Point of VIEW

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An Adrenaline (and Gold?) Rush for the GPCR Community

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ABSTRACT G-Protein-coupled receptors are one of the largest protein families found in metazoans. Using several novel strategies, the first atomic resolution structures of a receptor that is activated by a diffusible ligand have been determined.

In 1823, the music world was astounded by the publication of Ludwig van Beethoven's monumental *33 Variations on a Waltz by Anton Diabelli*. So it is today, eight years after Palczewski *et al*. unveiled the structure of the first G-protein-coupled receptor (GPCR), retinal rhodopsin (reviewed in ref 1), that we are now dazzled again by the beauty of the first variation on the GPCR theme revealed by publication of crystal structures of the β-2-adrenergic receptor (β2AR) (*2*–*4*), which responds to the catecholamine neurotransmitter, epinephrine (formerly adrenaline). What do the β2AR structures solved by Kobilka, Weis, Stevens, and their colleagues tell us that we did not know from rhodopsin? Much as the collection of Beethoven's *Diabelli Variations* illustrated the depth of his genius, the β2AR structures provide important insights that both reinforce our understanding of core mechanistic themes and illuminate the underlying basis for the astonishing functional diversity displayed by the GPCR family, the largest and most clinically important class of receptors in the eukaryotic kingdom (*5*). While intrinsically beautiful, the structural principles now established also hold practical promise for the design of new drugs targeted to specific GPCRs.

Similarities and differences between rhodopsin and β2AR biology make these receptors ideal for structural comparison. Like all vertebrate photoreceptors, rhodopsin contains a covalently linked chromophore (retinal) that undergoes light-driven *cis*–*trans* isomerization (*1*). In the dark, *cis*-retinal stabilizes rhodopsin in its inactive conformation, completely suppressing spontaneous activity. Upon photon absorption, retinal isomerizes to the *trans* form, releasing the constraints that hold rhodopsin in the inactive state and stabilizing its active conformation, producing a biological response within milliseconds. In contrast, like essentially all other GPCRs, β2AR binds a diffusible ligand noncovalently, producing biological responses on a slower time scale (seconds-to-minutes) (*6*). Despite such differences, rhodopsin and β2AR share with all GPCRs a common mechanism of action: catalyzing GDP−GTP exchange on the α-subunit of a membrane-bound receptor-associated ("coupled") heterotrimeric G protein. Thus, a central challenge has been to determine the structural changes evoked by photon absorption or by binding of a specific diffusible ligand and how they alter receptor conformation and bring about G protein activation.

Because most GPCRs are inherently conformationally flexible (*7*), especially when extracted with detergent from their native plasma membrane environment, and because β2AR in particular has limited polar surfaces available to form crystal contacts (*6*), determining its 3D structure by protein crystallography presented some enormous technical challenges. Kobilka, Weis, and Stevens have solved these problems using tactics that represent a *tour de force* in the biochemistry, engineering, and crystallogra-

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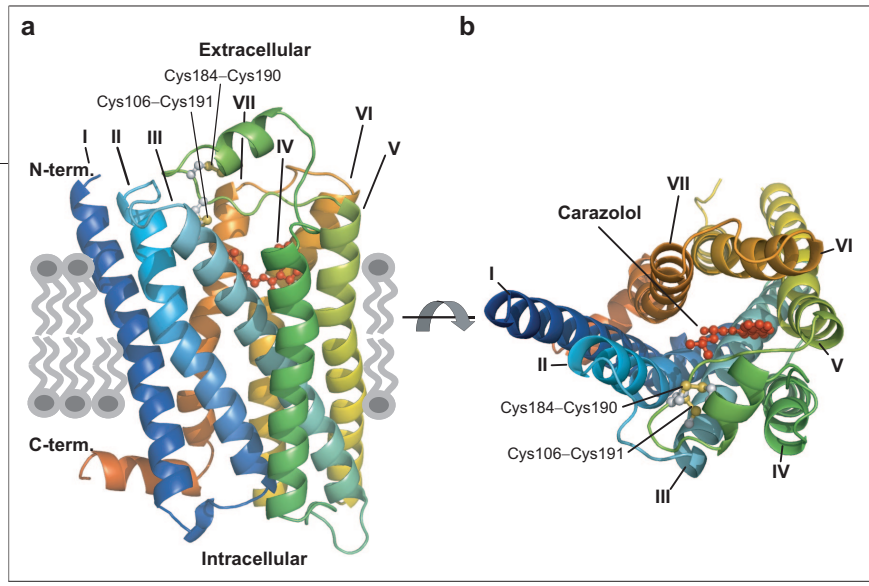

Figure 1. Two views of the carazolol-bound state of mammalian β2AR. Structure of β2AR, derived from the coordinates of the β2AR-T4 lysozyme chimera (*2*, *4*) (Protein Data Bank identification 2RH1) and rendered in PyMOL (www.pymol.org) with the lysozyme part of the chain removed: a) a side view in the plane of the plasma membrane with the exocellular and cytoplasmic sides indicated and b) from the top, viewing the receptor from the extracellular milieu. The seven transmembrane helices are labeled (in the amino to carboxyl direction): TM1 (dark blue), TM2 (light blue), TM3 (aquamarine), TM4 (dark green), TM5 (light green), TM6 (orange), and TM7 (rust). Two disulfide bonds between Cys106—Cys191 and Cys184—Cys190 (white and yellow) and the bound ligand (red) are shown in ball-and-stick representation.

