Structure of the dopamine D2 receptor in complex with the antipsychotic drug spiperone

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Structure of the dopamine D₂ receptor in complex with the antipsychotic drug spiperone

In addition to the serotonin 5-HT₂A receptor (5-HT₂AR), the dopamine D₂ receptor (D₂R) is a key therapeutic target of antipsychotics for the treatment of schizophrenia. The inactive state structures of D₂R have been described in complex with the inverse agonists risperidone (D₂Rris) and haloperidol (D₂Rhal). Here we describe the structure of human D₂R in complex with spiperone (D₂Rspi). In D₂Rspi, the conformation of the extracellular loop (ECL) 2, which composes the ligand-binding pocket, was substantially different from those in D₂Rris and D₂Rhal, demonstrating that ECL2 in D₂R is highly dynamic. Moreover, D₂Rspi exhibited an extended binding pocket to accommodate spiperone’s phenyl ring, which probably contributes to the selectivity of spiperone to D₂R and 5-HT₂AR. Together with D₂Rris and D₂Rhal, the structural information of D₂Rspi should be of value for designing novel antipsychotics with improved safety and efficacy.
Dopamine is a neurotransmitter that controls numerous physiologic functions in the brain and peripheral nervous system via dopamine receptors of the G-protein-coupled receptor (GPCR) superfamily. In humans, five dopamine receptors (D₁–D₅) have been identified and have been classified according to their sequence, intracellular signaling, pharmacology, and localization as D₁-class receptors (D₁R and D₅R) or D₂-class receptors (D₂R, D₃R, and D₄R)1–3. Thus, D₂R is similar to both D₁R and D₅R, with 80 and 54% sequence identities, respectively, in their transmembrane helices4–6. D₂R is highly distributed in the striatum, nucleus accumbens, and olfactory tubercle7,8, and it plays important pharmacologic roles in numerous human disorders related to dopaminergic dysfunction, including schizophrenia9–11 and Parkinson’s disease12–13.

D₂R antagonists have been developed as antipsychotics to block dopaminergic transmission for the treatment of schizophrenia14. Antipsychotics are either typical or atypical; typical antipsychotics generally antagonize D₂R, whereas atypical antipsychotics antagonize both D₂R and the serotonin 5-HT₂A receptor (5-HT₂AR). Both of these groups at least improve the positive symptoms of schizophrenia15. However, they are also associated with a wide range of severe side effects, such as extrapyramidal symptoms, weight gain, metabolic disorders, and constipation16,17. Extrapyramidal symptoms are caused by excessive inhibition of D₂R in the nigrostriatal pathway. Other side effects are primarily due to the undesired binding of antipsychotics to other aminergic receptors, such as the serotonin 5-HT₂C receptor (5-HT₂CR), which exhibits 46% sequence identity with the D₂R. Antipsychotics with improved receptor selectivity provide valuable information for the rational design of antipsychotics with improved receptor selectivity.

Overall structure of D₂Rspi. In this study, we describe the structure of D₂R in complex with spiperone (D₂Rspi), a butyrophenone antipsychotic23. D₂R, D₃R, and D₄R are practically identical, as shown by the RMSD values of Ca atoms between them (Supplementary Table 1). A G-protein-bound active conformation of D₂R also was reported in complex with an agonist, brotropine (D₂RbRIL)24. Interestingly, the conformation of the extracellular loop (ECL) 2 in D₂RbRIL and D₂Rhal is entirely different from those of D₃Rreti, D₄Rnem, and D₂Rspi (Supplementary Fig. 1). Additionally, while the conformation of ECL1 is relatively conserved among the structures of D₂-class receptors, Trp100I3.41 on ECL1 of D₂Rspi is uniquely directed to the binding pocket (Supplementary Fig. 1).

In this study, we describe the structure of D₂R in complex with spiperone (D₂Rspi), a butyrophenone typical antipsychotic that binds with high affinity to D₂R, D₃R, D₄R, and 5-HT₂A25. We also present a structural comparison of D₂Rspi with other D₂R structures, D₂Rreti and D₂Rnem, in addition to 5-HT₂AR complex with risperidone (5-HT₂ARi26) and 5-HT₂CR with ritanserin (5-HT₂CRi27). The structure of D₂Rspi given herein provides valuable information for the rational design of antipsychotics with improved receptor selectivity.

Results

Overall structure of D₂Rspi. Because wild-type D₂R is not expressed in Spodoptera frugiperda (Sf9) insect cells, we prepared a stable construct for crystallization trials. D₂R was stabilized by the truncation of 34 N-terminal residues and the replacement of the intracellular loop (ICL) 3 with the thermostabilized apocytochrome b562RIL28 (D₂R-bRIL). D₂R-bRIL was further stabilized by the mutations S121K3.39 and L123W3.41 (here, superscripts indicate residue numbers according to the Ballesteros–Weinstein scheme29 and the replacement of bRIL with mbIIG, the loop-modified cytochrome bs IIG30 (D₂R-mbIIG S121K3.39/L123W3.41, see Methods). The use of mbIIG instead of bRIL was essential to obtain D₂R crystals. S121K3.39 is a mutation of the allosteric sodium binding site of class A GPCRs that mimics the presence of the sodium ion, therefore stabilizing the inactive state30,31. The S121K3.39, L123W3.41, and S121K3.39/L123W3.41 mutants, in addition to the stabilized construct, showed similar affinities for spiperone to the wild-type human D₂R (Supplementary Table 2 and Supplementary Fig. 2), suggesting that these mutations did not substantially affect the binding of spiperone. Additionally, the L123W3.41 mutant showed similar agonist activity to that of the wild-type human D₂R by a transforming growth factor alpha (TGFα) shedding assay32, which measured the agonist activities of wild-type and mutant D₂R for spiperone against a fixed concentration of dopamine (Supplementary Table 3 and Supplementary Fig. 3). However, because the S121K3.39 mutation stabilizes the inactive state of D₂R, the agonist activities of the mutants with S121K3.39 could not be determined (Supplementary Table 3 and Supplementary Fig. 3). Etioclode and sulpiride enhance the affinity for D₂R in the presence of the sodium ion, whereas spiperone does not33. This enhancement is ascribed to an interaction network from the bound sodium ion in the allosteric binding site34. The S121K3.39 mutation decreased the affinity for etioclode (Supplementary Table 2), suggesting that the side chain of Lys1213.39 does not sufficiently mimic the sodium ion in the allosteric binding site for the binding of etioclode.

