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Structural Features for Functional Selectivity at Serotonin Receptors

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Abstract

Drugs active at G protein-coupled receptors (GPCRs) can differentially modulate either canonical or non-canonical signaling pathways via a phenomenon known as functional selectivity or biased signaling. We report biochemical studies that show that the hallucinogen lysergic acid diethylamide (LSD), its precursor ergotamine (ERG) and related ergolines display strong functional selectivity for β-arrestin signaling at the 5-hydroxytryptamine (5-HT) receptor 5-HT_{2B}, while being relatively unbiased at the 5-HT_{1B} receptor. To investigate the structural basis for biased signaling, we determined the crystal structure of the human 5-HT_{2B} receptor bound to ERG, and compared it with the 5-HT_{1B}/ERG structure. Given the relatively poor understanding of GPCR structure-function to date, insight into different GPCR signaling pathways are important to better understand both adverse and favorable therapeutic activities.

Apart from canonical G protein mediated signaling, G protein-coupled receptors (GPCRs) also activate non-canonical G protein-independent pathways, frequently mediated by β-arrestins (1, 2). So-called 'biased' GPCR agonists differentially activate both signaling pathways with distinct efficacies and potencies as compared to unbiased agonists that activate both pathways equally (3). This preferential activation of one pathway over the other has been termed "functional selectivity" or "signaling bias" (2–5). Depending on the receptor, biased signaling patterns are key for mediating inflammation (6), apoptosis (7), and many other processes (2). Biased ligands have been proposed to stabilize receptor conformations that are distinct from those induced by unbiased ligands, and selectively change the propensity of GPCR coupling to either G proteins or β-arrestin (2).

Agonist-induced changes in "trigger motifs" of GPCRs (8) near the binding pocket facilitate large-scale helical movements that are accompanied by rearrangements in highly conserved residues called "micro-switches" (9) that prime GPCRs for subsequent G protein binding and activation (10). The structural features of a signaling biased receptor state remain

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elusive, and although complexes of two β-arrestin-biased ligands with the β_1 adrenergic receptor (β_1AR) have been recently solved (11), they did not reveal activation-related changes in the receptor.

To elucidate molecular and structural details of biased signaling, we characterized G protein- and β-arrestin-mediated signaling at G protein-coupled serotonin (5-HT; 5-hydroxytryptamine) receptors with several representative ergolines like LSD and ERG, and solved the crystal structure of the 5-HT_{2B} receptor in complex with ERG, which was identified as a highly biased agonist for the 5-HT_{2B} receptor (12).

To investigate potential differences of ergoline signaling at 5-HT receptors, we examined three prototypical serotonin receptors that interact with distinct G proteins. The 5-HT_{1B} receptor inhibits cyclic adenosine monophosphate (cAMP) production through G_i, the 5-HT_{2B} receptor mediates phospholipase C activation through G_q, and the 5-HT_{7A} receptor stimulates cAMP production through G_s (13). We compared G protein- and β-arrestin-mediated signaling at cloned human 5-HT_{1B} and 5-HT_{2B} receptors, and G protein-mediated signaling at 5-HT_{7A} receptors stimulated by selective and non-selective ligands in HEK293 cells (Fig. 1, table S1) (14).

LSD, and especially ERG displayed bias for β-arrestin signaling at 5-HT_{2B} (Bias factors 101 and 228 respectively; Fig. 1D), minimal bias at 5-HT_{1B} (Bias factors 5 and 25 respectively; Fig. 1D), and G protein antagonism at 5-HT_{7A} receptors (Fig. 1B, table S1). We also found significant β-arrestin signaling bias for other ergolines, such as dihydroergotamine (DHE), methylergonovine (MTE), pergolide (PER), and cabergoline (CAB) at the 5-HT_{2B} receptor, whereas all other evaluated compounds showed no significant bias (Fig. 1D). ERG and DHE, both of which contain a large tripeptide moiety substitution at the amide scaffold, displayed more extreme signaling bias at the 5-HT_{2B} receptor compared to LSD.

To investigate the molecular details responsible for biased signaling, we crystallized an engineered 5-HT_{2B} receptor construct in complex with ERG, solved its structure at 2.7 Å (figure S1, S2, table S3), and compared it to the structure of 5-HT_{1B}/ERG reported in the companion manuscript (15), as well as to other known unbiased active-state GPCR structures.

