Biochemistry
Viewpoint
Cite This: Biochemistry 2019, 58, 137−139
pubs.acs.org/biochemistry

Structural Basis of Partial Agonism at the β2-Adrenergic Receptor

Arun K. Shukla*

Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur 208016, India

Approximately one-third of the currently prescribed drugs target G protein-coupled receptors (GPCRs), which comprise the largest and most versatile family of cell surface receptors in the human body. GPCR ligands are typically categorized as inverse agonists, neutral antagonists, partial agonists, full agonists, and biased agonists on the basis of their efficacy profile toward downstream signaling pathways and functional selectivity. Deciphering the structural details of ligand−receptor interaction, receptor activation, and effector coupling has been a central focus of GPCR research, not only for understanding the fundamental principles of GPCR signaling but also for leveraging this information for designing novel therapeutics. High-resolution structures of a large number of GPCRs in complex with different ligands and effector proteins have been obtained using X-ray crystallography and cryo-electron microscopy (cryo-EM) in recent years.[1,2] Taken together with a large body of pharmacological data in the literature, these structures have yielded unprecedented information about receptor activation and signaling.[3] However, our understanding of structural mechanisms driving ligand efficacy at GPCRs, partial agonism and biased agonism in particular, is still very much evolving.[4]

Partial agonism at GPCRs is defined as the ability of certain ligands to trigger submaximal effector coupling and downstream responses even at full receptor occupancy. It is important to understand the structural features at the level of ligand−receptor interaction and receptor conformation that drive partial agonism to fully appreciate the complex nature of GPCR activation, signaling, and regulation. Masureel et al. have now determined a crystal structure of the human β2-adrenergic receptor (β2AR) in complex with a partial agonist, salmeterol, stabilized by a conformationally specific nanobody[5] (Figure 1). This crystal structure, taken together with accompanying biophysical data, reveals a number of interesting mechanistic details about partial agonism of salmeterol at the β2AR.

β2AR, a prototypical GPCR, has been one of the most extensively studied receptor systems at the structural level, and a number of structures, including those with inverse agonists, full agonists, and heterotrimeric G proteins, are available. In addition, as a range of well-characterized partial agonists with varying efficacies are also available for the β2AR, it represents an optimal receptor system for investigating the structural details that determine partial agonism. Salmeterol, a high-affinity partial agonist for β2AR, is one of the long-acting β2AR agonists (LABAs), displays a high degree of receptor subtype selectivity (i.e., >1500-fold selectivity for β2AR vs β1AR), and is frequently prescribed for asthma and chronic obstructive pulmonary disorder. An interesting feature of salmeterol is its unique chemical structure involving a long aryloxyalkyl extension in addition to its pharmacophore moiety, and it has been suggested that the extended structure binds to a "non-orthosteric" binding site on the receptor, frequently termed the "exosite".

The crystal structure of salmeterol-bound β2AR now confirms this notion and reveals the precise nature of this exosite on the receptor (Figure 1B). The pharmacophore moiety of salmeterol occupies the orthosteric binding pocket, similar to the native agonist epinephrine. Interestingly, however, a set of residues from the extracellular ends of TM6 and TM7 together with extracellular loops (ECLs) 2 and 3 constitute the exosite to accommodate the extended structure of salmeterol. Interestingly, a comparison of β2AR with the other subtype of the β-adrenergic receptor, i.e., β1AR, reveals a significant divergence in the residues constituting the exosite for salmeterol, which in turn provides a previously lacking mechanistic framework for explainin the striking receptor subtype selectivity of salmeterol.

There are two key questions that are important for understanding what determines partial agonism of a given ligand at a GPCR. First, how does a partial agonist differ from a full agonist in terms of its interaction in the ligand binding pocket? Second, how do these differences manifest at the conformational level, on the intracellular side of the receptor in particular, to direct partial effector coupling? The crystal structure of the salmeterol-bound β2AR offers insights into both of these questions. First, a key difference between the binding of epinephrine and salmeterol in the orthosteric binding pocket is the lack of a hydrogen bond between the ligand and Asn293$^{6.55}$ in the salmeterol-bound structure. This leads to the absence of a polar network formed by Ser204$^{5.43}$, Asn293$^{6.55}$, and the ligand and a somewhat relaxed position of the extracellular end of TM5 (Figure 2). Interestingly, molecular dynamics simulation provides corroborating evidence for these differences observed in the ligand binding pocket of the salmeterol-bound β2AR structure. It is tempting to speculate that a relatively less extensive interaction network in the ligand binding pocket is translated into a somewhat less pronounced conformational rearrangement on the intracellular side of the receptor.