For crystallization, we generated an antibody recognizing the D₂R structure (IgG3089) and prepared a novel Fab fragment (Fab3089) of IgG3089. Using the stabilized construct and Fab3089, we successfully obtained crystals of D₂R (Supplementary Fig. 4a). The structure of D₂R was determined in complex with spiperone at 3.1 Å resolution using an X-ray free-electron laser (Fig. 1, Table 1, and Supplementary Fig. 4). D₂Rspi bound to Fab3089 at the extracellular region (Fig. 1a and Supplementary Fig. 4c), exhibiting a canonical GPCR fold with seven transmembrane helices (TM1–7) and an intracellular amphipathic helix 8 (H8) (Fig. 1). The D₂Rspi structure demonstrated the inactive conformation because the seven helical bundle structure and the conformations of the four activation microswitches are more similar to those of the inactive state conformation in D₂Rrel than those of the active state conformation in D₂Rhal (Supplementary Fig. 5 and Supplementary Table 1). We also compared the PIF motif of D₂Rspi with those of the inactive state and the active state structures of the β₂-adrenergic receptor to confirm D₂Rspi demonstrated the inactive conformation, because D₂Rhal contains the I122A3.40 mutation in the motif.

There were no secondary structures in the ECLs and ICL1; ICL2 in D₂Rspi was disordered. Spiperone was bound to the orthosteric binding site (Fig. 1 and Supplementary Fig. 4d). Like other class A GPCRs, the ligand-binding pocket was covered by the C-terminal segment of ECL2, which is stabilized by a disulfide bond between Cys1073.25 and Cys18245.50 on ECL2 and Cys18245.50 on ECL3 (Fig. 1).

Binding mode of spiperone. In D₂Rhal, spiperone was surrounded by residues from TM2, 3, 5, 6, and 7 and ECL2 (Figs. 1 and 2a, b). The binding mode of spiperone was consistent with the results of the TGFα shedding assay (Supplementary Table 3) and of the ligand-binding assay for spiperone using the mutants35–38.

The tertiary amine in the triazaspiro ring formed a salt bridge with Asp1143.32; this is strictly conserved in aminergic receptor structures (Fig. 2a, b). In D₂R, the mutant of Asp1143.32 loses its affinities to both agonists and antagonists22,36. This interaction...
may be stabilized by the conserved hydrogen bond between Asp114.32 and Tyr416.74. On the opposite side of spiperone from Asp114.32, there is hydrophobic contact between spiperone and Phe389.65. This contact is likely essential for spiperone binding, given that F389A.65 showed a 34-fold reduction in affinity for spiperone compared to that of wild-type D2R.35. Additionally, there was contact between the triazaspiro ring and Ile183.45, Ile184.45, and Cys182.45 on ECL2 (Fig. 2a, b). The loss of this contact with the I184A.45 mutation significantly reduced the antagonist activity for spiperone (Supplementary Table 3), suggesting that the contact with Ile184.45 is crucial for the antagonist activity of spiperone. The contact between Ile183.45 and spiperone was influenced by the binding of Fab3089, because the side-chain conformation of Ile183.45 was stabilized by Tyr55 of Fab3089 (Fig. 2c). The I183A.45 mutant slightly increased the antagonist activity for spiperone (Supplementary Table 3).

On the extracellular side of the salt bridge, the phenyl ring of spiperone was bound in an extended binding pocket (EBP) between TM2 and TM3 (Fig. 2a, d). The EBP was formed by residues Val87.25, Trp90.26, Val91.261, Leu94.26, Trp100.2350, Phe110.28, Val111.269, and Cys182.45. The drastic reduction of the antagonist activity of W100A.2350 for spiperone indicates that Trp100.2350 is crucial for maintaining EBP conformation (Supplementary Table 3). Because of the triazaspiro ring rigidity and the direction of the conserved salt bridge between the tertiary amine and Asp114.32, the EBP is likely essential for spiperone binding. In the EBP of D2Rspi, spiperone’s phenyl ring forms hydrophobic contacts with Trp90.26, Val91.261, Leu94.264, Phe110.28, and Val111.269 (Fig. 2a, b). Mutations of most of

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**Table 1 Data collections and structure refinement statistics.**

| Data collection                  | D2Rspi (PDB 7DFP) |
|----------------------------------|------------------|
| **Data collection**              |                  |
| Space group                      | C2               |
| Cell dimensions                  |                  |
| a, b, c (Å)                      | 161.9, 40.5, 165.9 |
| α, β, γ (°)                      | 90, 116.5, 90    |
| Resolution (Å)                   | 43.1-3.1 (3.2-3.1) |
| Rmerge (%)                       | 18.8 (73.2)      |
| CC1/2                            | 0.97 (0.58)      |
| Rwork / Refine                   | 4.4 (1.5)        |
| Completeness (%)                 | 100 (100)        |
| Redundancy                       | 99.8 (45.4)      |
| **Reefinement**                  |                  |
| Resolution (Å)                   | 43.1-3.1 (3.2-3.1) |
| No. reflections                  | 18,048           |
| Rwork / Refine                   | 18.5 / 21.7 (26.7/31.7) |
| No. atoms                        |                  |
| Protein                          | 5896             |
| Ligand                           | 29               |
| B factors                        |                  |
| Protein                          | 97.1             |
| Ligand                           | 105.9            |
| R.M.S. deviations                |                  |
| Bond lengths (Å)                 | 0.002            |
| Bond angles (°)                  | 0.56             |

*Values in parentheses are for highest-resolution shell.*
these residues resulted in considerably decreased antagonist activity for spiperone (Supplementary Table 3).

