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Structural Basis for Molecular Recognition at Serotonin Receptors

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Abstract

Serotonin or 5-hydroxytryptamine (5-HT) regulates a wide spectrum of human physiology through the 5-HT receptor family. We report the crystal structures of the human 5-HT_{1B} G protein-coupled receptor bound to the agonist anti-migraine medications ergotamine and dihydroergotamine. The structures reveal similar binding modes for these ligands, which occupy the orthosteric pocket and an extended binding pocket close to the extracellular loops. The orthosteric pocket is formed by residues conserved in the 5-HT receptor family, clarifying the family-wide agonist activity of 5-HT. Compared to the accompanying structure of the 5-HT_{2B} receptor, the 5-HT_{1B} receptor displays a 3 angstrom outward shift at the extracellular end of helix V, resulting in a more open extended pocket that explains subtype selectivity. Together with docking and mutagenesis studies, these structures provide a comprehensive structural basis for understanding receptor-ligand interactions and designing subtype-selective serotonergic drugs.

The neuromodulator serotonin (5-hydroxytryptamine; 5-HT) is essential for diverse functions at nearly every organ system in the human body (1–4). The activity of 5-HT is mediated through activation of members of a large family of 5-HT receptor proteins, which can be grouped into seven subfamilies (5-HT_{1-7}) on the basis of sequence homology and signaling mechanisms (5). Except for the 5-HT_3 receptor, which is a ligand-gated ion

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channel, the other twelve members are heterotrimeric guanine nucleotide binding protein (G protein) coupled receptors (GPCRs). The serotonergic system is a target of many widely prescribed drugs including atypical anti-psychotics, anti-migraine medications, anxiolytics and anti-depressants (1), and the recently approved anti-obesity medication lorcaserin (6, 7). However, clinical use of several serotonergic drugs caused unexpected side effects arising from off-target interactions with 5-HT receptor subtypes and related receptors for biogenic amine (1, 4, 8, 9).

The 5-HT_{1B} receptor couples to G protein alpha subunits G_i or G_o and is widely expressed in the brain and the cardiovascular system. In the CNS, the 5-HT_{1B} receptor functions as an inhibitory presynaptic receptor to modulate the release of 5-HT and many other neurotransmitters (1, 2). The 5-HT_{1B} receptor is a primary molecular target for the anti-migraine drugs ergotamine (ERG) and dihydroergotamine (DHE), which are efficacious 5-HT_{1B} receptor agonists (10). Off-target activation of the related 5-HT_{2B} receptor is responsible for the valvulopathic activity of many approved drugs and is the main reason for their withdrawal (9–12). We report two crystal structures of the human 5-HT_{1B} receptor bound to the full agonists, ERG and DHE (tables S1 and S2). Comparison with the accompanying structure of the human 5-HT_{2B} receptor bound to ERG (13) reveals critical structural determinants for ligand recognition and subtype selectivity, and provides a structural rationale for designing safer and better serotonergic drugs.

Crystallization studies of the 5-HT_{1B} receptor were done with engineered constructs, 5-HT_{1B}-1 and 5-HT_{1B}-2 (14), which crystallized with ERG and DHE at resolutions of 2.7 Å and 2.8 Å, respectively. Due to the high similarity between these two structures (figure S2), for brevity we focus on the structure of the 5-HT_{1B}-1/ERG complex for analysis and discussion of key structural features for ligand recognition and selectivity in 5-HT_{1B} versus 5-HT_{2B} receptors.

The main fold of the 5-HT_{1B} receptor consists of a canonical seven-transmembrane (7TM) α-helical bundle (Fig. 1A). The extracellular loop 2 (ECL2) that partially covers the ligand binding pocket is stabilized by a $C122^{3.25}$-$C199^{ECL2}$ disulfide bond, highly conserved in GPCRs. Part of the N terminus folds on top of the binding pocket where Y40 forms hydrogen-bond interactions with ligand binding residue $D352^{7.36}$ (figure S5) (15, 16). This feature suggests that the N terminus could have a role in ligand recognition in the 5-HT_{1B} receptor by interacting with residues within the binding pocket.

