Ligands Stabilize Specific GPCR Conformations: But How?

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Small molecules modulate the activity of G protein-coupled receptors in a number of different ways leading to distinct signaling outcomes, but the mechanisms behind this modulation remain unclear. In this issue of Structure, Zacher et al. have used dynamic single-molecule force spectroscopy to study this question by examining global thermodynamic properties of β2AR in the presence of different ligands.

G protein-coupled receptors (GPCRs) are a family of seven-transmembrane helix proteins found in almost all eukaryotic organisms that respond to a wide variety of external sensory or endogenous signals. Ligand binding is translated into conformational changes in the receptor that result in activation of intracellular G proteins, GPCR kinases, and arrestins, which, in turn, modulate the activity of downstream effectors inside the cell. GPCRs are essential in cell physiology, and their malfunction is commonly translated into pathological outcomes. As a result, GPCRs constitute one of the most important pharmaceutical targets.

The beta 2 adrenergic receptor (β2AR) is one of the most extensively characterized GPCR and serves as a model system for understanding general principles of GPCR structure, signaling, and physiology. This receptor has a rich pharmacology with ligands that increase the level of basal activity toward the G protein (partial and full agonists), decrease it (inverse agonists), or block the receptor without changing its activity (neutral antagonists). In addition, in recent years, some ligands have been found to modulate separately the different cellular pathways that can be activated by a GPCR, receiving the name of biased agonists. For instance, bucindolol, carvedilol, fentoterol, and terbutaline have been shown to display a bias toward beta-arrestin-mediated signaling pathways.

Ligands modulate the function of GPCRs by stabilizing specific conformations of the receptor, but the details of this general mechanism are not yet completely understood. The available crystal structures of GPCR-ligand complexes represent only snapshots generally obtained using heavily engineered proteins. For this reason, complementary data, particularly obtained through spectroscopic methods, are key to unravel the dynamic aspects of GPCRs activation.

The β2AR has been extensively used to measure ligand-induced conformational changes to different degrees of spatial and temporal resolution using a wide variety of spectroscopy techniques, mostly in detergent-solubilized receptors, but also in living cells. Single molecule spectroscopy experiments (Bockenhauer et al., 2011) have demonstrated that, even in the absence of ligands, the receptor exists in equilibrium between a number of discrete conformational states. Agonist binding (or other factors, e.g., oligomerization) promotes activation by shifting this equilibrium toward the active states, although binding of G protein is required for their full stabilization. Fluorescence resonance energy transfer (FRET) experiments in living cells have permitted analysis of the individual steps of the activation pathway, from ligand binding to the production of second messengers (Lohse et al., 2009). This technique is sensitive enough to detect, for instance, distinct conformational changes induced by epinephrine and norepinephrine and allows the calculation of activation kinetics (Reiner et al., 2010). Higher spatial resolution can be obtained by measuring fluorescence intensity and kinetics (see Kobilka, 2007 for a review). These experiments can discriminate between conformational changes induced by agonists or partial agonists (Swaminath et al., 2005) or even find specific switches that are triggered during activation (Yao et al., 2006). Finally, NMR spectroscopy has shown that there is a conformational coupling between the extracellular regions of the receptor and the orthosteric binding site (Bokoch et al., 2010) and has allowed us to propose specific conformational changes involved in biased signaling (Liu et al., 2012).

Spectroscopy measurements can be complemented by data that record structure-related information from the whole system simultaneously. For instance, quantitative mass spectrometry studies, which measure the reactivity of Cys and Lys to a labeling agent upon activation by different ligands, have found that ligands induce a significant variability in receptor conformations (Kahsai et al., 2011). Along this line, this issue of Structure presents a work where dynamic single-molecule force spectroscopy is used to study global thermodynamic properties of β2AR in the presence of different ligands (Zacher et al., 2012).

In these experiments, liposomes containing the receptor are attached to a mica surface. The tip of the atomic force microscope captures the N- or C-terminus of a single molecule of the receptor and pulls it at a constant velocity, which results in the sequential unfolding of secondary structural elements. The microscope records the force during the pulling, and the force-distance profiles allow determining a number of thermodynamic parameters related to the stability.
and flexibility of these elements. By comparing these parameters obtained in the presence of three agonists (adrenaline, BI-167107, and THRX-144877), the inverse agonist carazolol, and the neutral antagonist alprenolol, the authors have been able to observe which regions of the receptor are stabilized in the presence of each type of ligand.

All the experiments consistently detect a series of structural elements that unfold together. In some cases, these elements are part of a transmembrane helix, and in other cases, they are composed by several transmembrane segments and loops. One of these “unfolding structural elements” is roughly composed of TM3-TM4-TM5, and is labeled as “core segment”.

In the absence of ligand, the core segment has a relatively high flexibility that allows the receptor to sample many conformational states, possibly contributing to basal activity of β2AR. This heterogeneity agrees with single-molecule fluorescence spectroscopy data (Bockenhauer et al., 2011).

All ligands seem to bind “loosely,” without mediating strong stabilizing interhelical interactions. Agonists and inverse agonists increase the conformational variability of the core segment. In the case of agonists, this would allow this region to be easily sampled by the empty receptor, while inverse agonists must stabilize a different conformational space that is not part of conformational transitions of the empty receptor. FRET studies in β2AR reconstituted in lipid vesicles have shown that carazolol (but not alprenolol, see below) promotes the formation of high-order oligomers (Fung et al., 2009). Thus, the authors propose that the extensive changes in the conformational variability of the core segment induced by carazolol may be related to oligomerization, which remains to be confirmed by further experiments.

The neutral antagonist alprenolol only stabilizes part of TM1. This is a surprising and counterintuitive effect, as this ligand does not interact directly with this region (Wacker et al., 2010). The authors suggest that these modest changes support the idea that neutral antagonists simply block the receptor without changing substantially its thermodynamic properties. It is difficult to visualize in structural terms how alprenolol and carazolol can result in such dramatically different effects in receptor stability and tendency to oligomerize (Fung et al., 2009). Their binding poses are very similar, stabilizing comparable helix-helix interactions and differing only in the interaction with TM5. Thus, this may be an illustration of how crystal structures do not reflect the dynamic aspects of a protein. For instance, it is possible that the higher off-rate of alprenolol compared with carazolol (which, of course, cannot be accounted for in a crystal structure) results in the overall weaker interactions observed in this work.

In summary, single-molecule force spectroscopy provides a way of estimating thermodynamic properties of ligand-bound GPCRs. While it may be difficult to assess the relevance of thermodynamic parameters when the receptor has been mostly unfolded, this work convincingly shows that the technique can measure differences between ligands of different efficacies and how they affect specific structural elements of the receptor.

REFERENCES


