G PROTEIN-COUPLED RECEPTORS

Crystallizing how agonists bind

Our understanding of how agonists bind to and activate members of the superfamily of G protein-coupled receptors (GPCRs) has been boosted by three papers published in *Nature* that present ligand–receptor X-ray crystal structures for β-adrenergic receptors (β-ARs).

The different conformational states that β-ARs can adopt and the inherent instability of agonist–receptor complexes have contributed to the lack of crystal structures available to date. In these papers, different methods were used to produce crystals that diffracted well enough for the structures of various complexes to be determined.

Warne and colleagues used a thermostabilized turkey β_1-AR to explore the initial conformational changes induced upon binding of full and partial agonists. Together with other subtle structural changes observed inside and outside the binding pocket, the authors propose that the degree to which an agonist has an effect depends on whether it can fulfil three main criteria: ligand-induced conformational changes of Ser215$^{5.46}$ and Ser212$^{5.43}$ (numbers in superscript correspond to the Ballesteros–Weinstein numbering system for conserved GPCR residues) in transmembrane segment 5 (TM5), and contraction of the binding pocket. Accordingly, the full agonists used in this paper (such as carmoterol and isoprenaline) fulfil all three criteria, whereas partial agonists (for example, salbutamol and dobutamine) do not interact with Ser215$^{5.46}$, and the antagonist cyanopindolol does not induce any of these changes.

Rasmussen and colleagues generated a camelid antibody fragment (known as a nanobody) targeted to the human β_2-AR, which was able to mimic G protein-like behaviour and allowed the formation of a high-affinity agonist-bound active-state crystal structure. This structure revealed major changes in the cytoplasmic ends of TM5 and TM6, which are outwardly displaced, whereas TM3 and TM7 move inwards. In the ligand-binding pocket, the largest change seen is an inward bulge of TM5 that is focused around Ser207$^{5.46}$.

In a companion publication to the study by Rasmussen *et al.*, an irreversible agonist was designed that efficiently formed crystals when bound to the low-affinity conformation of a human β_2-AR. The covalent agonist formed hydrogen bonding contacts with Ser203$^{5.42}$ and Ser207$^{5.46}$ on TM5 in the binding pocket, but the structure of the cytoplasmic domains was more similar to the inverse agonist-bound, inactive-state β_2-AR than to the nanobody-stabilized active-state structure. Therefore, agonist binding alone is not enough to stabilize the active conformation at the cytoplasmic surface.

Overall, these data offer a valuable and new understanding of how GPCRs are activated; until now, the only seven-transmembrane domain receptor to be crystallized in different conformational states was rhodopsin, which is activated by light rather than ligand binding. Moreover, these insights could aid the rational design of ligands for this large and diverse family of receptors.

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ORIGINAL RESEARCH PAPERS Warne, T. *et al.* The structural basis for agonist and partial agonist action on a β_1-adrenergic receptor. *Nature* **469**, 241–244 (2011) | Rasmussen, S. G. F. *et al.* Structure of a nanobody-stabilized active state of the β_2 adrenoceptor. *Nature* **469**, 175–180 (2011) | Rosenbaum, D. M. *et al.* Structure and function of an irreversible agonist–β_2 adrenoceptor complex. *Nature* **469**, 236–240 (2011)