The 5-HT_{1B}/ERG complex structure revealed a large ligand binding cavity defined by residues from helices III, V, VI, VII and ECL2, comprising an orthosteric pocket embedded deep in the 7TM core and an extended binding pocket close to the extracellular entrance (Fig. 1). ERG adopts a binding mode with the ergoline ring system occupying the orthosteric binding pocket and the cyclic tripeptide moiety bound to the upper extended binding pocket (Fig. 2C). In the orthosteric pocket, the ergoline scaffold is anchored through the salt bridge interaction between its positively charged nitrogen and the carboxylate of $D129^{3.32}$ which is fully conserved in 5HT and other monoamine receptors. The side chain of $D129^{3.32}$ is further stabilized by a hydrogen bond to the hydroxyl of $Y359^{7.43}$. Side chains of $C133^{3.36}$, $I130^{3.33}$, $W327^{6.48}$, $F330^{6.51}$ and $F331^{6.52}$ form a narrow hydrophobic cleft, which packs tightly against the nearly planar ergoline ring system. In addition, the indole N-H hydrogen forms a hydrogen bond with $T134^{3.37}$ (Fig. 2A). Comparison with the ERG bound 5-HT_{2B} receptor structure revealed that the orthosteric binding pockets in two receptors are very similar, with the key interactions conserved (Fig. 2, A, D and E). The only difference is observed in the region where residues from helix V contact ERG: due to the lack of a side chain at $G221^{5.42}$, the side chain of $F217^{5.38}$ in the 5-HT_{2B} receptor reaches into the ligand binding pocket and packs on top of the ERG indole ring; by comparison, the corresponding

interaction in the 5-HT_{1B} receptor occurs between the side chain of $S212^{5.42}$ and ERG, while $Y208^{5.38}$ does not interact with the ligand due to the outward shift of helix V (Fig. 2, A and D). Significant differences are observed in the extended binding pockets: the extended binding pocket of the 5-HT_{1B} receptor is broader than that of the 5-HT_{2B} receptor (Fig. 1, B, C and D) due to the 3.0 Å outward shift of the top of helix V. Moreover, in contrast to the relatively bulky $M218^{5.39}$ of the 5-HT_{2B} receptor, the corresponding residue of the 5-HT_{1B} receptor is a smaller threonine, which results in a further expansion of this pocket (Fig. 2B). Despite these differences in contact residues between the two subtypes, the cyclic tripeptide moiety of ERG maintains a similar overall orientation relative to the ergoline moiety, likely stabilized by an intramolecular hydrogen bond (Fig. 2, D and E). The conformations of the phenyl group of the ERG cyclic tripeptide do differ: it contacts with $L347^{6.58}$ and $V348^{6.59}$ in the 5-HT_{2B} receptor, whereas in the 5-HT_{1B} receptor, it rotates to occupy a cavity close to $T209^{5.39}$ at helix V. Correspondingly, $M337^{6.58}$ turns into the pocket to contact with the phenyl ring of the ligand in the 5-HT_{1B} receptor. As shown in the accompanying paper, these differences in ERG interactions correlate with different functional states: at the 5-HT_{1B} receptor ERG causes full activation; whereas ERG induces intermediate G protein activation and β-arrestin biased signaling at the 5-HT_{2B} receptor (13).

Mutations of several residues in the orthosteric binding site of the 5-HT_{1B} receptor, including the conserved $D129^{3.32}$, abolished the binding of the radioligand lysergic acid diethylamide (LSD) (table S4). The prototypical hallucinogen LSD has the same ergoline moiety as ERG, and these interactions within the orthosteric pocket appear to be the main driving force for binding of ergolines. In contrast, none of the tested mutations of the extended binding pocket residues drastically reduced the binding affinity of ERG, suggesting that the cyclic tripeptide moiety of ERG is accommodated without contributing substantially to the binding affinity. This extended binding site partially overlaps with the sites inferred in interaction with allosteric modulators in the M_2 muscarinic acetylcholine receptor (17, 18) suggesting its potential role in mediating allosteric modulations at 5-HT receptors (13).

