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Structure watch

'FIGHT OR FLIGHT' RECEPTOR REVEALED

The 'fight or flight' hormone adrenaline — which is produced during times of stress — is an agonist of the β_2-adrenergic receptor (β_2AR), a G-protein-coupled receptor (GPCR) that mediates multiple downstream effects, including pupil dilation, blood vessel constriction and increased pulse rate. Three-dimensional structures of GPCRs are difficult to obtain because hydrophobic surfaces in the transmembrane (TM) domains and GPCR flexibility (caused by multiple conformational states that vary according to agonist binding) prevent the molecular contacts that enable protein crystallization. However, two 3D crystal structures of this 'fight or flight' receptor have now been determined using different receptor–ligand complexes.

Many GPCRs, including β_2AR, have some constitutive activity even in the absence of agonist binding. Hence, β_2AR activity was repressed using the inverse agonist carazolol to stabilize the β_2AR structure in its inactive state, although some basal activity remained. Two different techniques were used to obtain the receptor–ligand complex for crystallization, which both provided a polar surface that promoted crystallization without altering agonist binding or preventing the conformational changes that are induced by receptor activation. Rosenbaum *et al.* and Cherezov *et al.* engineered a β_2AR fusion protein in which they replaced most of the conformationally variable third intracellular loop (ICL3) of β_2AR with T4 lysozyme, whereas Rasmussen *et al.* stabilized β_2AR using an antibody that specifically binds to ICL3. Crystals of the β_2AR–T4 lysozyme fusion protein and the β_2AR–antibody complex were obtained under lipid conditions that mimic the membrane environment of the receptor and that also aid crystallization.

Similar to rhodopsin (the only GPCR structure that had previously been solved), β_2AR has seven transmembrane domains that form a helical bundle in which carazolol, and presumably other ligands, bind. β_2AR also contains the conserved E/DRY motif, which comprises three charged amino acids — known as the ionic lock — that tether the α-helical TM3 and TM6 together and stabilize β_2AR in the inactive state. Although carazolol binding should mainly induce the inactive conformation of β_2AR, surprisingly, this lock is broken in both structures of the carazolol-bound β_2AR complexes, similar to an active GPCR. TM3 and TM6 of β_2AR have a more open structure than their counterparts in the inactive rhodopsin structure, so that R131 and E268 of the β_2AR E/DRY motif are not close enough to each other to form a hydrogen bond. This finding is unlikely to be an artefact of the crystallization process because it is observed in both of the β_2AR structures, which were crystallized under different conditions. Thus, the authors suggest that the more open structure of carazolol-bound β_2AR may account for the residual activity that is observed, and propose that stronger inverse agonists might stabilize this lock and fully inactivate β_2AR.

ORIGINAL RESEARCH PAPERS Rosenbaum, D. M. *et al.* GPCR engineering yields high-resolution structural insights into β2-adrenergic receptor function. *Science* **318**, 1266–1273 (2007) | Cherezov, V. *et al.* High-resolution crystal structure of an engineered human β2-adrenergic G protein-coupled receptor. *Science* **318**, 1258–1265 (2007) | Rasmussen, S. G. F. *et al.* Crystal structure of the human β2 adrenergic G-protein-coupled receptor. *Nature* **450**, 383–387 (2007)