phy of an integral membrane protein. In each of the two β2AR structures (*3*, *4*) the receptor is bound to a partial inverse agonist (carazolol), stabilizing an inactive conformation. To provide further stability and increase polar surface for crystallization, Kobilka and Weis bound a monoclonal antibody to the third cytoplasmic loop of the receptor (*3*, *8*). In a second complementary approach, Kobilka, Weis, and Stevens replaced part of the third cytoplasmic loop with T4 lysozyme (*2*, *4*). Given the potential for introducing non-native perturbations that might have arisen from the use of these strategies and the other extraordinary measures (e.g., necessity for cholesterol) that were used for crystallization, it is important to emphasize and rather reassuring that the details of the two structures are remarkably congruent, an indication that the salient structural features of β2AR have been preserved. Nonetheless, even though their overall folds are quite similar, more of the transmembrane helices and loops of the receptor were resolved and the bound ligand was more clearly visible in the structure of the β2AR-T4 lysozyme chimera than in the structure of native β2AR that was immobilized by binding of a Fab antibody fragment directed against the third intracellular loop.

Tertiary Structure. Like rhodopsin, the tertiary structure of β2AR consists of an extracellular N-terminus, seven transmembrane α-helices linked by three intracellular and three extracellular loops, and a cytosolic C-terminal tail (Figure 1). This common architecture argues that structural organization of this kind underlies the ability of GPCRs to activate G proteins. However, relative to rhodopsin, the helices in β2AR are shifted, probably to provide a solvent-accessible entryway to permit access of its catecholamine ligand to its binding site.

Ligand-Binding Site. In rhodopsin and β2AR, respectively, the binding pockets for covalently attached retinal and for the diffusible small-molecule ligand (carazolol) are located toward the extracellular ends of transmembrane helices III, IV, V, and VII. However, the retinal-binding site of rhodopsin is capped by a β-sheet consisting of the N-terminus preceding helix I and a loop (EC2) that links helices IV and V, rendering retinal inaccessible to solvent. In contrast, the ligand-binding site of the β2AR is clearly more open and solvent-exposed, in part because the N-terminus of β2AR is disordered and does not interact with EC2. Instead, EC2 contains an extra helical region and disulfide bonds that clamp it and hold it out of the way, thus allowing the ligand access into the binding pocket. Structural differences also exist deeper in the ligand-binding pockets of rhodopsin and β2AR. In rhodopsin, the β-ionone ring of retinal interacts with a residue (Trp265) in helix VI, whose rotameric state is thought to constitute the "toggle switch" that is flipped by chromophore isomerization and initiates the shift from the inactive to active receptor conformation. In contrast, the carazolol ligand does not interact with the equivalent residue (Trp268) in β2AR; rather, Phe289 and Phe290 form an extended aromatic network with Trp268, presumably constraining its rotameric state.

The structure of the β2AR ligand-binding pocket provides insight into mechanisms that dictate the pharmacological selectivity of this receptor and its related adrenergic receptors, such as the β1AR (*9*). Strikingly, residues forming the carazolol-binding site in β2AR are conserved in β1AR, even though the affinities of these receptors for certain ligands differs by at least 100-fold. Therefore, other differences, such as side chains around the entrance to the ligand-binding pocket or nuances of the helical packing, are likely to provide the structural basis for the observed pharmacological discrimination of ligands by these receptors. Indeed, the potential effects of subtle differences in helical packing are supported by studies of chimeric receptors (*10*, *11*).

Importantly, the structure of the ligand-binding pocket in β2AR indicates that conformational changes are required to accommodate the binding of agonists, which stabilize the active conformation, relative to inverse agonists, which stabilize the inactive state (Box 1). When modeled into the ligand-binding pocket, the catechol hydroxyls of an agonist, isoproteronol, point toward two residues (Ser204 and Ser207)

Box 1. Classes of GPCR Ligands

Agonist: Any artificial or naturally occurring substance that binds to and produces full activation of a specific receptor and its consequent biological or biochemical response in cells or tissues.

Partial agonist: Any agonist that, regardless of the amount used, produces submaximal activation of a receptor and its consequent biological responses.

Inverse agonist (negative antagonist): Applicable only if a receptor displays spontaneous activity in the absence of an agonist. An inverse agonist is any substance that binds to a receptor and fully suppresses its spontaneous activity.

Partial inverse agonist: Any inverse agonist that, regardless of amount used, produces submaximal suppression of spontaneous receptor activity.

Antagonist (neutral antagonist): Any substance that binds to the same receptor as an agonist yet produces no effect other than to block the ability of an agonist to activate a receptor and its biological responses.

critical for catecholamine binding; however, in the inactive state, the distance is too long for hydrogen bonding. Likewise, residues implicated in selective binding of agonists (Asn293 and Tyr308) are also too distant to interact productively with the modeled isoproteronol ligand. Thus, significant conformational remodeling of β2AR presumably occurs to accommodate agonist occupancy of this binding pocket.

