Angewandte International Edition Chemie

GPCR Drug Design

DOI: 10.1002/anie.201307542

# Structures of Class B G Protein-Coupled Receptors: Prospects for Drug Discovery

*Felix Hausch**

*Dedicated to Professor Florian Holsboer*

drug design · GPCRs · glucagon · receptors · structure elucidation

G protein-coupled receptors (GPCRs) are the most important class of drug targets. GPCR structural biology has witnessed a revolution in the last five years, cumulating in the Nobel Prize in Chemistry 2012.[1] While a consensus for the structural framework for the majority of GPCRs (the class A GPCRs) has now emerged,[2] their more distantly related cousins of the class B GPCR family are structurally still poorly understood. Now two landmark publications by Hollenstein et al. and Siu et al. have presented the first class B GPCR structures providing the first highly resolved snapshots for this class of molecular signaling machines.[3,4]

Class B GPCRs are a small but clinically highly relevant subfamily of the GPCR kingdom. Humans have 15 class B receptors, which all respond to long peptide hormones. These endocrine factors are key regulators of glucose homeostasis, bone maintenance, or coping with stress. Parathyroid hormone, glucagon-like peptide, or analogues thereof are clinically used to treat osteoporosis or diabetes while antagonists of the receptors for glucagon, the calcitonin-gene-related peptide, or the corticotropin-releasing factor are pursued for the treatment of diabetes, migraine, or depression. Despite extensive efforts by the pharmaceutical industry it has been very challenging to develop drug-like nonpeptidic ligands for class B GPCRs. This was thought to be due to a rather flat binding pocket compared for example to monoaminergic GPCRs of the class A family, which are tailored by nature to recognize small molecules.

A major challenge in GPCR crystallography is to freeze the normally very dynamic proteins in a functional and stable state, and class B GPCRs are no exception. Hollenstein et al. and Siu et al. achieved this for the corticotropin-releasing factor receptor 1 ($CRF_1R$) and for glucagon receptor (GCGR), respectively, by truncating the transmembrane domain of these receptors (i.e., removing the extracellular domain that is typical for class B GPCRs) and by fusing them to well-crystallizable helper proteins. Both groups also stabilized the receptors with high-affinity antagonists and the availability high-affinity ligands for these two class B GPCRs was likely instrumental for the successful crystallization. Hollenstein et al. further introduced 12 thermostabilizing point mutations that stabilized the inactive conformation of $CRF_1R$.

The key lessons from the two class B crystal structures are class B specific structural details, which are common between $CRF_1R$ and GCGR and distinct from the typical class A GPCRs motifs. The two structures reveal a set of interactions between the transmembrane helices close to the intracellular part that are similar in both class B GPCR structures and are likely conserved within the class B GPCR family. The most striking difference to class A GPCRs is a more V-shape topology of the transmembrane helices, which creates a wide cleft open to the extracellular space and likely represents the binding pocket of the peptide agonist. This arrangement of the extracellular halves of the transmembrane helices was surprising and it could not be predicted by homology modeling based on crystallized class A GPCRs. The much larger binding pocket of the two class B GPCRs provides some explanation why it was so difficult to discover ligands for these proteins. However, the putative binding peptide cleft is also much deeper than anticipated and displays several subcavities that might serve as new starting points for a rational design or a refined in silico screening of class B GPCR ligands.

A particular illuminating aspect of the $CRF_1R$ structure is the detailed insight into the binding mode of the nonpeptide antagonist CP-376395 (Figure 1a). This antagonist binds in a cavity near the cytoplasmic side, far remote from the binding sites of class A GPCR ligands, and far from the putative binding site of the native ligand CRF. It proves that members of the predominant class of $CRF_1R$ antagonists represented by CP-376395 are indeed allosteric modulators. The occluded nature of the discovered binding site also explains the slow dissociation rates of many $CRF_1R$ ligands.[5] The binding mode of CP-376395 also calls for a note of caution when using mutagenesis data for homology modeling. The $CRF_1R$ field was blessed with a very defined pharmacophore for antagonists and a well-defined point mutation (His199Val), which was thought to disrupt an essential hydrogen bond to an invariant hydrogen acceptor of the antagonists.[6] It turns out that His199 is actually located rather remote from the antagonist and works indirectly by stabilizing the conformation of Tyr327, which contacts the isopentyl amino moiety of CP-376395. In this context note