Alignment of all human 5-HT GPCRs sequences shows that the residues in the orthosteric binding pockets are much more conserved than those in the extended binding pockets (figure S7). This likely reflects an evolutionary pressure to maintain the structure of the orthosteric binding pocket for recognition of the endogenous ligand 5-HT (19). Molecular docking of 5-HT into the orthosteric binding pocket of the 5-HT_{1B} and 5-HT_{2B} receptors revealed important residues involved in the recognition of 5-HT (Fig. 3A), many of which have been previously implicated by site-directed mutagenesis and molecular modeling studies (20, 21). 5-HT shares a common chemical scaffold with ergolines (figure S4) and recognizes the 5-HT receptors in a similar manner as ERG and DHE. $D^{3.32}$ forms a salt bridge with the positively charged amino group of 5-HT, while $T^{3.37}$, which is highly conserved in 5-HT receptors and most other aminergic GPCRs, forms a hydrogen bond with the indole N-H hydrogen. This hydrogen bond appears to be important for the recognition of 5-HT by 5-HT_{1B} and 5-HT_{2B} receptors because mutating $T^{3.37}$ to alanine reduces the affinity of 5-HT at both receptors by more than 10 fold (table S4). The indole ring points towards residues on helix V: $S212^{5.42}$ and $A216^{5.46}$ at the 5-HT_{1B} receptor; $G221^{5.42}$ and $A225^{5.46}$ at the 5-HT_{2B} receptor. This ligand-receptor interface is less polar in the 5-HT receptor family compared with those of other biogenic amine receptors (table S5), thereby perfectly matching the property of the 5-HT indole head group, which is less polar than those of other aminergic receptor native agonists, such as epinephrine, dopamine and histamine.

LSD promiscuously binds to 5-HT receptors: it has agonist activity at most 5-HT receptors (table S6), whereas it is a potent antagonist at 5-HT_{7A} receptor (13). Docking of LSD into

the 5-HT_{1B} and 5-HT_{2B} receptor structures suggests that binding of the LSD ergoline moiety in the orthosteric binding pocket is essentially identical to that of the ERG ergoline moiety (Fig. 3B). Many single point mutations within the orthosteric binding pocket reduce or abolish the binding of LSD at both 5-HT_{1B} and 5-HT_{2B} receptors (table S4). The conservation of the orthosteric binding pocket provides an atomic-level explanation for the observed promiscuous interactions between drugs like LSD and 5-HT receptors.

Triptans are 5-HT analogs that are among the most frequently prescribed anti-migraine medications which act primarily through 5-HT_{1B} and 5-HT_{1D} receptors (1, 2, 22). A common structural feature of triptans is the large substitution group at the 5′ position of the indole ring. Functional assays indicate that triptans act as agonists of the 5-HT_{1B} receptor, but not the 5-HT_{2B} receptor (table S7). To address the structural determinants of this selectivity, we performed docking simulations at both 5-HT_{1B} and 5-HT_{2B} receptors (figure S8). Whereas triptans were well accommodated in the 5-HT_{1B} receptor binding pocket, the narrower extended binding pocket in the helix V region of the 5-HT_{2B} receptor forced the ligands to adopt unfavorable positions (Fig. 3C and figure S8C). Among the tested triptans, donitriptan and eletriptan showed relatively higher potency in G_q –mediated signaling at the 5-HT_{2B} receptor (table S7). These two triptans have longer and more flexible linkers between 5′ substituents and the indole ring, enabling them to fit better in the narrow binding pocket of the 5-HT_{2B} receptor. The $M218^{5.39}$A mutation of the 5-HT_{2B} receptor, which increases the space in the binding pocket, significantly enhanced the potency of donitriptan and eletriptan (figure S9), although their potency was still lower than that at the 5-HT_{1B} receptor. Thus, the broader opening near the extracellular end of helix V in the 5-HT_{1B} receptor appears to be important for selectively accommodating the 5′ substituents of triptan ligands, whereas the narrower pocket in the 5-HT_{2B} receptor shows reduced binding to these compounds.