Residues $P^{5.50}$, $I^{3.40}$ and $F^{6.44}$ (16, 17), the "P-I-F" motif, form an interface between helix V, helix III and helix VI near the base of the ligand binding pocket in the β_2 adrenergic receptor (β_2AR) and many other aminergic receptors, including all 5-HT GPCRs. In the active-state structures of β_2AR (8, 18), a chain of conformational rearrangements occur in the P-I-F residues, in which an inward shift of helix V residue $P211^{5.50}$ is coupled with: (i) a rotamer switch in $I121^{3.40}$, (ii) a large movement of the $F282^{6.44}$ side chain, and (iii) a corresponding rotation of helix VI on the cytoplasmic side (8). The 5-HT_{1B} and 5-HT_{2B} receptor structures display two different conformations of the P-I-F motif (Fig. 2). For the 5-HT_{1B} receptor, we observe that the P-I-F configuration is essentially identical to that of the active-state of β_2AR (β_2AR-R*, PDB ID: 3SN6, (18)) (Fig. 2B). Whereas the 5-HT_{2B} receptor adopts a similar conformation of $P229^{5.50}$ and $I143^{3.40}$, the side-chain conformation of $F333^{6.44}$ was similar to that observed in the inactive β_2AR (β_2AR-R, PDB ID: 2RH1, (19)) (Fig. 2C, figure S2B). The P-I-F motif, therefore, appears to be in an active-like conformation in the 5-HT_{1B} structure, but only in an intermediate active conformation in the 5-HT_{2B} receptor structure.

At the cytoplasmic side of the receptors, GPCR activation is generally characterized by the displacement of helices V, VI and VII (10). The magnitude of the helical motions depends on the activation-state; for example, the outward displacement of the intracellular tip of helix VI ranges between 3 and 14 Å, whereas helix VII shifts between 3 to 5 Å towards the

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receptor core (10, 18–22). These concerted rearrangements induce an opening of the helical bundle at the cytoplasmic side, which facilitates the binding and subsequent activation of G proteins. Analysis of the 5-HT_{1B} and 5-HT_{2B} receptor structures shows that the conformation in the intracellular half of the helical bundle is notably shifted towards that seen in active-state GPCR structures (Fig. 3, figures S3 and S4), and distinct from those of inactive-state GPCR structures (Fig. 3, figures S5 and S6). In the 5-HT_{1B} and 5-HT_{2B} receptor structures, the helix VI intracellular part is located at least 2 to 4 Å further away from the receptor core than in the inactive-state structures of other aminergic GPCRs (figure S5). This conformation of helix VI is close to the one observed in active-state structures of the A_{2A} adenosine receptor (A_{2A}AR) (figure S3), and rhodopsin (Rho) (figure S4), though the outward shift of the helix is smaller in magnitude compared to that of the G protein-bound β_2AR (Fig. 3A, 3C). The only difference that we observed in helix VI between 5-HT receptor subtypes was a small clockwise rotation in the 5-HT_{1B} receptor towards the active-state that is absent in the 5-HT_{2B} structure (Fig. 2B, 2C, 3A, 3C). Helix VII in both 5-HT receptor structures also displays intermediate active-states when compared to β_2AR (Fig. 3B, 3D), where it is shifted further towards the receptor core than in inactive-state structures of other aminergic GPCRs (figure S6). Whereas the 5-HT_{2B}/ERG receptor structure shows less pronounced active-like changes in helix VI, helix VII appears to be in a more active conformation than it is in the 5-HT_{1B}/ERG receptor structure (Fig. 3B, 3D).

Another important aspect of GPCR activation are rearrangements of side chains in highly conserved motifs D(E)/RY (helix III) and NPxxY (helix VII), which are referred to as “micro-switches” (9). Thus, the D(E)/RY motif of all inactive-state and most active-state GPCR structures shows an intact salt bridge between the side chains of $D(E)^{3.49}$ and $R^{3.50}$. Importantly, this salt bridge is broken only in the active-state structures of β_2AR-G$\alpha\beta\gamma$ (18) and Opsin-GαCT (21), where $R^{3.50}$ interacts instead with the G protein and Gα peptide, respectively. The salt bridge is preserved between the side chains of $R153^{3.50}$ and $D152^{3.49}$ in the 5-HT_{2B} receptor structure, but it is broken in the 5-HT_{1B} receptor structure (Fig. 4A, 4B, 4D, figure S2C). In the 5-HT_{1B} receptor structure, the $D146^{3.49}$ side chain forms a hydrogen bond to Y157 in ICL2, and the $R147^{3.50}$ side chain interacts with the main chain carbonyl in a loop of the fusion protein BRIL (residue L1048), which is partially inserted into the G protein binding crevice (Fig. 4A). Thus, the conformation of the D(E)RY motif mimics the active state of β_2AR in the 5-HT_{1B} structure, but resembles the inactive state in the 5-HT_{2B} structure (Fig. 4A, 4C and figure S7).

