PERSPECTIVE

Agonist Binding: A Multistep Process

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Received February 10, 2004; accepted February 10, 2004. This article is available online at http://molpharm.aspetjournals.org

The structural basis of G protein-coupled receptor (GPCR) activation by agonists has been the focus of much experimental research and has inspired the generation of numerous kinetic and molecular models. In this issue, Liapakis et al. (2004) provide new mechanistic insight with an elegantly simple set of studies on a well characterized experimental system, the β2 adrenergic receptor (β2AR). They provide evidence for the existence of one or more intermediate conformational states linking the inactive receptor to the fully active receptor.

The authors determine the binding affinities and efficacies of a panel of ligands that represents the structural constituents of the catecholamine epinephrine (a subset is shown in Fig. 1A). By comparing the affinities of the different compounds, it is possible to determine the contribution of each chemical substituent of the ligand (catechol hydroxyls, N-hydroxyl, or N-methyl) to the binding affinity. The results suggest that there is no preformed binding site for the agonist epinephrine in the unliganded β2AR. Figure 1B illustrates what is meant by a preformed binding site. In this simple lock-and-key model, all of the amino acid side chains that contribute to the binding site for epinephrine are in the optimal position to engage the chemical substituents of the ligand. The contribution of each substituent to the binding affinity will depend on the type of interaction between the substituent and the receptor (aromatic, van der Waal’s, hydrogen bonding, ion pairs). The interaction of each substituent with the binding site should make a significant contribution to ligand affinity, and this contribution should be relatively independent of contributions made by the other substituents. Based on these assumptions, binding affinity should increase in independent of the energetic costs of receptor activation are paid in installments. Based on these assumptions, binding affinity should increase in

Phenethylamine did not increase the binding affinity. However, the authors observed that the catechol hydroxyls, the β-hydroxyl, or the N-methyl group increased the affinity by ~10-fold when added to a ligand having any one of the other three substituents (considering the two catechol hydroxyls as one substituent). For example, adding the N-methyl group to dopamine makes methyldopamine. The affinity of methyldopamine for the β2AR is approximately 10 times greater than the affinity of dopamine. Moreover, each of these substituents contributed 60- to 120-fold to binding affinity in ligands having both of the other two substituents. These binding studies do not support the simple model shown in Fig. 1B. One possible way to explain these results is a model whereby the agonist binds through a series of conformational intermediates as shown in Fig. 1C. In the unliganded state, there is a minimal, low-affinity binding site that permits interactions between the receptor and a few structural features on the agonist (e.g., the aromatic ring and the amine). Binding to this site increases the probability of a conformational transition that is stabilized by an interaction between the receptor and the catechol hydroxyls. The binding energy gained by interactions between the receptor and the catechol hydroxyls pays for the energetic costs of the conformational change. This conformational transition increases the probability of yet another conformational change stabilized by interactions between the receptor and the N-methyl and/or the N-methyl. Thus, the receptor becomes activated through a series of conformational intermediates, and the energetic costs of receptor activation are paid in installments.

This model is consistent with the results of studies using fluorescence spectroscopy to monitor agonist-induced conformational changes in the β2AR (Ghanouni et al., 2001; Swaminath et al., 2004). Fluorescence lifetime studies provide evidence for at least one intermediate conformational state in agonist bound receptor (Ghanouni et al., 2001). More recent time-resolved studies show that epinephrine induces a biphasic conformational change in purified β2AR, consistent with an intermediate conformational state (Swaminath et al., 2004).

ABBREVIATIONS: GPCR, G protein-coupled receptor; β2AR, β2-adrenergic receptor.
Time-resolved peptide binding studies on the neurokinin receptor revealed that an agonist peptide binds with biphasic kinetics (Palanche et al., 2001). Thus, it is likely that agonist binding through intermediate conformational states will be generalizable to other GPCRs, particularly those activated by peptides and protein hormones, where there are a larger number of sites of interaction between the receptor and the agonist.

What are the practical implications of agonist binding and activation of GPCRs through a multistep process? For those interested in drug development, it should influence the approach to structure-based drug design and the use of molecular models for in silico screening of chemical libraries. Most current GPCR models are based on the inactive structure of rhodopsin. Thus, it may not be possible to accurately dock an agonist into an inactive GPCR structure.

Multistep agonist binding may also have implications for cellular signaling. Evidence suggests that the intermediate conformational states generated during agonist binding may have unique functional properties. The rapid binding component of neurokinin A was associated with a cellular calcium response, whereas the slow component was required for cAMP signaling (Palanche et al., 2001). In the case of the β2AR, the rapid conformational change is sufficient for activating Gs, whereas the slow conformational change is required for efficient agonist-induced internalization (Swaminath et al., 2004). The β2AR is known to activate both Gs and Gi, as well as non-G protein-dependent signaling pathways (Luttrell and Lefkowitz, 2002; Azzi et al., 2003). Thus, a single agonist may initiate a specific series of signaling and/or regulatory events by inducing or stabilizing a sequence of conformational states.

References
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