On the intracellular side of the salt bridge, spiperone’s fluorophenyl ring penetrated deeply into the ligand-binding pocket, binding in the bottom hydrophobic cleft (Fig. 2a, b, d). Similar bottom hydrophobic clefts have been observed in the structures of the histamine H1 receptor40, 5-HT2AR26, and 5-HT3CR27. In the cleft, the fluorophenyl ring formed a CH–π interaction with Cys1183.36; hydrophobic interactions with Thr1193.37, Ile1223.40, Ser1975.46, Phe1985.47, and Phe3826.44; and edge-to-face π interactions with Trp3866.48 and Phe3906.52 (Fig. 2a, b). Trp3866.48 is a microswitch in the CWxP motif. Indeed, W386L6.48 affected activation by dopamine and showed no antagonist activity for spiperone (Supplementary Table 3 and Supplementary Fig. 5). Thus, the direct interactions of spiperone with Ile1223.40 (3.7 Å distance) and Phe3826.44 (3.6 Å distance) in the PIF motif may block the conformational rearrangements of the PIF motif and help to stabilize the inactive conformation, as has been observed in the structural studies of 5-HT2AR26 and 5-HT3CR27. Of the 21 contact residues, 20 were conserved between structures of other aminergic receptors, including D3R, D4R, and D5R (Supplementary Table 4), reflecting the similarly high affinity of these receptors for spiperone42.

**Comparison with D2Rtra, D2Rtab, and D2Rbro.** There are striking structural differences in the ligand-binding pocket of D2Rtra and the other inactive state structures of D2R: D2Rris, and D2Rhal (Fig. 3a, b and Supplementary Table 1). In D2Rris, the ligand-binding pocket was covered by the C-terminal segment of ECL2 (Fig. 3a–c), on which the side chains Ile1183.40 and Ile18445.52 pointed to the entrance and the bottom of the ligand-binding pocket, respectively. This conformation was conserved in the structures of other aminergic receptors, including D3R, D4R, and 5-HT2AR (Supplementary Fig. 6). In D2Rris and D2Rhal, however, ECL2 extended away from the top of the receptor core (Fig. 3a, b, d). In this conformation, Ile18445.52 was buried in the hydrophobic core outside the ligand-binding pocket, and Ile18445.52 reached the top of the ligand-binding pocket (Fig. 3a, b). Thus, Ile18445.52 did not contact with risperidone in D2Rris, while Ile18445.52 contacted with spiperone in D2Rtra. These findings...
were consistent with I184A showing similar antagonist activity for risperidone with the wild-type (Supplementary Table 3).

On ECL1, D2R demonstrated the diverse side-chain conformation of Trp100. In D2Rspi, Trp100 interacted with the conserved disulfide bond (Fig. 3b). The conformation was highly conserved in the structures of class A GPCRs (Supplementary Fig. 6). The interaction between a disulfide bond and tryptophan is often observed in protein structures; this may contribute to protecting the disulfide bond and stabilizing the structure.

Fig. 3 Comparison of D2R structures. a Extracellular view of the superposition of D2Rspi and D2Rlis. The side chains of disulfide bridge, I183 and I184 are shown as sticks. Red arrows indicate the shift of helices in D2Rlis with the distance relative to D2Rspi. b Extracellular view of ECL1 and ECL2 of D2Rspi, D2Rlis, D2Rhal, and D2Rbro. The side chains of the disulfide bridge, W100 and I183 and I184 are shown as sticks. Surface representation of D2Rspi (green), D2Rlis (cyan), D2Rhal (purple), D2Rbro (olive), spiperone (orange), risperidone (magenta), haloperidol (ivory), and bromocriptine (lightblue) are shown.

Trp100 in D2Rlis exhibited similar side-chain conformation with that of D2Rspi, although it did not contact with the disulfide bond because of ELC2 flipping (Fig. 3b). In D2Rlis, Trp100 moves to the ligand-binding pocket and forms a T-stacking interaction with risperidone’s tetrahydropyridopyrimidinone ring (Fig. 3b). Trp100 in D2Rlis forms a hydrophobic patch with Leu94 and Ile184, covering the ligand-binding pocket. Despite this, the ligand-binding pocket in D2Rlis was more exposed to the extracellular solution compared with that in D2Rspi (Fig. 3c, d). W100A in D2R has been shown to reduce
the residence times of several antipsychotics22. Based on these results, it was hypothesized that the hydrophobic patch in D2Rsis contributes to the slow dissociation of antipsychotics22. However, the results were also consistent with the conformation observed in D2Rspi, in which Trp10023.50 stabilized the conformation of ECL2 and EBP.

The EBP is uniquely observed in D2Rspi among the inactive state structures of D2R. In D2Rspi, the side chain of Phe11023.28 that creates the EBP was flipped compared with those of D2Rsis and D2Rhal (Fig. 3e), D2Rreti, and the 5-HT2 receptor. In D2Rspi, the flipped Phe11023.28 side chain formed a stacking interaction with Trp902.60 (Fig. 3e).