The highly conserved NPxxY motif at the cytoplasmic end of helix VII is another key micro-switch of GPCR activation (9). Upon GPCR activation, (i) the intracellular end of helix VII moves towards the receptor core and (ii) a rotation of $Y^{7.53}$ around the helical axis moves the side chain further into the 7TM bundle (10). Both 5-HT receptor structures show active-state conformations of the NPxxY motif when compared to β_2AR, A_{2A}AR and Rho (Fig. 4D–E and figures S2D and S7), with more pronounced activation features in the 5-HT_{2B} receptor.

Our analysis indicates that the 5-HT_{1B}/ERG structure has most of the attributes of a classical agonist-induced active-like state, consistent with our biochemical findings that ERG is a comparatively unbiased agonist at the 5-HT_{1B} receptor. In contrast, the 5-HT_{2B}/ERG structure exhibits conformational characteristics of both the active- and inactive-state. The structure of β_2AR-G$\alpha\beta\gamma$ complex, along with recent NMR and fluorescence studies of β_2AR, implicate helix VI predominantly in G protein signaling, whereas conformational changes in helix VII are associated with enhanced β-arrestin signaling (18, 23, 24). Thus, an active-like state in the helix VII conformation of the 5-HT_{2B} receptor, but only partial changes in helix VI, mirrors the strong β-arrestin bias of ERG at 5-HT_{2B} receptors observed in pharmacological assays.

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A likely structural explanation for the distinct conformational features and biased pharmacology of ERG between 5-HT_{1B} and 5-HT_{2B} receptors can be found in the region of the extracellular loop 2 (ECL2) junction with helix V. In the 5-HT_{2B} receptor structure, E212-R213-F214 form an additional helical turn which is stabilized by a structured water molecule and forms a sharp angle to the extracellular tip of helix V (Fig. 5). As a result, the segment of ECL2 that connect helices III and V via the conserved disulfide bond is shortened in the 5-HT_{2B} receptor inducing an inward shift and creating a conformational constraint on the position of the extracellular tip of helix V. Because of these rearrangements, ERG forms additional hydrophobic contacts with $M218^{5.39}$, $L347^{6.58}$, $V348^{6.59}$, $L362^{7.35}$ and $K211^{ECL2}$ (figure S8A) and stabilizes a closer distance between helices V and VI, which form hydrophobic contacts between $L219^{5.40}$, $V348^{6.59}$ and $L349^{6.60}$, along with a hydrogen bond between $S222^{5.43}$ and $N344^{6.45}$ (figure S8B). These extensive ligand-mediated interactions between helices V and VI in the 5-HT_{2B}/ERG complex may be inferred to prevent rearrangements in helix VI and the corresponding rotation of $F^{6.44}$ (8), observed in the 5-HT_{1B} receptor and structures of other active-state GPCRs. The strengthened interactions of helix V and VI through ligand-mediated hydrogen bonds to both helices have also been linked to inhibition of G protein signaling at β_2AR by the most efficacious inverse agonist (25).

