0026-895X/04/6505-1060–1062$20.00
MOLECULAR PHARMACOLOGY
Copyright © 2004 The American Society for Pharmacology and Experimental Therapeutics
Mol Pharmacol 65:1060–1062, 2004

Vol. 65, No. 5
3269/1147671
Printed in U.S.A.

PERSPECTIVE

Agonist Binding: A Multistep Process

Brian Kobilka

Department of Molecular and Cellular Physiology, Stanford Medical Center, Stanford, Palo Alto, California

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 β_2 adrenergic receptor (β_2AR). 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, β-hydroxyl, *N*-methyl) to the binding affinity. The results suggest that there is no preformed binding site for the agonist epinephrine in the unliganded β_2AR. 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 by adding the catechol hydroxyls to phenethylamine to make dopamine, by adding the *N*-methyl to phenethylamine to make methylphenethlamine, or by adding the β-hydroxyl to phenethylamine to make hydroxyphenethylamine. Yet the authors found similar binding affinities for all four compounds (phenethylamine, dopamine, methylphenethlamine, and hydroxyphenethylamine). Adding any one of the substituents (catechol hydroxyls, β-hydroxyl, or *N*-methyl) to 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 β_2AR 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 receptor and the β-hydroxyl 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 β_2AR (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 β_2AR, consistent with an intermediate conformational state (Swaminath et al.,

ABBREVIATIONS: GPCR, G protein-coupled receptor; β_2AR, β_2-adrenergic receptor.

