. The traditional approach to drug discovery has been to target the endogenous ligand (orthosteric)-binding site, to either mimic or block the actions of the endogenous neurotransmitter or hormone in a competitive manner. However, this approach has suffered from a paucity of suitably subtype-selective ligands. This is not surprising given that orthosteric binding sites are often highly conserved between subtypes of a single GPCR subfamily. An alternative approach is to target allosteric sites that are topographically distinct from the orthosteric site, to either enhance or inhibit receptor activation. This approach has been highly successful for ligand-gated ion channels. For example benzodiazepines, positive allosteric modulators (PAMs) of $GABA_A$ receptors, are an effective and safe treatment for anxiety and sleep disorders (Mohler et al., 2002). Discovery and characterization of allosteric modulators of GPCRs has gained significant momentum over the last few years, especially since the clinical validity of GPCR allosteric modulators was demonstrated with two allosteric modulators entering the market. In 2004, cinacalcet (an allosteric enhancer of the Calcium-sensing receptor (CaSR)) was approved for the treatment of hyperparathyroidism, a disease associated with CaSR deficiency (Lindberg et al., 2005). In 2007, maraviroc (an allosteric inhibitor of the chemokine receptor CCR5) was approved for the treatment of HIV infections. This drug stabilizes CCR5 receptor conformations that have a lower affinity for the HIV virus, blocking CCR5-dependent entry of HIV-1 into cells (Dorr et al., 2005). Thus, allosteric modulation represents an exciting novel means of targeting GPCRs particularly for CNS disorders, a therapeutic area with one of the highest rates of attrition in drug discovery (Kola and Landis, 2004).

2. Allosteric modulation of metabotropic glutamate receptors

2.1. Quantifying allosteric interactions

The binding of an allosteric ligand to its site will change the conformation of the receptor, meaning that the "geography" of the orthosteric site and any other potential receptor-ligand/protein interfaces, also have the potential to change. As a consequence, the binding affinity and/or signaling efficacy of the orthosteric ligand are likely to be modulated, either in a positive or negative manner. The simplest allosteric GPCR model assumes that the binding of an allosteric ligand to its site modulates only the affinity of the orthosteric ligand and vice versa; this model is referred to as the allosteric ternary complex model (ATCM; Fig. 1A). Within the framework of an ATCM, the interaction is governed by the concentration of each ligand, the equilibrium dissociation constants of the orthosteric and allosteric ligands (K_A and K_B, respectively), and the "cooperativity factor" α, a measure of the magnitude and direction of the allosteric interaction between the two conformationally linked sites (Stockton et al., 1983; Ehlert, 1988). A value of $\alpha < 1$ (but greater than 0) indicates negative cooperativity, such that the binding of an allosteric ligand inhibits the binding of the orthosteric ligand. Values of $\alpha > 1$ indicate positive cooperativity, such that the allosteric modulator promotes the binding of orthosteric ligand, whereas values of $\alpha = 1$ indicate neutral cooperativity, i.e. no net change in binding affinity at equilibrium. Because the two sites are conformationally linked, the allosteric interaction is reciprocal, i.e., the orthosteric ligand will modulate the binding of the allosteric ligand in the same manner and to the same extent.

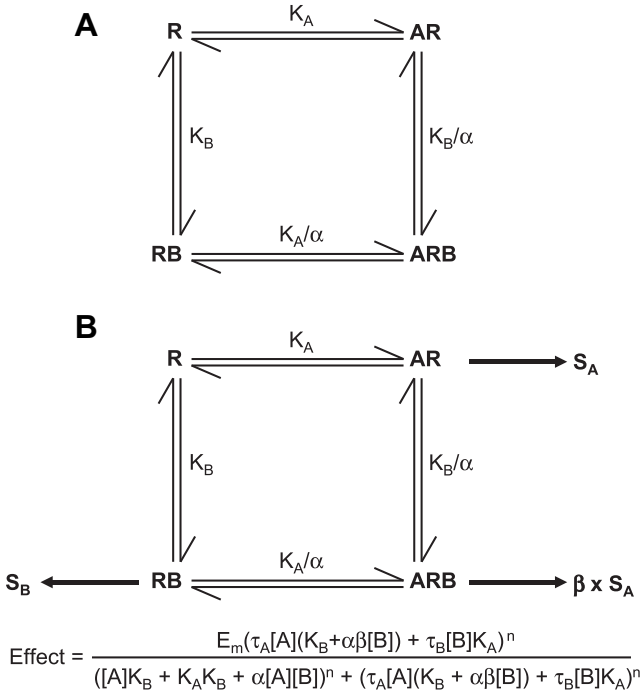

Fig. 1. Models of allosteric interactions. A) Allosteric Ternary Complex Model, B) Operational Model of Allosterism. Refer to text for definitions of parameters.

The simple ATCM describes the effect of the modulator only in terms of changes in orthosteric ligand affinity, and vice versa, thus the stimulus that is generated by the ARB ternary complex (a receptor (R) simultaneously occupied by both agonist (A) and modulator (B)) is assumed to be no different to that imparted by the binary AR complex. In general, many allosteric modulators studied to date, particularly those interacting with class A GPCRs, appear to behave in a manner consistent with this simple ATCM. However, there is no *a priori* reason why the conformational change engendered by an allosteric modulator in the GPCR does not perturb signaling efficacy in addition to, or independently of, any effects on orthosteric ligand binding affinity. Indeed, for mGlus the majority of allosteric modulators influence orthosteric ligand efficacy in the absence of effects on affinity. This is most likely a reflection of the fact that the orthosteric and allosteric binding sites are located in very distinct regions of the receptor i.e. the extracellular N-terminus and the transmembrane-spanning domains respectively (Conn et al., 2009a,b; see later for discussion). It is also important to note that an allosteric modulator can have differential effects on affinity versus efficacy. A striking example of this is the cannabinoid CB_1 receptor allosteric modulator, Org27569, which is an allosteric enhancer of [^{3}H]CP 55940 binding but an allosteric inhibitor of CP 55940 function (Price et al., 2005). This potential for differential effects on efficacy as well as affinity has necessitated the development of alternative models to describe allosteric interactions.

To account for such allosteric effects on efficacy, the ATCM has been extended into an allosteric "two-state" model (ATSM)