Norfenfluramine is the active metabolite of fenfluramine, which is one of the two components of the infamous ‘Fen-Phen’ anti-obesity cocktail. The anti-obesity effect of norfenfluramine occurs mainly through activation of the 5-HT_{2C} receptor (1), but due to its high potency and efficacy as a 5-HT_{2B} receptor agonist, it can cause life-threatening side effects including pulmonary hypertension and heart valve disease (9, 23–25). To elucidate the structural basis for norfenfluramine’s subtype selectivity, we simulated its binding to the 5-HT_{1B} and 5-HT_{2B} receptor structures (Fig. 3D). Norfenfluramine tightly fit the orthosteric binding pocket of the 5-HT_{2B} receptor with $F217^{5.38}$ and $M218^{5.39}$ forming a hydrophobic cap that interacted with the trifluoromethyl group. The $F217^{5.38}$A mutation (table S8) and the $M218^{5.39}$V mutation (26), both reduce the potency of norfenfluramine compared to that for the wild type 5-HT_{2B} receptor. In the docking model of the 5-HT_{1B} receptor, these close contacts are missing, thus the potency of norfenfluramine is reduced compared to that at the 5-HT_{2B} receptor (table S8).

Species-specific differences in the ligand binding properties of 5-HT_{1B} and other 5-HT receptors from rodents and humans have impeded the extrapolation of findings from animal model to humans (27, 28). The rodent 5-HT_{1B} receptors, for instance, have a much higher binding affinity than the human 5-HT_{1B} receptor for certain adrenergic compounds caused by a difference at position 7.39 (N351 for rat and mouse, T355 for human) (27, 29). Modeling of the T355N mutation into the binding pocket of human 5-HT_{1B} receptor revealed that $N355^{7.39}$, together with $D129^{3.32}$ and $Y359^{7.43}$, form a polar interaction network that anchors the propanolamine moiety of adrenergic antagonists, propranolol and cyanopindolol, thereby mimicking the recognition modes observed in both the β_1 and β_2 adrenergic receptors (Fig. 4). Hydrophobic interactions of $I130^{3.33}$, $W327^{6.48}$, $F330^{6.51}$ and $F331^{6.52}$ with the aromatic rings of propranolol and cyanopindolol are largely conserved between 5-HT_{1B} and β-adrenergic receptors (30, 31), allowing high affinity binding of these

antagonists in the human 5-HT_{1B} $T355^{7.39}N$ and rodent 5-HT_{1B} receptors. These findings thereby provide a structural explanation for the pharmacological differences between these rodent and human GPCRs.

In conclusion, comparative analysis of the 5-HT_{1B} and 5-HT_{2B} receptor structures and functions, together with specific mutagenesis studies, provided a comprehensive framework for understanding ligand promiscuity and selectivity, thereby aiding development of safer and more effective medications that target the GPCR superfamily.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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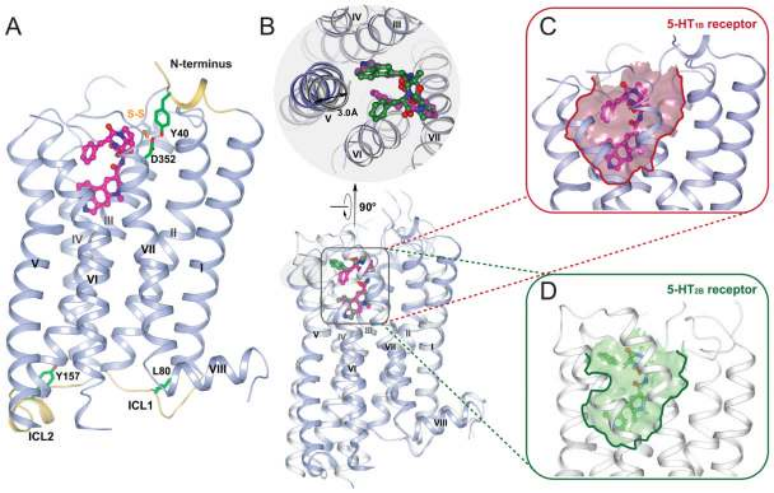

Fig. 1.