D2Rspi and D2Rhal also possessed the bottom hydrophobic cleft (Fig. 3f). However, the conformation of this cleft in D2Rsis and D2Rhal was altered by the shift of the extracellular half of TM5 in the ligand-binding pocket relative to that in D2Rspi (Fig. 3a, i). Resultantly, Phe1895.38 and Ser1935.42 contacted risperidone in D2Rspi, though these residues did not contact haloperidol in D2Rhal (Fig. 3f). The shift of TM5 observed in D2Rsis and D2Rhal is likely inhibited in D2Rreti by steric contact between the extracellular end of TM5 and ECL2. Indeed, Phe1895.38 in D2Rsis and D2Rhal occupied a similar position with Ile18445.52 in D2Rspi in the ligand-binding pocket (Fig. 3f). The shift can also be affected by the I122A3.40 mutation introduced to stabilize the receptor in D2Rsis and D2Rhal22,23. In D2Rsis and D2Rhal, the Cβ atom of Ala1223.40 was in contact (3.8 Å distance) with the carbonyl oxygen atom of Ser1975.46 (Supplementary Fig. 7a); when the side chain of Ala1223.40 was replaced by isoleucine using Coot4, the resulting side chain formed steric contacts (less than 3.0 Å distance) with the surrounding residues, including Ser1975.46 and Pro2015.50 on TM5, and with risperidone or haloperidol in any of the seven allowed side-chain rotamers for isoleucine (Supplementary Fig. 7b).

Unlike inactive state structures, D2Rbro showed a typical active state conformation in the microswitches and the seven helical bundles (Supplementary Fig. 5). Conformations of ECL2 and the extracellular end of TM5 in D2Rbro were more similar to those of D2Rspi than those of D2Rsis and D2Rhal (Fig. 3b, i). Trp10023.50 of D2Rspi existed at a similar position with those in D2Rspi and D2Rhal but with a different side-chain rotamer (Fig. 3b). EBP in D2Rspi was not observed in D2Rbro (Fig. 3e).

Comparison with 5-HT2AR and 5-HT2CR. The conformation of the extracellular end of TM5, the conserved Trp3.30 on ECL1, the C-terminal segment of ECL2, and the disulfide bridge between ECL2 and TM3 in 5-HT2AR and 5-HT2CR were similar to those of D2Rspi (Fig. 4a, b and Supplementary Fig. 6). On the C-terminal segment of ECL2, the Leu22845.51 and Leu22945.52 residues of 5-HT2AR contacted risperidone and zotepine, respectively26, and the Val20845.52 residue of 5-HT2CR interacted with ritanserin27. The high conservation of the residues in the ligand-binding pocket (Supplementary Table 4) and the structural similarity of 5-HT2AR and 5-HT2CR in D2Rspi and D2Rhal explain why antipsychotics often bind to D2R, 5-HT2AR, and 5-HT2CR with high affinity.

Phe11022.38 and Trp902.60 forming EBP in D2Rspi corresponded with Trp1513.28 and Val1302.60 in 5-HT2AR and Trp3.28 and Leu1092.60 in 5-HT2CR, respectively. In D2R, W90L2.60 reduced the antagonist activity more than F110W3.38 and similarly with W90L2.60/F110W3.38 (Supplementary Table 3). Risperidone shows a high affinity to D2R and 5-HT2AR but a low affinity to 5-HT2CR25. A potential explanation of this difference in affinity is that while Trp1513.28 in 5-HT2AR can be flipped to form the EBP without any steric hindrance, Trp1303.28 in 5-HT2CR is challenging to flip because of the steric contact with Leu1092.60 (Fig. 4c, d). Thus, the EBP of D2Rspi and the putative EBP of 5-HT2AR may contribute to spiperone’s higher selectivity for these receptors than for 5-HT2CR. The binding mode of spiperone in the EBP may be useful for designing selective D2R and 5-HT2AR antipsychotics.

A unique side-extended cavity was previously observed in the structure of 5-HT2AR between TM4 and TM5 that was suggested to contribute to the binding site of 5-HT2AR-selective drugs26. D2Rspi did not possess the side-extended cavity between TM4 and TM5 (Fig. 4e).

Comparison with D2Rreti and D2Rnem. The conformation of D2Rspi was similar to that of D2Rreti and D2Rnem except for the extracellular half of TM6 (Fig. 5a, b). The conformation of the C-terminal segment of ECL2 in D2Rspi was almost identical to that of D2Rreti and D2Rnem (Fig. 5a, b). On ECL2, Ile18445.52 in D2Rreti and Leu18745.52 in D2Rnem contacted eticlopride20 and nemonapride21, respectively, similar to Ile18445.52 in D2Rspi, which contacts spiperone. A previous study showed that eticlopride and nemonapride bind just above the bottom hydrophobic cleft in D2Rreti and D2Rnem22 (Supplementary Fig. 8a), but this is different from the binding of spiperone in D2Rspi, risperidone in D2Rris, and haloperidol in D2Rhal. To interact with these benzamide antipsychotics, the extracellular half of TM6 exhibited a greater tilt toward TM5 in D2Rreti and D2Rnem than in D2Rspi, D2Rreti, and D2Rnem (Fig. 5a, b and Supplementary Fig. 8b). Thus, His34945.55 in D2Rreti and His41445.55 in D2Rnem interacted with eticlopride and nemonapride, respectively, whereas no contact was made between spiperone and His39545.55 in D2Rspi (Supplementary Fig. 8b). Due to the large tilt of TM6, the distances between Cys3.36 and Phe6.52 in D2Rreti and D2Rnem were approximately 1.5 and 2.0 Å closer, respectively, than in D2Rspi (Fig. 5a, b and Supplementary Fig. 8b). Together, the side-chain flip of Cys1183.36 and the tilt of TM6 resulted in the closure of the bottom hydrophobic cleft in D2Rreti and D2Rnem (Fig. 5a, b and Supplementary Fig. 8a, b). Conversely, the insertion of spiperone’s fluorophenyl ring between Cys3.36 and Phe6.52 created the bottom hydrophobic cleft and inhibited the large tilt of TM6 in D2Rspi. D2-class receptors show high affinities for eticlopride, nemonapride, spiperone, and risperidone25, and display conserved residues in the ligand-binding pocket. Therefore, it is likely that these receptors show a similar conformation with D2Rreti and D2Rnem when they bind to benzamide antipsychotics and with D2Rspi, D2Rnem, and D2Rhal when they bind to butyrophenone or pyridopyrimidine antipsychotics (Supplementary Fig. 8c).