Ergolines predominantly signal through β-arrestin pathways at 5-HT_{2B} receptors, whereas signaling at 5-HT_{1B} receptors appears non-biased. The differential signaling patterns are mirrored in the crystal structures, which show features of an intermediate active-state for the 5-HT_{1B} receptor, and a β-arrestin-biased activation-state for the 5-HT_{2B} receptor. We propose a mechanism by which ERG stabilizes a conformation of the 5-HT_{2B} receptor that selectively interferes with G protein signaling (figure S9). The tripeptide moiety of ERG appears to function as a negative allosteric regulator of G protein signaling at the 5-HT_{2B} receptor, as ERG exhibits strongly increased β-arrestin signaling bias compared to LSD and MTE. Because both therapeutic (26) and adverse (27) drug effects have been associated with β-arrestin recruitment by GPCRs, identifying the features of biased GPCR states by known and yet to be discovered intracellular signaling proteins may facilitate the development of safer and more effective therapeutics with selective signaling profiles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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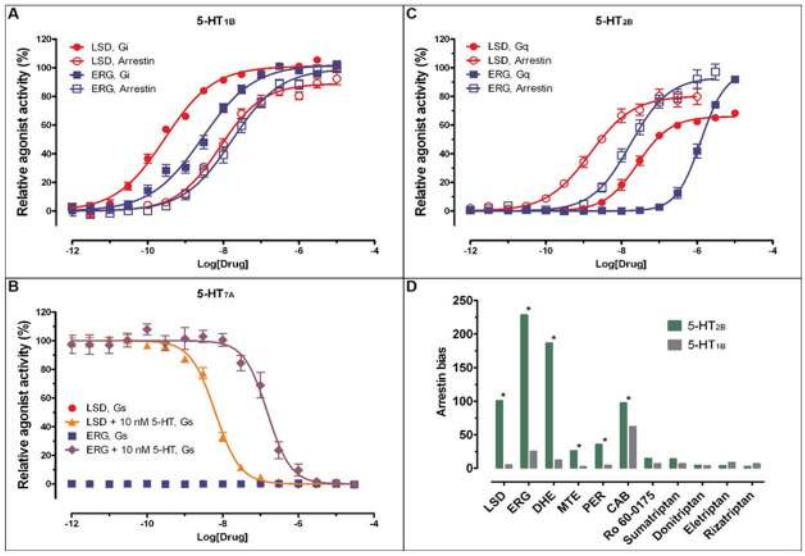

Fig. 1.

Distinct signaling properties of lysergic acid diethylamide (LSD) and ergotamine (ERG) at 5-HT_{1B}, 5-HT_{7A} and 5-HT_{2B} receptors. We used luminescence-based assays to measure 5-HT_{1B} receptor mediated G_i activation and cAMP production; fluorescence-based calcium mobilization assays to measure 5-HT_{2B} receptor mediated G_q activation and β-arrestin translocation-dependent luciferase reporter assays to measure 5-HT_{1B} and 5-HT_{2B} receptors mediated β-arrestin recruitment, all in HEK293 derived cells. (A) Normalized concentration-response studies for LSD and ERG at human cloned 5-HT_{1B} receptor-mediated activation of G_i and non-canonical (Arrestin) signaling. (B) Normalized concentration-response studies for LSD and ERG at human cloned 5-HT_{7A} receptor-mediated activation of G_s signaling in the presence and absence of 5-Hydroxytryptamine (5-HT). (C) Normalized concentration-response studies for LSD and ERG at human cloned 5-HT_{2B} receptor-mediated activation of G_q and non-canonical (Arrestin) signaling. The solid circles for LSD-G_s signals are superimposed by the solid squares for ERG-G_s signals and thus, not visible. (D) Mean β-arrestin bias factors were calculated for serotonergic agonists (dihydroergotamine (DHE), methylergonovine (MTE), pergolide (PER) and cabergoline (CAB)), at 5-HT_{2B} and 5-HT_{1B} receptors. Concentration-responses curves were fit to the Black and Leff operational model to obtain transduction coefficients [Log(τ/K_A)] for each ligand at each corresponding pathway. The ΔLog(τ/K_A) was then calculated with 5-HT as a reference agonist for each pathway, and the $\Delta\Delta$Log(τ/K_A) was calculated between two pathways for each ligand. The bias factor is unit-less and defined as $10^{\Delta\Delta Log(\tau/KA)}$ (28). Compounds with values close to one represent unbiased agonists while compounds with large numerical values, typically >100, represent extremely biased agonists. *$p<0.0001$ via two-way ANOVA comparing 5-HT_{2B} vs 5-HT_{1B} bias factors; N=3–6 separate experiments. ERG, DHE and, to a lesser extent, LSD, MTE and PER show strong β-arrestin bias at 5-HT_{2B} receptor, but not 5-HT_{1B} receptor.

