G PROTEIN-COUPLED RECEPTORS

Pioneering peptide GPCR structure determined

A report in *Nature* has presented the first crystal structure of a peptide G protein-coupled receptor (GPCR) from the β group of the class A GPCR family: neurotensin receptor 1 (NTSR1) bound to a peptide agonist. As the endogenous ligand of NTSR1, the 13-amino-acid peptide neurotensin (NTS), exerts a range of cellular activities — from modulating dopamine transmission to promoting cancer cell growth — elucidation of how NTS binds to its receptor could aid in the design of potential drugs.

Wild-type NTSR1 is unstable in detergent solution, and so conformational thermostabilization through the introduction of amino acid mutations at six locations was used to produce crystals of rat mutant NTSR1 (termed NTSR1-GW5) in the presence of the carboxy-terminal portion of NTS ($NTS_{8–13}$). NTSR1-GW5 then had T4 lysozyme (T4L) engineered into intracellular loop 3 (ICL3) to improve the likelihood of obtaining well-diffracting crystals. NTSR1-GW5-T4L was expressed in insect cells, solubilized and then crystallized using the lipidic cubic phase methodology. The structure was determined at a resolution of 2.8 Å using molecular replacement.

The overall structure of NTSR1-GW5-T4L is similar to other GPCR structures determined so far, except that it has no helix 8, which has been observed in all GPCR structures apart from CXC chemokine receptor 4. This may be because NTSR1-GW5-T4L does not have this helix or because of an artefact of the crystallization procedure. Moreover, transmembrane helix 7 (TM7) extends by three helical turns beyond the conserved NPXXY motif, in contrast to other GPCRs, which extend only by one turn.

By comparing the NTSR1-GW5-T4L structure with rhodopsin and β_2-adrenergic receptor structures in active and inactive conformations, the authors show that NTSR1-GW5-T4L is in an active-like conformation. Co-crystallization of NTSR1-GW5-T4L with $NTS_{8–13}$ shows that the ligand binding pocket is composed of amino acid residues in the amino terminal, the three extracellular loops (ECL1–ECL3) and TM2–TM7. The authors also show that the binding mode of $NTS_{8–13}$ to NTSR1 is markedly different to the binding of agonists to rhodopsin, β_1-adrenergic receptor and adenosine A_{2A} receptor, as $NTS_{8–13}$ does not penetrate as deeply into the receptor as agonists for the other three GPCRs.

Together, these data could support the development of non-peptide ligands for NTSR1 that could be useful in the treatment of neurological disorders and cancer, as well as aiding homology modelling of other peptide GPCRs.

Man Tsuey Tse

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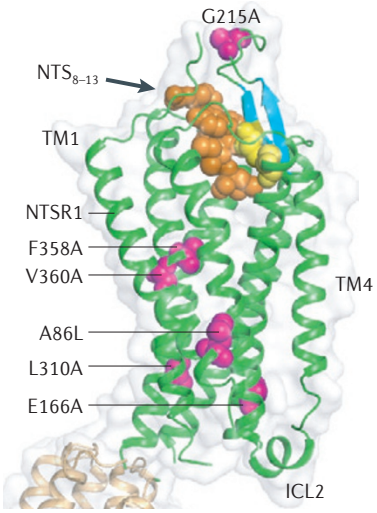

Side view of the NTSR1 structure bound to the peptide agonist $NTS_{8–13}$