The Structure of Neuropeptide Y bound to its G protein-coupled Y2 receptor

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In order to influence the numerous pharmacological important signal transduction pathways involving G protein-coupled receptors (GPCRs), agonists or antagonists with high specificity and selectivity have to be developed to avoid adverse reactions. This is accomplished by large scale screening or considerably more efficiently by structure based drug design. The latter requires detailed information about the atomistic structure and dynamics of the natural ligand in its receptor bound state. NMR spectroscopy is an excellent method to study ligand/receptor interaction in their native lipid environment.

Here, we determined the structure and binding sites of the 36 amino acids comprising Neuropeptide Y (NPY) bound to the Y2 receptor. The interaction of NPY with the Y2 receptor plays, among others, an essential role in food intake and in the regulation of the circadian rhythm. Our approach was applying solution as well as solid-state NMR spectroscopy as complimentary methods. While backbone angle information were obtained from 13C-13C correlation MAS NMR spectra, interaction sites could be identified by comparing 1H-15N chemical shifts of NPY in presence and in the absence of the receptor, acquired from HSQC solution NMR spectra.

The required milligram amounts of the Y2 receptor were obtained by recombinant expression in *E.coli* as inclusion bodies and subsequent refolding of the GPCR into lipid membranes. Full functionality of the prepared receptor was shown by ligand binding and G protein activation. Several variants of NPY with four 15N/13C labelled amino acids each for easy signal assignment were produced by solid-phase syntheses.

Using the obtained restraints from NMR measurements, the structure of receptor bound NPY was calculated and modelled into the receptor binding pocket. The result revealed a structural change for the last five C-terminal amino acids of NPY from α-helix to random-coil upon receptor binding. The known C-terminal binding site could be confirmed. Further, a second binding site was identified, that interacts with the second extracellular loop of the Y2 receptor, which was finally proven in cell culture assays.