Sipiperone and nemonapride show high affinities for D2-class receptors25. In D2R, the putative EBP was closed by the side chain of Phe1062.28 (Fig. 5c). If the Phe1062.28 is flipped, D2R can form an EBP similar to that of D2Rspi with the conserved residues around this region. The EBP in D2Rspi was observed at a position similar to that of the EBP in D2Rnem that binds the phenyl ring of nemonapride21 (Fig. 5d), although the contact residues were not conserved between D2R and D2R (Supplementary Table 4).

Discussion

D2Rspi was observed to differ substantially from the other inactive state structures of D2R, D2Rsis, and D2Rhal, especially in ECL2, forming the entrance part of the ligand-binding pocket. This suggests that ECL2 is highly dynamic in the inactive state of D2R. The residues on ECL2 in D2R have been mapped by the substituted-cysteine accessibility method40. In that study, the sulphydryl groups of 1183C45.51 and 1184C45.52 reacted with charged sulphydryl-specific reagents, indicating that these residues are water-accessible. The binding of N-methylspiperone to 1183C45.51 and 1184C45.52 was inhibited by the reaction with

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sulfhydryl-specific reagents, indicating that Ile183\(^{45.51}\) and Ile184\(^{45.52}\) were directed toward the ligand-binding pocket. Ile183\(^{45.51}\) is less likely to contact the ligand because N-methylspiperone binding to I183C\(^{45.51}\) was inhibited only by a bulkier sulfhydryl-specific reagent, MTSET. I184C\(^{45.52}\) reduced the affinities for nemonapride and N-methylspiperone, suggesting that Ile184\(^{45.52}\) contacts nemonapride and N-methylspiperone. These results are consistent with the conformation of ECL2 observed for D2R\(_{spi}\) but not with that of D2R\(_{ris}\) or D2R\(_{hal}\). The difference of the ECL2 conformation can be caused by the bound ligand, although D2R\(_{ris}\) and D2R\(_{hal}\) show very similar ECL2 conformation. Indeed, ECL2 in 5-HT\(_{2A}\)R moves slightly to bind a different ligand\(^{26}\). An MD simulation study also suggested these dynamics of the ECL2 of D2R\(^{47}\) and reported that the helical conformation of ECL2 observed in D2R\(_{ris}\) tended to unwind toward an extended conformation, similar to that of D3R\(_{spi}\), regardless of the bound ligand, including spiperone, risperidone, or eticlopride\(^{47}\). The unwinding involves a drastic rearrangement of the side chain of Ile183\(^{45.51}\), dissociating from a hydrophobic pocket. The study also suggested that the ECL2 conformation observed in D2R\(_{ris}\) represents a higher energy state than the extended conformation. Considering the structural similarity between D2R\(_{spi}\) and D3R\(_{eti}\), the conformation of ECL2 in D2R\(_{spi}\) likely corresponds to a lower energy state conformation.

Currently, there is a need for novel, safer antipsychotics that bind selectively to 5-HT\(_{2A}\)R and D2R. In this study, we revealed that the ligand-binding pocket of D2R forms more than two different conformations in the inactive state. Moreover, we showed that the EBP in D2R\(_{spi}\) and the putative EBP in 5-HT\(_{2C}\)R\(_{ris}\) could be used as the binding site for selective atypical antipsychotics. D2R\(_{hal}\) was used for the structure-based discovery of selective ligand\(^{23}\). The use of multiple different conformations

Fig. 4 Comparison of D2R\(_{spi}\) and 5-HT\(_{2}\) receptors. Extracellular view of the superpositions of D2R\(_{spi}\) and either 5-HT\(_{2A}\)R\(_{ris}\) (a) or 5-HT\(_{2C}\)R\(_{ris}\) (b). c The EBP of D2R\(_{spi}\) and the corresponding part of 5-HT\(_{2A}\)R\(_{ris}\). d The EBP of D2R\(_{spi}\) and the corresponding part of 5-HT\(_{2C}\)R\(_{ris}\). e Vertical cross sections of D2R\(_{spi}\) and 5-HT\(_{2A}\)R\(_{ris}\). In this figure, D2R\(_{spi}\) (green), 5-HT\(_{2A}\)R\(_{ris}\) (blue), 5-HT\(_{2C}\)R\(_{ris}\) (brown), spiperone (orange), risperidone (yellow), and ritanserin (gray) are shown.
spiperone (orange), eticlopride (blue), and nemonapride (red) are shown.

Together with D2Rris, D2Rhal, and 5-HT2ARris, the human D2R (UniProt ID P14416) was synthesized by TAKARA Bio. Protein engineering for structure determination clearly increased the possibility of design of new antipsychotics with low side effects.

Methods

Protein engineering for structure determination. The coding sequence of human D2R (UniProt ID P14416) was synthesized by TAKARA Bio. D2R was stabilized by removing the N-terminal 34 residues, by introducing two mutations (S121K3.39 and L123W3.41) and by replacing ICL3 (Lys2215.70 to Leu3636.25) stabilized by removing the N-terminal 34 residues, by introducing two mutations (S121K3.39 and L123W3.41)29. mbIIG contains four mutations (M7W, R98I, H102I, and R106G), with the loop-modifications of the EBP of D2Rspi and D4Rnem. In this figure, D2Rspi (green), D2Rhal (yellow), and D4Rnem (pink) are shown.

in the structure-based design instead of a single conformation clearly increased the possibility of finding high-affinity compounds. Together with D2Rhal, D2Rhal, and 5-HT2ARris, the structure of D2Rspi can be utilized for a rational, structure-based design of new antipsychotics with low side effects.

