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RECEPTOR PHARMACOLOGY

The many faces of G protein-coupled receptors

The ability of different agonists to activate different signalling pathways from a G protein-coupled receptor (GPCR), despite binding the same receptor pocket, is a well-known phenomenon, but the underlying mechanisms have not been clear. Now, Lefkowitz and colleagues have used single-domain antibodies (nanobodies) to probe GPCR allostery, broadening our knowledge about the dynamic nature of ligand-dependent GPCR activation.

GPCR allostery refers to the change in receptor affinity for its intracellular G protein transducer upon binding of a ligand to the extracellular ligand-binding pocket, owing to conformational changes in the receptor. Moreover, G protein binding can alter GPCR affinity for a ligand. These interactions have given rise to the ternary complex model that describes interactions between a ligand, receptor and G protein.

In the current study, the authors sought to understand the structural basis for these allosteric relationships, using the β_2-adrenergic receptor (β_2AR) as a model system. In a competitive radioligand binding assay, they measured the affinity of the β_2AR agonist isoprenaline for the receptor in the presence of nanobodies, which hold the receptor in a particular conformation. Presence of the nanobody Nb80, which stabilizes an active conformation of the β_2AR, increased isoprenaline affinity for the receptor by 75-fold. Conversely, presence of the nanobody N60, which binds an inactive conformation of the receptor, reduced isoprenaline affinity by 70-fold.

This dramatic decrease represents a 'very-low-affinity' receptor state and suggests that the low-affinity state associated with an uncoupled GPCR (traditionally considered the receptor 'at rest') could actually be a rapidly exchanging ensemble of conformations. The large free energy difference between the very-low-affinity state and the activated state describes the full allosteric potential of the receptor, which is orders of magnitude greater than previously thought.

NMR spectroscopy and X-ray crystallography confirmed previous work showing two inactive states of the β_2AR, and revealed the structural basis by which Nb60 preferentially stabilizes one of these states.

Last, the authors measured the positive allosteric effect of Nb80 and the negative cooperative effect of Nb60 on the affinity of 17 ligands (comprising agonists, partial agonists and antagonists) for the β_2AR in a competitive radioligand binding assay. The surprisingly complex relationship that emerged between the effects of each nanobody on ligand affinity suggested that ligands 'perceive' specific receptor conformations differently.

The authors propose a three-state model of receptor activation in which a ligand can differentially affect two equilibria: one between unbound receptor and 'active' Nb80-bound receptor, and one between unbound receptor and 'inactive' Nb60-bound receptor. Efficacy can be viewed as the sum total of a ligand's effect on these two equilibria, with partial agonists potentially achieving similar efficacies via different component effects. The authors note that many unresolved states are likely to exist.

Such work could assist the design of drugs such as 'biased ligands', which are currently under investigation to provide more specific drugs with reduced side effects, such as cleaner pain management via opioid receptor signalling.

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ORIGINAL ARTICLE Staus, D. P. *et al.* Allosteric nanobodies reveal the dynamic range and diverse mechanisms of G-protein-coupled receptor activation. *Nature* **535**, 448–452 (2016)
FURTHER READING Kingwell, K. Pioneering biased ligand offers efficacy with reduced on-target toxicity. *Nat. Rev. Drug Discov.* **14**, 809–810 (2015)