In fact, on the intracellular side, an interesting feature observed in the salmeterol-bound structure is a relatively smaller outward movement of TM6, in comparison to the epinephrine-bound receptor structure and the β2AR−Gαs complex (Figure 3). This outward movement of TM6 is considered one of the hallmarks of receptor activation as it provides a docking interface for the α-subunit of heterotrimeric G proteins. Thus, a smaller movement of TM6 may indicate a somewhat intermediate active state of the β2AR that in turn is

Special Issue: Future of Biochemistry: The International Issue

Received: November 29, 2018
Published: December 31, 2018

DOI: 10.1021/acs.biochem.8b01237
Biochemistry 2019, 58, 137−139

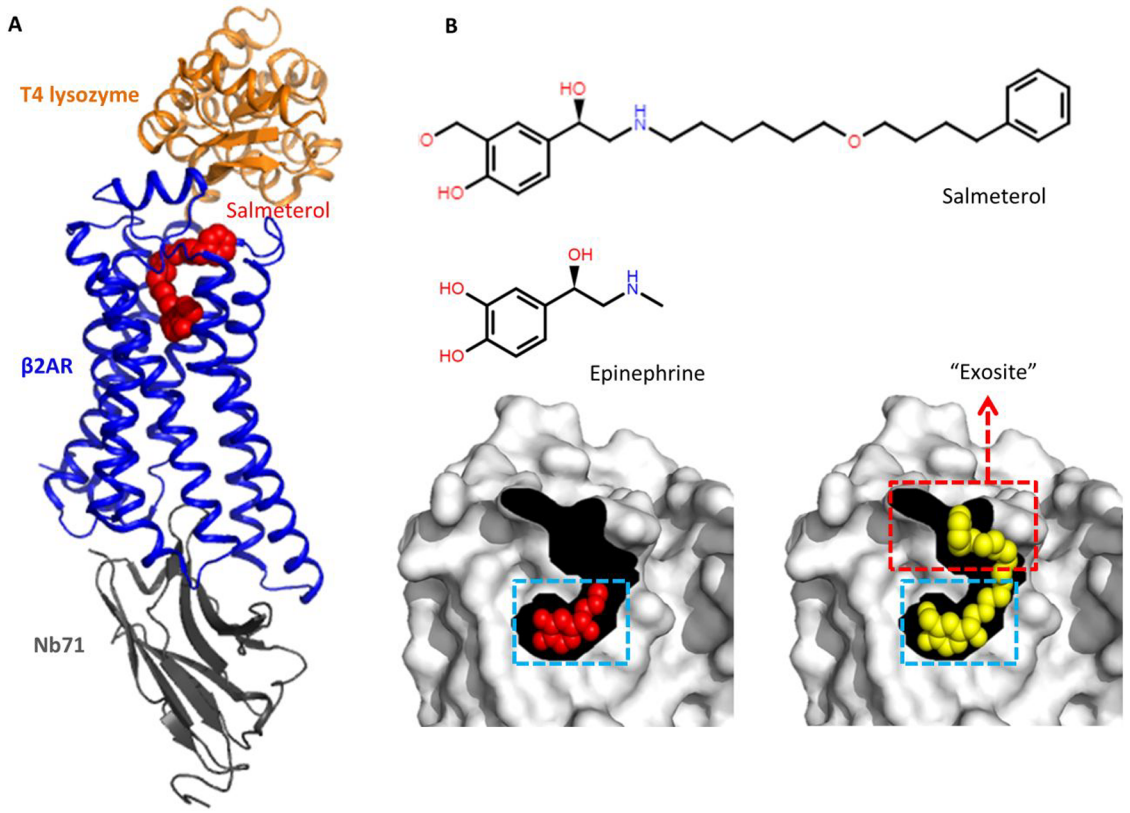

Figure 1. Overall crystal structure of the salmeterol-bound β2-adrenergic receptor (β2AR). (A) Cartoon representation of the crystal strucuture of the human β2AR in complex with salmeterol stabilized by a nanobody, termed Nb71. The N-terminal T4 lysozyme is colored orange, β2AR blue, Nb71 gray, and salmeterol red. The image is generated from the Protein Data Bank (PDB) coordinates of the salmeterol-bound β2AR (PDB entry 6MXT) using Pymol. (B) Chemical structures of the native β2AR agonist epinephrine (also termed adrenaline) and salmeterol and comparison of their modes of binding to the β2AR. The "exosite" occupied by the aryloxyalkyl tail of salmeterol is indicated. The binding mode of epinephrine is derived from a previously determined crystal structure of β2AR in complex with epinephrine (PDB entry 4LDO).