Overall architecture of the 5-HT_{1B} receptor bound to ERG and comparison of the ligand binding pocket shapes of the 5-HT_{1B} receptor and the 5-HT_{2B} receptor. (A) The 5-HT_{1B} receptor is shown as a light blue colored ribbon cartoon, with N terminus, ICL1 and ICL2 highlighted in yellow. Ligand ERG is colored magenta. The disulfide bond between C122 and C199 is shown as orange sticks. The Y40 and $D352^{7.36}$ side chains, which mediate the interaction between the N terminus and the ligand binding pocket, are shown as green sticks with a dashed line indicating the hydrogen bond interaction. $L80^{ICL1}$ and $Y157^{ICL2}$ interact with residues of the 7TM bundle stabilizing the local structures of ICL1 and ICL2. (B) Shown at the bottom is the superposition of the 5-HT_{1B}/ERG structure (light blue) and the 5-HT_{2B}/ERG structure (white). The ligands are colored magenta for the 5-HT_{1B} receptor and green for the 5-HT_{2B} receptor. The top panel shows an extracellular view of the ligand binding sites. The arrow indicates a 3.0 Å shift (distance measured between the α-carbons of $T209^{5.39}$ in the 5-HT_{1B} receptor and $M218^{5.39}$ in the 5-HT_{2B} receptor) at the extracellular end of helix V. (C) and (D) The surface representation of the ligand binding pockets of the 5-HT_{1B} receptor and the 5-HT_{2B} receptor are shown in transparent pink and transparent green, respectively.

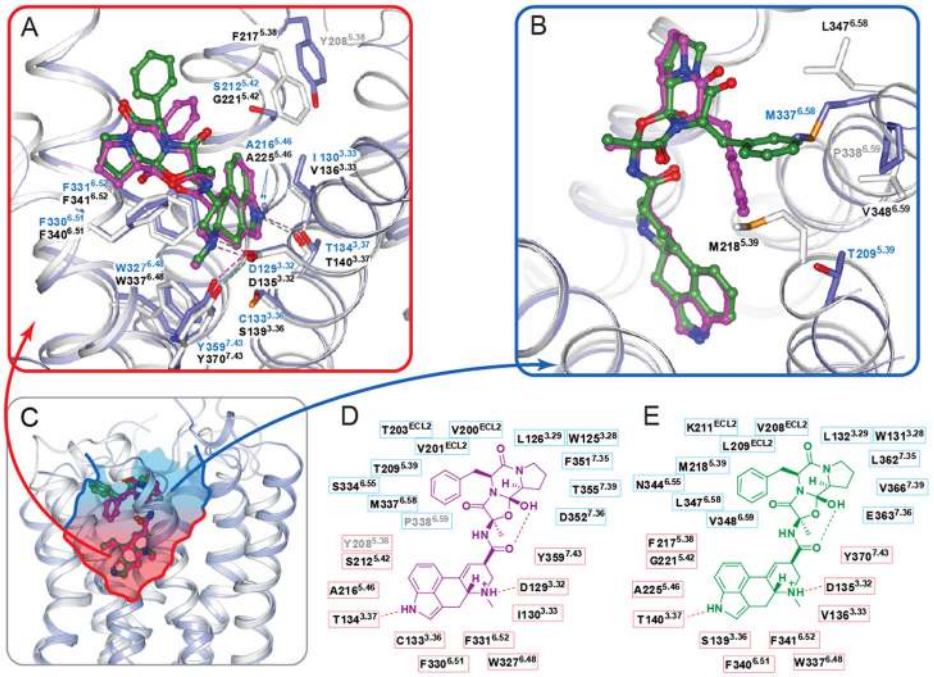

Fig. 2.

Comparison of ligand-receptor interactions in 5-HT_{1B}/ERG and 5-HT_{2B}/ERG structures. (A), (B) and (C) Superposition of the ligand binding pockets of 5-HT_{1B} receptor (light blue) and 5-HT_{2B} receptor (white). The carbons of ligand ERG in the 5-HT_{1B} and the 5-HT_{2B} receptor structures are shown as magenta and green, respectively. (A) Residues forming the orthosteric binding sites are shown as sticks and labeled in blue for 5-HT_{1B} receptor and black for 5-HT_{2B} receptor. The salt bridge interactions between $D^{3.32}$ and ERG, as well as the hydrogen bond interactions between $T^{3.37}$ and ERG, $Y^{7.43}$ and $D^{3.32}$, are shown as red dashed lines for 5-HT_{1B} receptor and green dashed lines for 5-HT_{2B} receptor. (B) Substantial differences in the extended ligand binding pockets resulted in different conformations of the ligand ERG. (C) The overall binding pockets with the orthosteric and the extended ligand binding sites shaded red and blue, respectively. (D) and (E) Diagram representation of ligand interactions in the binding pockets of 5-HT_{1B} and 5-HT_{2B} receptors, respectively. Intramolecular hydrogen bonds within the cyclic tripeptide moiety are indicated as dashed lines. Residues in the orthosteric binding pockets are shown in red boxes, while extended binding pocket residues are shown in blue boxes. The hydrogen bond interaction between $T^{3.37}$ and ERG and the salt bridge interactions between $D^{3.32}$ and ERG are indicated by red dashed lines. In (A), (B) and (D), $Y208^{5.38}$ and $P338^{6.59}$ which do not interact with ERG are labeled in grey.