GPCR Activation. Spectroscopy studies provide evidence that both rhodopsin (*12*) and β2AR (*13*) undergo activation-associated conformational changes. Despite a wealth of such biophysical data, the structural mechanisms of GPCR activation remain poorly understood. Results obtained by electron paramagnetic resonance and fluorescence spectroscopy (*14*) suggest that rhodopsin activation involves large rigid-body movements of transmembrane helices III and VI. In contrast, much smaller transmembrane domain movements are suggested when crystal structures of rhodopsin in its inactive state versus a photoactivated intermediate conformation are compared (*1*), although the structural relationship between this intermediate and fully active (meta II) rhodopsin is not clear.

Although the structures of β2AR bound to an inverse agonist define an inactive conformation of this receptor, they nevertheless provide some insight into potential mechanisms whereby agonist binding could change the conformation of the cytoplasmic domains of the receptor to cause G protein activation. In this regard, one interesting feature that can be inferred from the structures is that side chain interactions involving transmembrane helices in the intracellular half of the receptor form a network that is more tightly packed than the network of interactions involving transmembrane helices within the extracellular half of the receptor. Thus, receptor activation may involve rearrangement of the contacts within this network of interactions rather than isolated rigid body movement of transmembrane helices *per se*. Indeed, fluorescence spectroscopy data indicate that various agonists stabilize β2AR in structurally distinct active states, indicative of conformational plasticity (*13*).

Support for the conclusion that subtle rather than gross structural rearrangements accompany β2AR activation is provided by amino acid substitutions that lead to elevated agonist-independent activity (i.e., constitutively active mutations, CAMs) or to impaired agonist-induced G-protein activation (i.e., uncoupling mutations, UCMs). CAMs are thought to define residues whose interactions stabilize the inactive conformation of the receptor. In the β2AR structure, residues of the CAM class cluster centrally on helices III and VI (*15*). UCMs identify residues whose interactions are not required for high-affinity ligand binding but are nonetheless required to stabilize (or for the function of) the active state of the receptor (*16*). Indeed, UCMs include a cluster of residues near the cytoplasmic end of helix VII. Intriguingly, the structures reveal that the residues pinpointed as CAMs and UCMs seem to be linked *via* packing interactions, such that agonist-induced movements of one would be propagated through a chain of interactions to affect the others. One such network of side chains packs near the toggle switch residue (Trp286), such that changes in the rotameric state of this side chain could be propagated to the cytoplasmic ends of the helices by affecting the packing of the residues defined as CAMs and UCMs. Regrettably, the disposition of parts of the intracellular loops and the C-terminal cytosolic tail are unknown because they were disordered in the crystal structures. They may be revealed if crystals of complexes between β2AR and its cognate heterotrimeric G-protein (G_s) can now be obtained and analyzed by X-ray diffraction methods.

Another intriguing hypothesis suggested by the rhodopsin and now the β2AR structures is that a relatively loosely packed, water-filled region facilitates conformational transitions in response to agonist binding. This kind of environment presumably limits constraints on and thus lowers the energy barrier for changes in side chain repacking. In β2AR, this region contains a network of potential hydrogen bond interactions that link the indole ring proton of Trp268 with several other conserved residues (Asn322, Pro323, and Tyr326) that extend toward the cytoplasmic ends of transmembrane helices. The potential importance of this water-filled network is underscored by the identification of UCMs that correspond to these conserved residues (*6*).

GPCR Dimers. β2AR, rhodopsin, and other GPCRs appear to have the capacity to form dimers or higher-order oligomers, and evidence indicates that such associations are of physiological relevance (*17–19*). However, the β2AR crystal structures show that, at least when held in its inactive conformation by binding to a partial inverse agonist, the receptor exists in a stably folded monomeric state that shows only minimal interactions with other receptors in the lattice. Of course, this observation could mean that one effect of such a ligand is to prevent receptor−receptor association. Until the structure of β2AR bound to a full agonist is obtained, the role of receptor−receptor contact in β2AR function and how the agonist-occupied form of the receptor stimulates G-protein activation will both remain matters of conjecture. Thus, although the recently determined 3D views of β2AR are exciting and important, determination of the structure of an agonist−receptor-G-protein complex remains the "holy grail" of GPCR biology.

Future Prospects. Because the vast majority of GPCRs bind diffusible ligands, these receptors must either have ligand-binding pockets that are always solvent-accessible or must display sufficient conformational flexibility that they "flicker" frequently into a state where the entrance to the pocket is transiently opened. The properties of β2AR and the features revealed by the recent determination of many of its structural elements at atomic resolution strongly suggest that, in the short term, it will be a much better template than rhodopsin for generating models of clinically relevant GPCRs whose structures have not been solved. Furthermore and in the long term, the path-finding approaches used by Kobilka, Weiss, and Stevens to acquire structures for β2AR will undoubtedly be applied to other GPCRs. Hence, we can all look forward to viewing other equally stirring variations on the GPCR theme.

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