[*] Dr. F. Hausch
AG Chemical Genomics, Max Planck Institute of Psychiatry
Kraepelinstr. 2, München (Germany)
E-mail: hausch@mpipsykl.mpg.de
Homepage: http://www.mpipsykl.mpg.de/en/research/groups/hausch/index.html

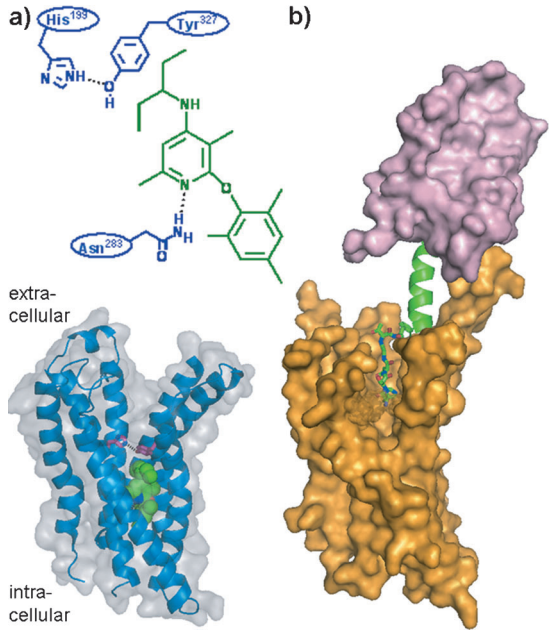


***Figure 1.*** Crystal structures of class B GPCR transmembrane domains. a) The corticotropin-releasing factor receptor 1 ($CRF_1R$) is shown as blue cartoons, the surface indicated transparent in gray. The bound allosteric nonpeptide antagonist CP-376395 is highlighted in green spheres. Residues His199 and Tyr327 separating the antagonist binding site from the putative peptide binding site are shown as pink sticks. The chemical structure of CP-376395 and its key interactions with $CRF_1R$ are shown at the top. b) The surface of the crystallized transmembrane domain of the glucagon receptor (GCGR) is shown in light orange. Residues W304, R308, F365, and A366 are rendered transparent to allow a view on the putative peptide binding mode in the interior of GCGR. The modeled extracellular domain is indicated in light pink. The C terminus of the modeled bound ligand glucagon is shown as a green α helix, the N terminus, which activates the transmembrane domain, is shown as green sticks. The GCGR–glucagon model described in Ref. [4] was kindly provided by Stevens and coworkers.

that the substituents in this latter "top-group" position are rather variable in $CRF_1R$ antagonists,[6] thus suggesting that with other ligands other conformations around His199 and Tyr327 may be possible.

While the conformations of the transmembrane helices close to the cytoplasm are fairly similar between $CRF_1R$ and GCGR, in line with their shared function to activate the same G proteins, more substantial deviations are apparent in the extracellular loops and the attached α-helical segments. Additional crystal structures of other class B GPCRs are required to address whether this reflects a higher flexibility in the extracellular half of class B GPCRs (possibly influenced by crystal contacts) or genuine differences between individual receptors.

The present structures likely represent $CRF_1R$ and GCGR in their inactive conformation. The most important open question is how these receptors are turned on. As a first step to clarify this, Siu et al. engaged on an extensive modeling based on their GCGR structure, (co)crystal structures of the extracellular domains with and without bound peptide ligand, crosslinking constraints, and an impressive set of mutagenesis data. Their model suggests that the part of glucagon that is responsible for GCGR activation adopts an extended conformation reminiscent of the way neurotensin binds to its cognate class A GPCR (Figure 1b).[7] However, the N-terminal histidine is predicted to dip deeper into the receptor to a similar level as typical small-molecule ligands of class A GPCRs.[2] While this model is plausible for GCGR, the activation mode of $CRF_1R$ might be slightly different. Compared to the GCGR agonist glucagon, CRF has an N-terminal extension,[8] which is variable and does not contribute to $CRF_1R$ activation.[9a] The N terminus of CRF is thus unlikely to bind deeply in the interior of the receptor but rather to fold back outside. Consistent with this hypothesis, the putative peptide binding cleft of $CRF_1R$ is wider than that of GCGR and would allow such a hairpin-like conformation.