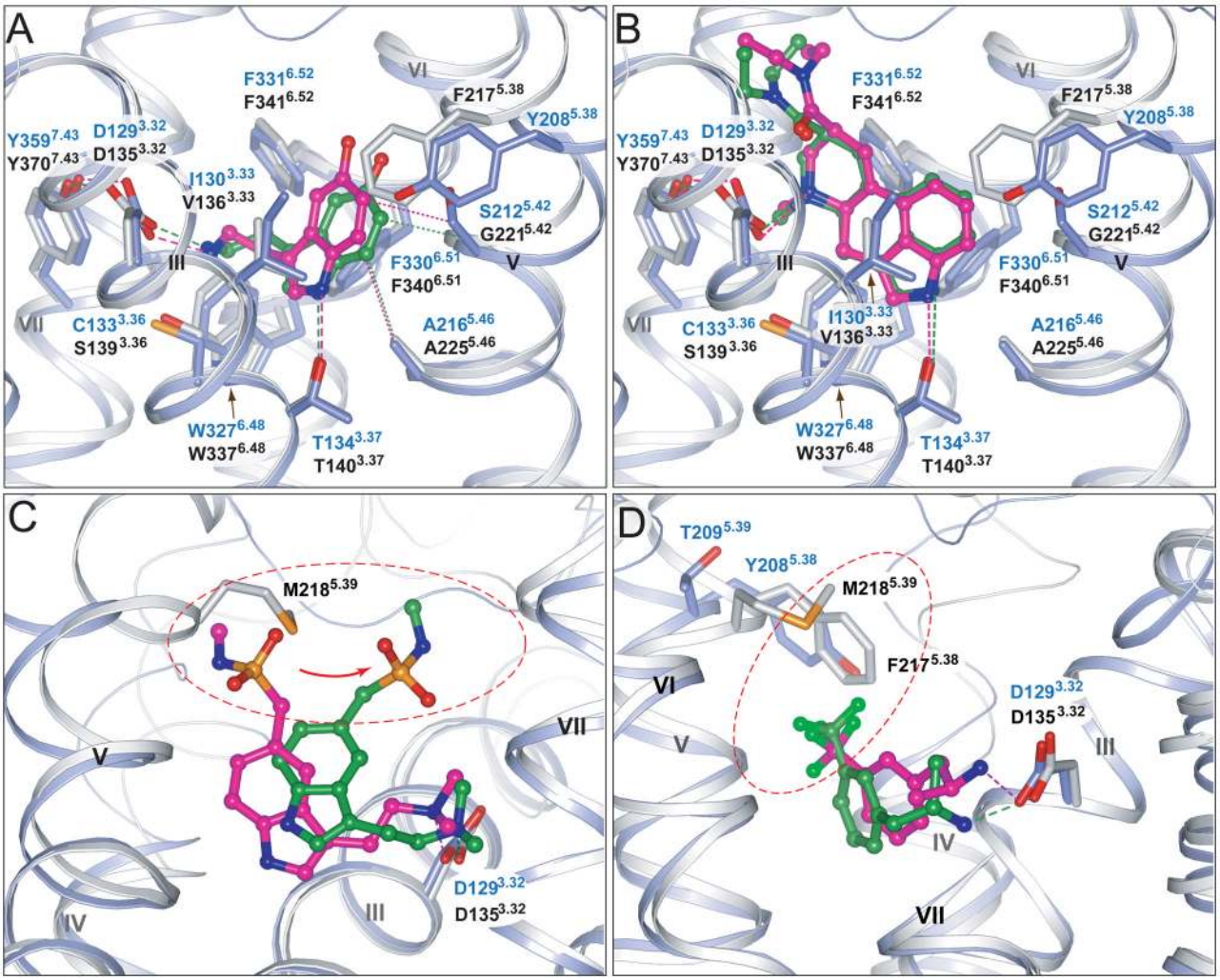

Fig. 3.