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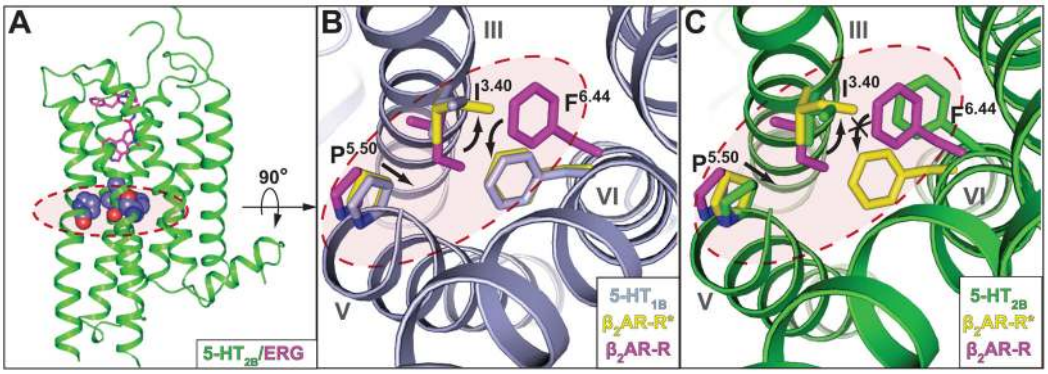

Fig. 2.

Trigger motif P-I-F displays active- and intermediate active-state for 5-HT_{1B} and 5-HT_{2B} receptors, respectively. Residues of the P-I-F motif are highlighted in a dashed red circle. (A) Overall architecture of the 5-HT_{2B} receptor (green) bound to ERG (magenta); residues of the $P^{5.50}$ $I^{3.40}$ $F^{6.44}$ motif are illustrated in space-filling representation. (B) Alignment between 5-HT_{1B} receptor (grey), β_2AR-R (magenta; PDB ID: 2RH1) and β_2AR-R*(yellow; PDB ID: 3SN6) indicates an activated P-I-F motif in the 5-HT_{1B}/ERG structure. Black arrows indicate likely rearrangements upon 5-HT_{1B} receptor activation according to analysis of β_2AR. (C) Alignment between 5-HT_{2B} receptor (green), β_2AR-R (magenta) and β_2AR-R* (yellow) suggests intermediate active-state of the P-I-F motif in the 5-HT_{2B}/ERG structure, with $F^{6.44}$ in an inactive conformation. Black arrows indicate likely rearrangements upon 5-HT_{2B} receptor activation according to analysis of β_2AR.

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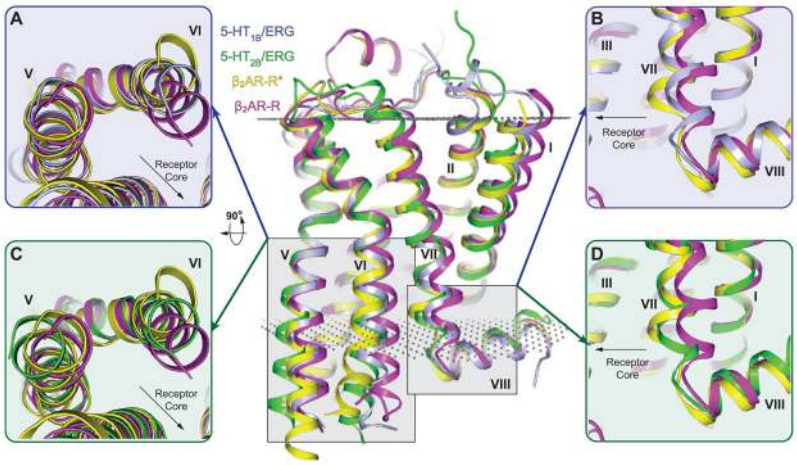

Fig. 3.