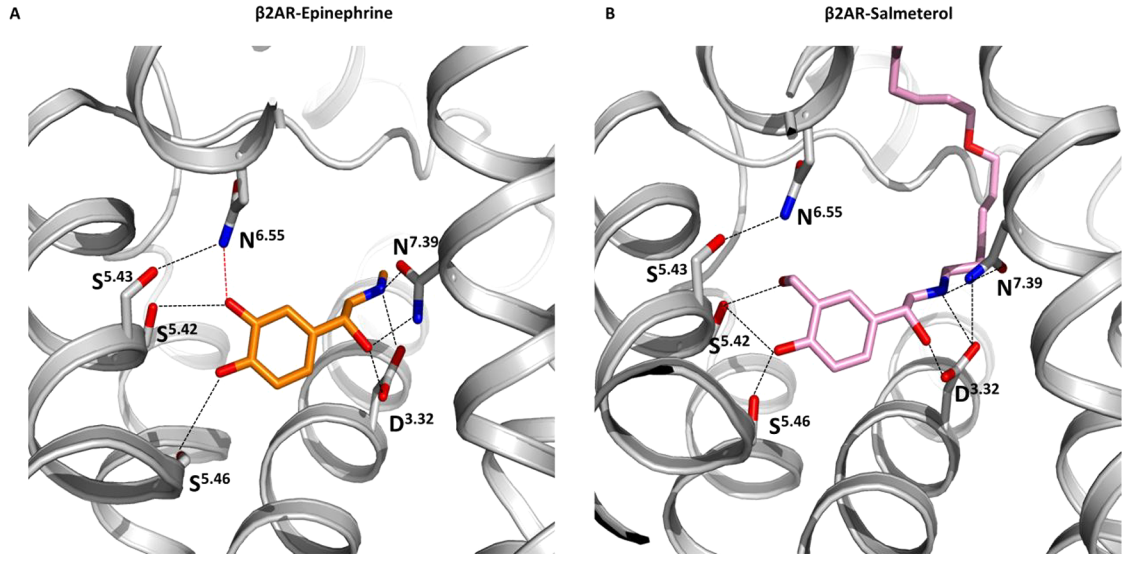

Figure 2. Key structural differences between the binding of epinephrine and salmeterol. (A) Interaction of epinephrine and (B) salmeterol in the orthosteric binding pocket of the β2AR derived from their respective crystal structures. A key difference in the salmeterol-bound structure is the lack of a hydrogen bond between the ligand and Asn293$^{6.55}$ that leads to a disrupted polar network involving Ser204$^{5.43}$, Asn293$^{6.55}$, and the ligand.

responsible for the partial G protein coupling and, hence, partial efficacy of salmeterol. An important consideration in interpreting the conformational features observed in the salmeterol-bound β2AR structure, and previously determined active-state structures, especially on the intracellular surface, is the contribution of stabilizing nanobodies used for crystallization. This is particularly important considering that the binding interface of the nanobody involves a significant