Protein expression and purification. The stabilized D2R was expressed in Sf9 cells and concentrated to 2.5 ml with a 100-kDa molecular weight cutoff Amicon Ultra-15 centrifugation resin (Clontech) for 10 h at 4 °C. The resin was washed with 10 column volumes (CV) of wash buffer I (50 mM HEPES, pH 7.5, 500 mM NaCl, 10% (v/v) n-dodecyl-ß-D-maltopyranoside (DDM, Anatec), 0.2% (v/v) cholesterol hemisuccinate (CHS, Sigma-Aldrich), and 20% (v/v) glycerol) supplemented with 2 mg/ml iodoacetamide (Wako Pure Chemical Industries, Ltd), 200 µM spiperone (Sigma-Aldrich), and the protease inhibitor cocktail. Insoluble materials were removed by centrifugation, and the supernatants were incubated with TALON metal affinity resin (Chromek) at 10 °C for 4 h. The plate without uracil [0.192% (w/v) yeast synthetic dropout media without uracil, 0.671% (w/v) DDM, 0.2% (w/v) cholesteryl hemi-succinate (CHS, Sigma-Aldrich), and 20% (v/v) glycerol] was then plated on an agar plate without uracil [0.192% (w/v) yeast synthetic dropout media without uracil, 0.671% (w/v) DDM, 0.2% (w/v) cholesteryl hemi-succinate (CHS, Sigma-Aldrich), and 20% (v/v) glycerol] at 30 °C for 24 h.

The generated plasmid encoding the receptor was isolated from the Miniprep Kit (Qiagen) by disrupting cells with 0.5 mm glass beads50. The construct was subcloned into the pFastBac1 vector (Invitrogen), with a C-terminus His-tagged TEV protease (expressed and purified from insect cells). To select antibodies that were generated by the Kyoto University Animal Experimentation Committee (approval no. Med-kyo16043). As the antigen, we used a stabilized D2R (D2R-mbIIG S121K3.39/L123W3.41). Purified antigen was reconstituted into liposomes containing chicken egg yolk phosphatidylcholine (Avanti) and monophosphoryl lipid (Sigma-Aldrich). MRL/lpr mice were immunized three times at two-week intervals with 0.1 mg of the proteoliposome D2R antigen. Single cells were harvested from mice spleens and were fused with NS-1 myeloma cells. To select antibodies that were generated by the Kyoto University Animal Experimentation Committee (approval no. Med-kyo16043). As the antigen, we used a stabilized D2R (D2R-mbIIG S121K3.39/L123W3.41). Purified antigen was reconstituted into liposomes containing chicken egg yolk phosphatidylcholine (Avanti) and monophosphoryl lipid (Sigma-Aldrich). MRL/lpr mice were immunized three times at two-week intervals with 0.1 mg of the proteoliposome D2R antigen. Single cells were harvested from mice spleens and were fused with NS-1 myeloma cells. To select antibodies that were generated by the Kyoto University Animal Experimentation Committee (approval no. Med-kyo16043). As the antigen, we used a stabilized D2R (D2R-mbIIG S121K3.39/L123W3.41). Purified antigen was reconstituted into liposomes containing chicken egg yolk phosphatidylcholine (Avanti) and monophosphoryl lipid (Sigma-Aldrich). MRL/lpr mice were immunized three times at two-week intervals with 0.1 mg of the proteoliposome D2R antigen. Single cells were harvested from mice spleens and were fused with NS-1 myeloma cells. To select antibodies that were generated by the Kyoto University Animal Experimentation Committee (approval no. Med-kyo16043). As the antigen, we used a stabilized D2R (D2R-mbIIG S121K3.39/L123W3.41). Purified antigen was reconstituted into liposomes containing chicken egg yolk phosphatidylcholine (Avanti) and monophosphoryl lipid (Sigma-Aldrich). MRL/lpr mice were immunized three times at two-week intervals with 0.1 mg of the proteoliposome D2R antigen. Single cells were harvested from mice spleens and were fused with NS-1 myeloma cells. To select antibodies that were generated by the Kyoto University Animal Experimentation Committee (approval no. Med-kyo16043). As the antigen, we used a stabilized D2R (D2R-mbIIG S121K3.39/L123W3.41). Purified antigen was reconstituted into liposomes containing chicken egg yolk phosphatidylcholine (Avanti) and monophosphoryl lipid (Sigma-Aldrich). MRL/lpr mice were immunized three times at two-week intervals with 0.1 mg of the proteoliposome D2R antigen. Single cells were harvested from mice spleens and were fused with NS-1 myeloma cells. To select antibodies that.
recognized the 3D structure of human D2R, we performed a multi-step screening method, using D2R-3d, which lacks residues of the N-terminal and ICL3, at each step, with included HEK293 cells, were pre-processed with Biacore T100 protein interaction analysis system (GE Healthcare) and were subsequently isolated by limiting dilution to establish monoclonal hybridoma cell lines. The resulting immunoglobulin-G (IgG3089) was purified with HiTrap Protein G HP (GE Healthcare) followed by electroelution chromatography. The Fab fragment (Fab3089) was then purified by size-exclusion chromatography (Superdex 200 10/300 GL, GE Healthcare) and affinity chromatography with a Protein A Sepharose 4 Fast-Flow column (GE Healthcare). The sequence of Fab3089 was determined via standard 5'-RACE using total RNA isolated from hybridoma cells.

**Crystallization.** The D2R-Fab3089 complex was prepared by mixing the purified D2R-mbIIG S121K/L123W and Fab3089 at a molar ratio of 1:1.2 for 1 h on ice. The mixture was injected onto a Superdex 200 10/300 GL column (GE Healthcare), and the fractions containing the complex were concentrated to approximately 30 mg/ml with a 50-kDa molecular weight cutoff Amicon Ultra-15 concentrator (Millipore). The D2R-Fab3089 complex in simple with spermine was reconstituted in LCP by mixing approximately 30 mg/ml protein solution with monolinol and 10% w/w cholesterol at a volume ratio of 2.3 (protein:lipid) using two 100-μl Hamilton syringes and a syringe coupler. One syringe and a coupler were then removed, and a cleaning wire was inserted into the protein-laden LCP in the other syringe. Approximately 10 μl of the protein-laden LCP was extruded from the needle with a 300-μm tube fitted to a capillary in a precipitant solution (0.1 M Tris-HCl, pH 8.0, 0.1 M CH₃COOLi, 28–32% PEG400, 5% dimethyl sulfoxide, 0.01 M ATP, and 1 mM spermine) and incubated at 20 °C. Microcrystals appeared after 2 days, growing to a maximum size of 20 × 2 × 2 μm within a week.