Structural alignment with β_2AR-R and β_2AR-R* reveals distinct active-state 7TM conformations of the 5-HT_{1B} and 5-HT_{2B} receptor structures. All structures were analyzed based on the last membrane embedded residue to minimize the effect of G proteins and fusion partners on the relative helix positions (see supplementary section). Center panel shows overall 7TM configuration of β_2AR-R (magenta; PDB ID: 2RH1), β_2AR-R*(yellow; PDB ID: 3SN6), 5-HT_{1B} (grey) and 5-HT_{2B} (green) receptors aligned through helices I–IV. Membrane boundaries are indicated by grey dots according to the OPM database (29). (A, C) Intracellular view of helix V and helix VI in β_2AR-R, β_2AR-R* compared to (A) the 5-HT_{1B}, or (C) the 5-HT_{2B} receptor. Residues on the intracellular side of the membrane have been removed for better comparison of ligand-induced helical rearrangements (see main text). Helix VI of the 5-HT_{1B} and 5-HT_{2B} receptors is in an intermediate active-state compared to β_2AR. (B, D) Side view of helix VII and helix VIII in β_2AR-R, β_2AR-R* and (B) 5-HT_{1B} receptor, or (D) 5-HT_{2B} receptor. Helix VII of the 5-HT_{1B} and 5-HT_{2B} receptors is in an intermediate active-state compared to β_2AR, with more pronounced activation features for the 5-HT_{2B} receptor.

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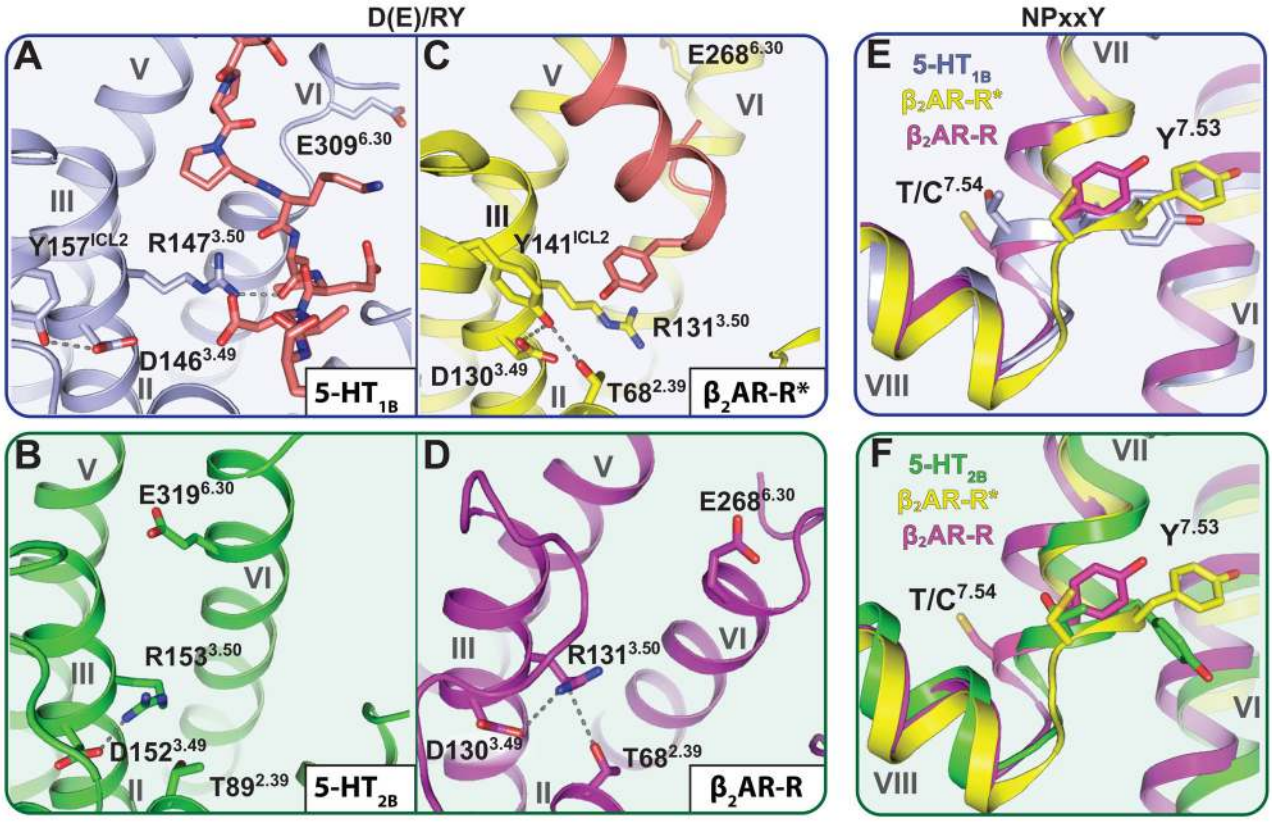

Fig. 4.