DOI: 10.1021/acs.biochem.8b01237
Biochemistry 2019, 58, 137−139

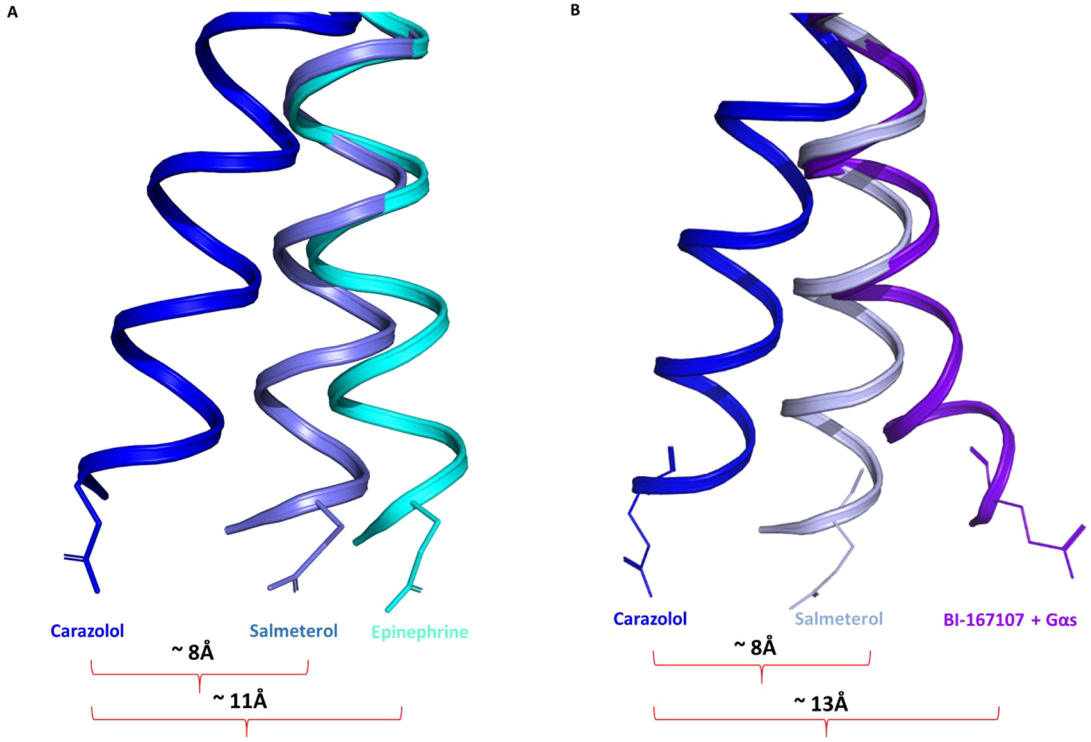

Figure 3. Outward movement of TM6 in various active β2AR structures. (A) Superimposition of the crystal structures of carazolol, salmeterol, and epinephrine-bound β2AR reveals a somewhat intermediate outward movement of TM6 (approximately 8 Å) in the salmeterol-bound structure compared to the epinephrine-bound structure (approximately 11 Å). (B) Similarly, the outward movement of TM6 in salmeterol-bound β2AR is significantly smaller than that in the fully active receptor conformation in the β2AR−Gαs complex (~13 Å). The images are generated using PyMol on the basis of previously determined crystal structures (2RH1 for carazolol-bound β2AR and 3SN6 for the β2AR−Gαs complex). The side chain of Glu296 present at the far cytoplasmic end of TM6 is highlighted.

contribution from TM6. However, extensive spectroscopic studies using the purified receptor in solution, in the absence of stabilizing nanobodies, further corroborate the partial opening on the intracellular surface of the receptor in the salmeterol-bound conformation compared to the full agonist-bound receptor conformation.

In conclusion, the crystal structure of salmeterol-bound β2AR allows direct visualization of a previously conceived exosite on the receptor and reveals structural features at the level of the ligand binding pocket and receptor conformation that potentially determine partial agonism of salmeterol. An interesting aspect that should be explored in the future is the extent to which these features are conserved for other partial agonists of β2AR and whether a similar structural mechanism also exists for other members of the GPCR family. Furthermore, the structural template of salmeterol-bound β2AR may catalyze focused efforts in the future to design allosteric modulators and novel bitopic ligands by targeting the exosite revealed in this crystal structure.

AUTHOR INFORMATION

Corresponding Author

*E-mail: arshukla@iitk.ac.in.

ORCID

Arun K. Shukla: 0000-0003-2910-8288

Funding

The research program in A.K.S.'s laboratory is supported by the Wellcome Trust/DBT India Alliance (IA/I/14/1/501285), the Department of Science and Technology (SERB/EMR/2017/003804), the Department of Biotechnology (BT/08/IYBA/2014-3), and the Indian Institute of Technology Kanpur. A.K.S. is an Intermediate Fellow of the Wellcome Trust/DBT India Alliance (IA/I/14/1/501285) and an EMBO Young Investigator.

Notes

The author declares no competing financial interest.

ACKNOWLEDGMENTS

The author thanks Eshan Ghosh and Madhu Chaturvedi for their assistance in preparing the figures.

REFERENCES

(1) Hilger, D., Masureel, M., and Kobilka, B. K. (2018) Structure and dynamics of GPCR signaling complexes. *Nat. Struct. Mol. Biol. 25*, 4−12.

(2) Wootten, D., Christopoulos, A., Marti-Solano, M., Babu, M. M., and Sexton, P. M. (2018) Mechanisms of signalling and biased agonism in G protein-coupled receptors. *Nat. Rev. Mol. Cell Biol. 19*, 638−653.

(3) Wacker, D., Stevens, R. C., and Roth, B. L. (2017) How Ligands Illuminate GPCR Molecular Pharmacology. *Cell 170*, 414−427.

(4) Shukla, A. K., Singh, G., and Ghosh, E. (2014) Emerging structural insights into biased GPCR signaling. *Trends Biochem. Sci. 39*, 594−602.

(5) Masureel, M., Zou, Y., Picard, L. P., van der Westhuizen, E., Mahoney, J. P., Rodrigues, J., Mildorf, T. J., Dror, R. O., Shaw, D. E., Bouvier, M., Pardon, E., Steyaert, J., Sunahara, R. K., Weis, W. I., Zhang, C., and Kobilka, B. K. (2018) Structural insights into binding specificity, efficacy and bias of a beta2AR partial agonist. *Nat. Chem. Biol. 14*, 1059−1066.

DOI: 10.1021/acs.biochem.8b01237
Biochemistry 2019, 58, 137−139