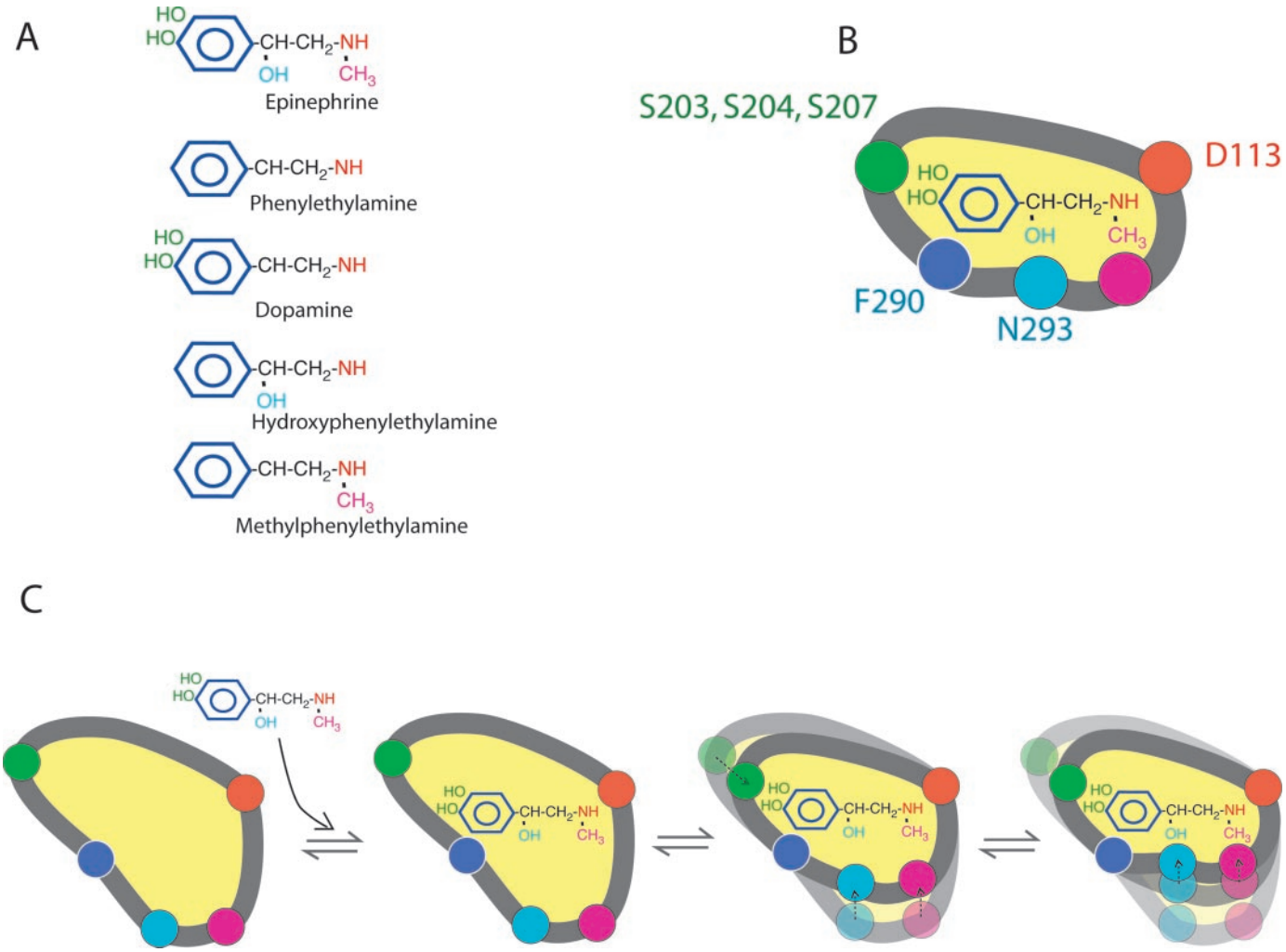

Fig. 1. A, structures of epinephrine, phenylethylamine, dopamine, hydroxyphenylethylamine and methylphenylethylamine. B and C, models of agonist binding. B, lock-and-key agonist binding model. Receptor sites that interact with specific substituents of the ligand are shown as colored circles. The amine nitrogen interacts with Asp113 in transmembrane segment 3 (Strader et al., 1989), the catechol hydroxyls interact with serines 203, 204, and 207 in transmembrane segment 5 (Strader et al., 1989; Wieland et al., 1996; Liapakis et al., 2000). Interactions with the aromatic ring and the chiral β-hydroxyl have both been mapped to transmembrane segment 6 (Strader et al., 1989; Wieland et al., 1996). C, sequential agonist binding model.

2004). 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 β_2AR, 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 β_2AR 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

Azzi M, Charest PG, Angers S, Rousseau G, Kohout T, Bouvier M, and Pineyro G (2003) Beta-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc Natl Acad Sci USA* **100:**11406–11411.

Ghanouni P, Gryczynski Z, Steenhuis JJ, Lee TW, Farrens DL, Lakowicz JR, and Kobilka BK (2001) Functionally different agonists induce distinct conformations in the G protein coupling domain of the β2 adrenergic receptor. *J Biol Chem* **276:** 24433–24436.

Liapakis G, Ballesteros JA, Papachristou S, Chan WC, Chen X, and Javitch JA (2000) The forgotten serine. A critical role for Ser-2035.42 in ligans binding to and activation of the β2-adrenergic receptor. *J Biol Chem* **275:**37779–37788.

Liapakis G, Chan WC, Papadokostaki M, and Javitch JA (2004) Synergistic contri-

butions of the functional groups of epinephrine to its affinity and efficacy at the β2 adrenergic receptor. *Mol Pharmacol* **65:**1181–1190.
Luttrell LM and Lefkowitz RJ (2002) The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals. *J Cell Sci* **115:**455–465.
Palanche T, Ilien B, Zoffmann S, Reck MP, Bucher B, Edelstein SJ, and Galzi JL (2001) The neurokinin A receptor activates calcium and cAMP responses through distinct conformational states. *J Biol Chem* **276:**34853–34861.
Strader C, Sigal I, and Dixon R (1989) Structural basis of β-adrenergic receptor function. *FASEB J* **3:**1825–1832.
Swaminath G, Xiang Y, Lee TW, Steenhuis J, Parnot C, and Kobilka BK (2004) Sequential binding of agonists to the β2 adrenoceptor: kinetic evidence for intermediate conformational states. *J Biol Chem* **279:**686–691.
Wieland K, Zuurmond HM, Krasel C, Ijzerman AP, and Lohse MJ (1996) Involvement of Asn-293 in stereospecific agonist recognition and in activation of the beta 2-adrenergic receptor. *Proc Natl Acad Sci USA* **93:**9276–9281.

Address correspondence to: Brian Kobilka, Stanford University School of Medicine, 157 Beckman Center, 279 Campus Dr., Stanford, CA 94305. E-mail: kobilka@cmgm.stanford.edu