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PERSPECTIVE

Seven Transmembrane Receptors as Nature's Prototype Allosteric Protein: De-emphasizing the Geography of Binding

Terry P. Kenakin

GlaxoSmithKline Research and Development, Research Triangle Park, North Carolina

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ABSTRACT

The article in this issue by Redka et al. (p. 834) illustrates some interesting interactions between classified orthosteric (bind to the same recognition site as endogenous agonist) and allosteric (bind to a different site) ligands. Of particular interest are the methods used to deal with an obfuscating factor in these kinds of studies, namely the propensity of seven transmembrane receptors to form dimers and thus demonstrate allosteric effects through binding at the orthosteric site. The judicious use of kinetics to detect and quantify allosteric action also is demonstrated. The various unique properties of allosteric modulators are discussed in the context of the increasing prevalence of allosteric ligands as investigational drugs.

The article by Redka et al. (2008) in this issue illustrates the emergence of important allosteric concepts relating to the function of seven transmembrane (7TM) receptors. In particular, the primarily allosteric nature of these proteins is highlighted by unexpected interactions between previously classified orthosteric and allosteric ligands. Thus, the interaction of an orthosteric ligand, *N*-methyl scopolamine, at the allosteric site for gallamine, is revealed through elegant kinetic studies on defined monomeric muscarinic M2 receptors. Several useful general observations from these studies are relevant to receptor pharmacology in general and the experimental study and quantification of allosteric receptor mechanisms in particular.

Although allosteric drug mechanisms have been described for 7TM receptors (notably benzodiazepine, GABA, and acetylcholine), numerically, they have been in the minority. The majority of 7TM agonists and antagonists are orthosteric in nature as defined by their apparent geography of binding (binding to the same site as the endogenous agonist). One reason for this disparity is that, historically, pharmacology and medicinal chemistry have biased detection assays toward the orthosteric site; i.e., screening with assays designed to detect interference with binding of the natural ligand have conspired to produce drugs that interact orthosterically with 7TM receptors. Another reason for a historical orthosteric bias may be related to kinetics. This idea relates to the fact that the rate of binding of a ligand will depend on the ligand's rate of onset, offset, and the mean lifetime that the binding site is exposed for potential binding. In the case of the orthosteric site, the receptor probably keeps this site accessible for most of the protein lifetime (an open orthosteric binding site conformation is highly preferred) to make the protein maximally sensitive to chemical transmission. Under these circumstances, the binding site is highly accessible to compounds in the drug discovery screening process. In the case of an allosteric site, this may not be true, and allosteric binding sites may have a shorter mean lifetime. This, in turn, necessitates a longer equilibration time with allosteric ligands for detectable binding. Thus, a rapid conventional high-throughput screen with endogenous agonist present may not necessarily be optimal for detection of allosteric ligands. Once the allosteric ligand is bound, the lifetime of the complex is correspondingly longer, making many allosteric ligands optimal for target coverage; i.e., allosteric ligands such as CCR5 HIV entry inhibitors often have slow rates of receptor offset, on the order of hundreds of hours (Watson et al., 2005). Similarly persistent offset kinetics have been reported for allosteric modulators of muscarinic receptors (Jakubík et al., 2002; Machová et al., 2007) and p38 mitogen-activated protein kinase inhibitors (Pargellis et al., 2002).

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ABBREVIATIONS: 7TM, seven transmembrane; NMDA, *N*-methyl-D-aspartate.

At present, reports of allosteric 7TM ligands are on the rise in the literature. There could be two reasons for this increase. One is that 7TM receptors are nature's prototype allosteric proteins. This is shown by the fact that they bind neurotransmitters and hormones in one region, change their shape accordingly (actually, a shape that the protein probably already knows how to make is selectively stabilized) to affect a protein-protein interaction (i.e., G-protein or β-arrestin) in another region. Seven transmembrane receptors, like all proteins, constantly undergo conformational changes according to the thermal energy of the system (Frauenfelder et al., 1988, 1991) to yield an "ensemble" of conformations (Onaran and Costa, 1997; Onaran et al., 2000). Ligands can selectively stabilize preferred conformations through selective binding (Burgen, 1981), and the resulting biased ensemble can then interact with the cell to provide various pharmacological signals. Within this thermodynamic scheme, the location of ligand binding is not particularly predestined. In fact, theoretically, any part of the protein can be a ligand binding site, and the thermodynamic outcome of the binding may be to bias the ensemble to a pharmacologically relevant endpoint.