Activation state of the D(E)/RY and NPxxY motifs in 5-HT_{1B} and 5-HT_{2B} receptors compared to β_2AR-R and β_2AR-R*. Configuration of the D(E)/RY motif in (A) the 5-HT_{1B} receptor (grey), (B) the 5-HT_{2B} receptor (green), (C) β_2AR-R* (yellow; PDB ID: 3SN6) and (D) β_2AR-R (magenta; PDB ID: 2RH1). Residues of the G protein in β_2AR-R* (C) and G protein mimicking BRIL loop (A) are highlighted in orange. The conformation of the D(E)/RY motif in the 5-HT_{1B} receptor is similar to that observed in β_2AR-R*, whereas the configuration of the 5-HT_{2B} receptor compares to that of β_2AR-R. (E, F) Conformational states of $Y^{7.53}$ of the NPxxY motif and the proceeding residue 7.54 in β_2AR-R, β_2AR-R* and (E) the 5-HT_{1B}, or (F) 5-HT_{2B} receptor. When compared to β_2AR, the conformation of the NPxxY motif in the 5-HT_{1B} receptor is in an intermediate active-state, whereas the configuration of the 5-HT_{2B} receptor is similar to β_2AR-R*.

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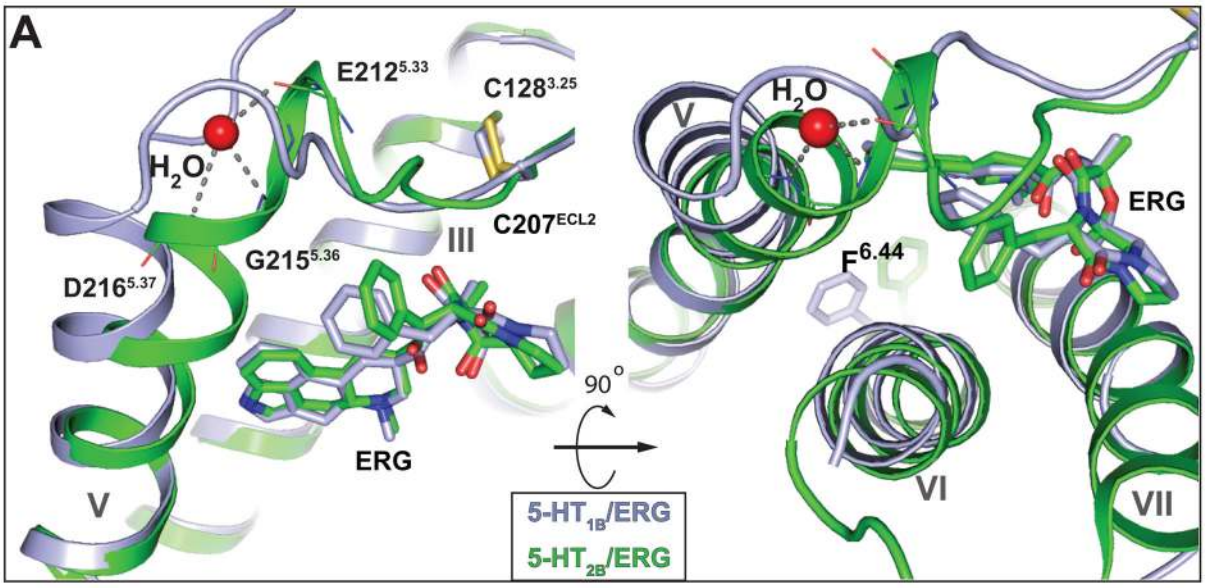

Fig. 5.

Structural differences in extracellular configuration of helix V between 5-HT_{1B} and 5-HT_{2B} receptors likely explain β-arrestin functional selectivity at the 5-HT_{2B} receptor. Side view (left) and top view (right) of 5-HT_{1B} (grey) and 5-HT_{2B} (green) receptors shows a kink in the extracellular end of helix V in the 5-HT_{2B} receptor. A water molecule (red sphere) was found to stabilize the kink through hydrogen bonds (grey dashed lines) with the $E212^{5.33}$ main-chain carbonyl oxygen and the main-chain nitrogens of $D216^{5.37}$ and $G215^{5.36}$. Main-chain atoms of both residues are shown as lines.