**Data collection using an X-ray free-electron laser.** The data were collected at beamline BLS-6 of the Spring-8 Compact Free-Electron Laser (SACLASS) (Hyogo, Japan) by the serial femtosecond crystallography technique using 1.5 × 1.5 μm² microbeams focused by Kirkpatrick–Baez mirrors with a short-working-distance octal port CCD detector with eight sensor modules. The data were collected at 7 keV with a pulse duration of approximately 10 fs and a repetition rate of 30 Hz. To inject the microcrystals, LCP was loaded into a sample cartridge through a needle connected to a syringe.

After centrifugation at 2000g for 10 s, the cartridge was mounted in a high-voltage micro-extrusion injector with a nozzle diameter of 100 μm13,52,58. The injector was set in a chamber filled with helium gas in the Diverse Application Platform for Hard X-ray Diffraction in SACLA (DAPHENIS) set-up and was maintained at a constant 20 °C. A total volume of 60 μl of LCP was injected at a flow rate of 420 nl/min. Data collection was guided by a real-time data processing pipeline based on Cheetah59. Data processing and indexing were performed using CrystFEL 0.8.060,61 and XGANDALF (https://onlinelibrary.wiley.com/journal/10.1002/crdi). The total number of collected, hit, and outliers. Figures were prepared using Cuemol (http://www.cuemol.org/) and PyMOL (https://www.pymol.org/).

**Radioligand-binding assay.** The mutants were prepared using the primers listed in Supplementary Table 5. The radioligand-binding assay was performed using HEK293 cell or S9 cell membranes that expressed the receptor. The wild-type or a mutant D2R was transfected with a pCAGGS plasmid into HEK293 cells using a FuGENE HD transfection reagent (10 μg of plasmid, 50 μl of FuGENE HD solution per 10-cm culture dish). The membranes were prepared as described in "Protein expression and purification" section. The protein concentration of the membrane was determined by the bichinchonic acid (BCA) method (Thermo Fisher Scientific) with bovine serum albumin as a standard. The membranes were stored at -80 °C until use. All the experiments were performed in triplicate (with independent experiments) in a total volume of 200 μl, added to the cell. For all the experiments, Unilifter-96 GF/B filter plates and the unbound ligand was washed three times with distilled water using a FilterMate Cell Harvester system (Perkin Elmer).

To determine KD value of the receptors for spermine, we used the equation accounting for ligand depletion. Binding data are reported as the mean ± SEM.

**TGFRs shedding assay.** The antagonist activity of spermine for the mutant D2Rs was determined by the TGFRs shedding assay52. Briefly, a pCAGGS plasmid encoding the human wild-type or a mutant D2R (full-length, untagged), together with pCAGGS plasmids that encoded the chimeric Gαq53 subunit and alkaline phosphatase-tagged TGFR-α (AP-TGFRa; human codon optimized), were transfected into HEK293A cells that were negative for mycoplasma contamination (MycoAlert Mycoplasma detection kit, Lonza) by using a PEI transfection reagent (PEI MAX MW 40,000, PolySciences). The chimeric Gαq53 subunit comprises the Gαq back-bone and the Gαs-derived 6-amino acid C-terminus, and it couples with Gz coupled D2R, but induces a Gq-dependent TGFRα shedding response32. For each 10-cm culture dish, we used 1 μg of D2R plasmid, 0.5 μg of Gαs plasmid, 2.5 μg of AP-TGFRα plasmid and 25 μl of 1 mg/ml PEI solution. After culturing for one day at 37 °C in a 5% CO2 incubator, the transfected cells were harvested by trypsinization, washed once with Hank’s balanced salt solution (HBSS) containing 5 mM HEPES (pH 7.4), and resuspended in 30 ml of the HBSS-containing HEPES. The cell suspension was seeded in a 96-well culture plate (“cell plate”) at a volume of 80 μl per well and incubated for 30 min in the CO2 incubator. To determine the antagonist activity of spermine or risperidone, cells were pretreated with 3.2-fold-titrated concentration of the antagonists (final concentrations of 32 pM–1 μM for spermine or 320 pM–10 μM for risperidone; 10 μl per well) for 5 min and stimulated with dopamine (final concentration of 1 μM; 10 μl per well). To determine the agonist activity of dopamine, vehicle (10 μl per well) were predispensed before cell seeding and 3.2-fold-titrated concentration of dopamine (final concentration of 1 μM, 320 pM–10 μl per well) were transferred to the cells. The compounds were diluted in 0.01% bovine serum albumin- and HBS-containing HEPES. After incubation with dopamine for 1 h, the cell plate was spun at 190 × g for 2 min and conditioned medium (CM; 80 μl per well) was transferred to an empty 96-well plate (“CM plate”). Alkaline phosphatase reaction solution (10 mM p-nitrophenylphosphate, 120 mM Tris-HCl, pH 9.5, 40 mM NaCl, and 10 mM MgCl₂) was dispensed into the cell plates and CM plates (80 μl). The absorbance of the plates at 405 nm was measured using a microplate reader (SpectraMax 340 PC384, Molecular Devices) before and after incubation for 1 or 2 h at room temperature. Ligand-induced AP-TGFα release was measured using a AP activity in conditioned media and subtracting a vehicle-treated spontaneous AP-TGFRα signal32. Using Prism 8 software (GraphPad Prism), the AP-TGFRα release signals were fitted with a four-parameter sigmoidal concentration–response curve, from which IC50 and Emax values were obtained. Negative logarithmic values of EC50 (IC50) were calculated to calculate the mean ± SEM.