The second reason allosterism is now observed more frequently may relate to the fact that there are more pharmacological assays available to detect it. Technological advances have allowed an increase in the use of functional high-throughput screens that are more suitable for detection of allosteric effect (Rees et al., 2002). With a functional assay, many more probes are available (such as G-proteins, β-arrestin, etc.) to report changes in receptor conformation. With a binding assay, only changes that affect the affinity for the radioactive ligand will be seen. Historically, there has been an emphasis on describing binding and function in terms of the relative geography of endogenous ligand sites on the receptor and sites used by synthetic ligands. However, there is no particular virtue in pharmacologically modifying this process through orthosteric interaction at the natural ligand binding site (other than perhaps simplicity of the mathematical models to describe the effect). In fact, from a control point of view, it can be argued that this is a rather clumsy mechanism to use therapeutically. In contrast, allosteric interactions, whereby a molecule stabilizes a particular receptor conformation through binding at a different site, is a far more flexible and possibly therapeutically rewarding process (Christopoulos, 2002; Christopoulos and Kenakin, 2002; Kenakin, 2004a, 2005; May et al., 2007). For example, an orthosteric antagonist with no positive efficacy reduces the receptor signal to zero for all agonists; an allosteric ligand may modulate to any level (not necessarily zero). Likewise, allosteric modulators can distinguish between affinity and efficacy and can have opposite effects on each. For example, ifenprodil actually increases the affinity of the NMDA receptor for NMDA but decreases its efficacy. Because allosteric effects are reciprocal, this results in an interesting profile whereby ifenprodil becomes more potent (increased affinity) at higher NMDA concentrations (Kew et al., 1996). This illustrates a unique property of allosteric modulators: they can adjust their potency with the level of activity of the system. Yet another unique property of allosteric ligands is that they can be probe dependent; i.e., in the case of antagonism, they can block some agonists but not others. For example, the allosteric muscarinic receptor modulator eburnamonine decreases the affinity of the agonist arecoline but *increases* the affinity of the agonist pilocarpine (Jakubík et al., 1997). This opens the possibility of permissive antagonism whereby the effects of some endogenous ligands are preserved but pathologically related effects are blocked. For example, a CCR5 HIV-1 entry inhibitor that allows the natural chemokine CCL3L1 to function, may preserve the demonstrated beneficial effects of CCL3L1 (Gonzalez et al., 2005) in progression to AIDS after HIV infection (Kenakin, 2005). In general, allosteric ligands offer more flexibility for therapeutic applications than do orthosteric ligands.

The article by Redka et al. (2008) demonstrates a crossover between orthosteric and allosteric receptor sites. Orthosteric interaction has been defined as a steric hindrance of access of the endogenous ligand to its natural binding site; the geography of binding is coincident. In contrast, allosteric binding may occur some number of angstroms away from this site, the interaction occurring through a conformational change in the receptor. The implication is that allosteric binding is associated with conformational change whereas orthsteric is not. However, it is now known from the discovery of inverse agonism for 7TM receptors (Costa and Herz, 1989) that any binding of a ligand, orthosteric or allosteric, stabilizes a biased ensemble of receptor conformations and results in a change in the macroconformation of the receptor. This can result in a pharmacological effect (i.e., inverse agonism). For example, a survey of 380 previously classified orthosteric antagonists for 73 receptors shows that 85% are inverse agonists (produce conformational change in the receptor; Kenakin, 2004b). The close relationship between binding and receptor conformational change is supported by thermodynamic simulations showing that binding is an active process, not a passive one, that results in receptor conformational change (Kenakin and Onaran, 2002).

The study by Redka et al. (2008) adds new knowledge to this field and highlights the allosteric nature of 7TM receptor dimers. The authors describe previously reported cooperative effects through binding to orthosteric sites (with respect to the natural endogenous agonist) sites and the production of receptor oligomers and how this effect can be nullified experimentally. This is important because the interaction of ligands at only orthosteric sites can produce cooperative effects (allosterism) through 7TM receptor dimerization. Therefore, without specifically ruling out this possibility, it would not be possible to discern ligand interactions at allosteric versus orthosteric sites. The experimental procedures in this present work allowed the observed kinetics to be ascribed to interactions at the allosteric site (albeit at higher concentrations than those required for orthosteric binding). It is interesting to note this crossover activity considering the documented probe-dependent antagonism of some reputed orthosteric antagonists (see Baker and Hill, 2007). In light of the data presented in this volume, it may be useful to consider crossover activities of other previously classified orthosteric ligands to interactions at allosteric sites. The work by Redka et al. (2008) underscores the use of receptor kinetics to detect and quantify allosteric effects. It also reveals the relative unimportance of receptor binding geography in terms of relating ligand effect with the endogenous ligand binding site; in essence, the complete surface of the 7TM receptor protein should be considered a possible ligand binding site for allosteric control of receptors.

In general, the emergence of functional screening assays,

over binding assays, seems to be relieving the bias toward finding orthosteric ligands, and increasing numbers of allosteric ligands have emerged from pharmacological screens over the past decade. With this increase in the availability of allosteric modulators has come a great deal of knowledge about the nature of allosteric effects. It is incumbent on pharmacologists to assimilate this information to facilitate the development of allosteric drugs.

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Address correspondence to: Dr. Terry Kenakin, Department of Biological Reagents and Assay Development, GlaxoSmithKline Research and Development, 5 Moore Drive, Research Triangle Park, NC 27709. E-mail: terry.p.kenakin@gsk.com