Docking of the promiscuous (5-HT and LSD) and selective (sumatriptan and norfenfluoramine) ligands into the binding pockets of the 5-HT_{1B} and 5-HT_{2B} receptor structures. Docking of 5-HT (A), LSD (B), sumatriptan (C) and norfenfluramine (D) into the orthosteric binding pockets of 5-HT_{1B} (receptor colored light blue; ligands colored magenta) and 5-HT_{2B} (receptor colored white; ligands colored green) receptors. In (A) and (B), the polar interactions between $Y^{7.43}$ and $D^{3.32}$, and between $D^{3.32}$, $T^{3.37}$ with the ligands are shown as dashed lines. In (A), the non-polar interactions between the indole ring of 5-HT and residues at positions 5.42 and 5.46 are shown as dotted lines. In (C), steric hindrance from $M218^{5.39}$ forced a reorientation of the sulfonamide group and a shift of the indole core structure of sumatriptan when docked into 5-HT_{2B} receptor. In (D), in the 5-HT_{2B} receptor, $F217^{5.38}$ and $M218^{5.39}$ form closed contacts with the trifluoromethyl group of the ligand, which are absent in 5-HT_{1B} receptor.

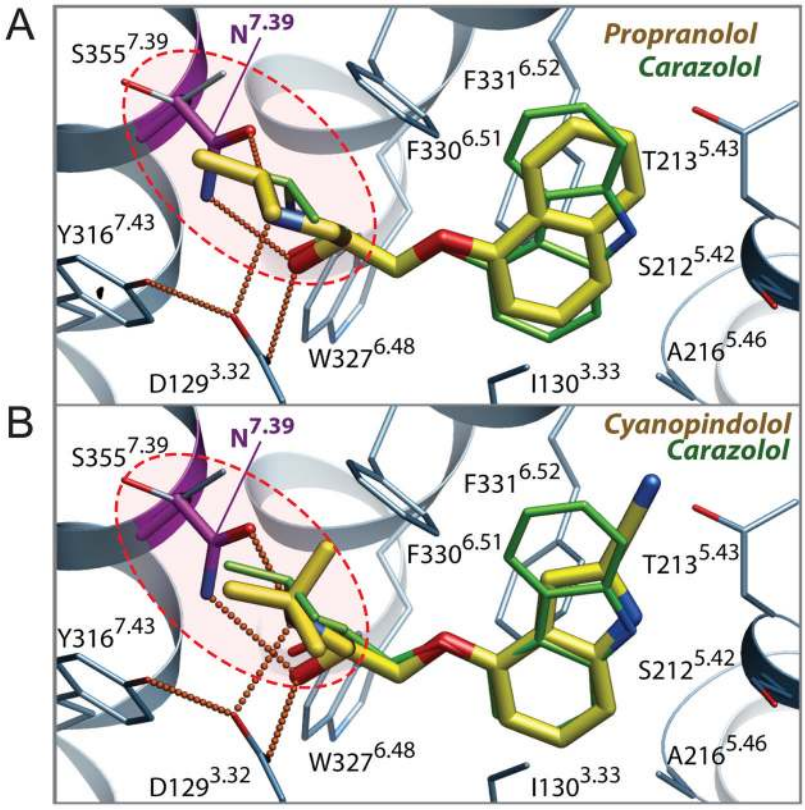

Fig. 4.

Structural basis for differences in the pharmacological properties between human and rodent 5-HT_{1B} receptors. The high affinity β-adrenergic antagonists propranolol (A) and cyanopindolol (B), both shown in yellow colored carbons, are docked in the model based on the human 5-HT_{1B}/ERG structure with $T^{7.39}$ mutated to N, as found in 5-HT_{1B} rat and mouse orthologs. The $N^{7.39}$ side chain (magenta carbons) remained flexible in the docking procedure. The hydrogen bond network involving $N^{7.39}$, $D^{3.32}$ and $Y^{7.43}$ and propanolamine moieties of the ligands is shown as orange dots. Carazolol (green carbons) from the superimposed β_2-adrenergic receptor structure (PDB ID: 2RH1) is shown for comparison.