Nonpeptidic agonists for class B GPCRs such as the glucagon-like peptide receptor 1 would be clinically extremely useful. For their precise rational design the structure of an agonist-bound class B GPCR is needed. This task is complicated by the special mechanism of class B GPCRs, which are activated in a two-step fashion.[9] First, the C-terminal part of the peptide ligands binds with high affinity to the extracellular domain (which is the hallmark of all class B GPCRs and which were deleted in the present crystallization constructs). The second N-terminal part of the peptide ligands then binds to the transmembrane domain and induces the structural rearrangements that lead to G protein activation inside the cell. This second interaction is less strong and usually only a fraction of GPCRs are activated at a time. To obtain a homogeneously activated class B GPCR it will thus be necessary to stabilize this activated state, for example by mutations specific for the active state or by complexing with G proteins. To further tackle the specific challenges of class B GPCRs, stabilizing the active orientation of the extracellular versus the transmembrane domain, rigidification of the peptide ligand, and/or enhancing its affinity for the transmembrane domain might be necessary to obtain a structure of a full-length active state like class B GPCR.

Received: August 27, 2013
Published online: October 11, 2013

[1] a) F. Hausch, *Angew. Chem.* **2008**, *120*, 3360 – 3362; *Angew. Chem. Int. Ed.* **2008**, *47*, 3314 – 3316; b) F. Hausch, F. Holsboer, *Angew. Chem.* **2012**, *124*, 12338 – 12341; *Angew. Chem. Int. Ed.* **2012**, *51*, 12172 – 12175; c) R. J. Lefkowitz, *Angew. Chem.* **2013**, *125*, 6494 – 6507; *Angew. Chem. Int. Ed.* **2013**, *52*, 6366 – 6378; d) B. Kobilka, *Angew. Chem.* **2013**, *125*, 6508 – 6517; *Angew. Chem. Int. Ed.* **2013**, *52*, 6380 – 6388.

[2] a) A. J. Venkatakrishnan, X. Deupi, G. Lebon, C. G. Tate, G. F. Schertler, M. M. Babu, *Nature* **2013**, *494*, 185 – 194; b) V. Katritch, V. Cherezov, R. C. Stevens, *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 531 – 556.

[3] K. Hollenstein, J. Kean, A. Bortolato, R. K. Y. Cheng, A. S. Dore, A. Jazayeri, R. M. Cooke, M. Weir, F. H. Marshall, *Nature* **2013**, *499*, 438 – 443.

[4] F. Y. Siu, M. He, C. de Graaf, G. W. Han, D. Yang, Z. Zhang, C. Zhou, Q. Xu, D. Wacker, J. S. Joseph, W. Liu, J. Lau, V. Cherezov, V. Katritch, M.-W. Wang, R. C. Stevens, *Nature* **2013**, *499*, 444 – 449.

  

[5] a) S. J. Ramsey, N. J. Attkins, R. Fish, P. H. van der Graaf, *Br. J. Pharmacol.* **2011**, *164*, 992 – 1007; b) B. A. Fleck, S. R. Hoare, R. R. Pick, M. J. Bradbury, D. E. Grigoriadis, *J. Pharmacol. Exp. Ther.* **2012**, *341*, 518 – 531.
[6] a) E. P. Zorrilla, G. F. Koob, *Drug Discovery Today* **2010**, *15*, 371 – 383; b) M. Paez-Pereda, F. Hausch, F. Holsboer, *Expert Opin. Invest. Drugs* **2011**, *20*, 519 – 535; c) J. Williams, *Expert Opin. Ther. Pat.* **2013**, *23*, 1057 – 1068.
[7] a) J. F. White, N. Noinaj, Y. Shibata, J. Love, B. Kloss, F. Xu, J. Gvozdenovic-Jeremic, P. Shah, J. Shiloach, C. G. Tate, R. Grisshammer, *Nature* **2012**, *490*, 508 – 513; b) F. Hausch, F. Holsboer, *Nature* **2012**, *490*, 492 – 493.
[8] C. Parthier, S. Reedtz-Runge, R. Rudolph, M. T. Stubbs, *Trends Biochem. Sci.* **2009**, *34*, 303 – 310.
[9] a) C. Devigny, F. Perez-Balderas, B. Hoogeland, S. Cuboni, R. Wachtel, C. P. Mauch, K. J. Webb, J. M. Deussing, F. Hausch, *J. Am. Chem. Soc.* **2011**, *133*, 8927 – 8933; b) S. R. J. Hoare, *Drug Discovery Today* **2005**, *10*, 417 – 427.



  