The equilibrium dissociation constant (KD) was calculated for each experiment performed in parallel from the IC50 values (for spermine and risperidone), an EC50 value (for dopamine), a Hill slope (K), and the tested concentration of dopamine (A; 1 µM), as follows:

\[
K_D = \frac{I_{50}}{1 + \frac{A}{E_{max}}} 
\]

(1)

The resulting KD values were logarithmically transformed and their negative values (pKD) were used to calculate the difference between the pKD values (ΔpKD) for a mutant (MT) and the wild-type (WT) receptor, derived from parallelly conducted experiments, as follows:

\[
\Delta pKD = pKD_{MT} - pKD_{WT} 
\]

(2)

Mean and SEM values of the pKD and ΔpKD values were calculated.

**Reporting summary.** Further information on research design in the Nature Research Reporting Summary linked to this article.

**Data availability**

The coding sequence of human D2R is available in UniProt with accession code P14416. The protein coordinate and atomic structure factor have been deposited in the Protein Data Bank (PDB) with accession code 7ZDP. The raw diffraction images have been deposited to CSDDB (https://csdadb.org/) with accession code 110. Other data are available from the corresponding authors upon reasonable request. Source data are provided with this paper.
References

1. Keinan, J. W. Multiple classes of dopamine receptors in mammalian central nervous system involvement of dopamine-sensitive adenylyl cyclase. *Life Sci. 23*, 479–483 (1978).

2. Sano, P. F., Gionvoni, S. & Trabucchi, M. Studies on the pharmacological properties of dopamine receptors in various parts of the central nervous system. *Adv. Biochemical Psychopharmacol. 19*, 155–165 (1978).

3. Schwartz, J. C., Girod, B., Martres, M.-P. & Sokoloff, P. The dopamine receptor family: molecular biology and pharmacology. *Neurosci. Neuropharmacol.* 9, 108–192 (1998).

4. Grandy, D. K. et al. Cloning of the cDNA and gene for a human D2 dopamine receptor. *Proc. Natl. Acad. Sci. USA 86*, 9762–9766 (1989).

5. Sokoloff, P., Girod, B., Martres, M.-P., Bouthenet, M. L. & Schwartz, J. C. Molecular-cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature 347*, 146–151 (1990).

6. Vanot, H. H. M. et al. Cloning of the gene for a human dopamine D4 receptor with high-affinity for the antipsychotic clozapine. *Nature 350*, 610–614 (1991).

7. Missale, C., Nash, S. R., Robinson, S. W., Jaber, M. & Caron, M. G. Dopamine receptors: from structure to function. *Physiol. Rev. 78*, 189–225 (1998).

8. Gerfen, C. R. Molecular effects of dopamine on striatal-projection pathways. *Trends Neurosci.* 23, 564–570 (2000).

9. Snyder, S. H., Taylor, K. M., Coyle, J. T. & Meyerhoff, J. J. The role of brain dopamine in behavioral regulation and the actions of psychotropic drugs. *Am. J. Psychiatry 127*, 199–207 (1970).

10. Creese, I., Burt, D. R. & Snyder, S. H. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science 192*, 481–483 (1976).

11. Howes, O. D. & Kapur, S. The dopamine hypothesis of schizophrenia: version III—the final common pathway. *Schizophr. Bull. 35*, 549–562 (2009).

12. Lang, A. E. & Lozano, A. M. Parkinson’s disease. First of two parts. *N. Engl. J. Med. 339*, 1044–1053 (1998).

13. Lang, A. E. & Lozano, A. M. Parkinson’s disease. Second of two parts. *N. Engl. J. Med. 339*, 1130–1143 (1998).

14. Seeman, P., Chau-Wong, M., Tedesco, J. & Wong, K. Brain receptors for antipsychotic drugs and dopamine: direct binding assays. *Proc. Natl. Acad. Sci. USA 72*, 4376–4380 (1975).

15. Meltzer, H. Y., Matsubara, S. & Lee, C. Classification of typical and atypical antipsychotic drugs—the basis of dopamine D1, D2 and serotonin2-pK(2) values. *J. Pharm. Exp. Ther. 251*, 238–246 (1989).

16. Roth, B. L., Sheller, D. J. & Kroese, W. K. Magic inefficient versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Disc. 3*, 353–359 (2004).

17. Muench, J. & Hamer, A. M. Adverse effects of antipsychotic medications. *Am. Fam. Physician 81*, 617–622 (2010).

18. de Graaf, C. et al. Crystal structure-based virtual screening for fragment-like ligands of the human histamine H1 receptor. *J. Med. Chem. 54*, 8195–8206 (2011).

19. Roth, B. L., Irwin, J. I. & Shoichet, B. K. Discovery of new GPCR ligands to illuminate new biology. *Nat. Chem. Biol. 13*, 1143–1151 (2017).

20. Chien, E. Y. et al. Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science 330*, 1091–1095 (2010).

21. Wang, S. et al. D3 dopamine receptor high-resolution structures enable the discovery of selective agonists. *Science 358*, 381–386 (2017).

22. Wang, S. et al. Structure of the D3 dopamine receptor bound to the atypical antipsychotic drug risperidone. *Nature 555*, 269 (2018).

23. Fan, L. et al. Haloperidol bound D2 dopamine receptor structure illuminated the discovery of subtype selective ligands. *Nat. Commun. 11*, 1074 (2020).

24. Yin, J. et al. Structure of a D2 dopamine receptor-G-protein complex in a lipid membrane. *Nature 584*, 125–129 (2020).

25. Roth, B. L., Lopez, E., Patel, S. & Kroese, W. K. The multiplicity of serotonin receptors: uselessly diverse molecules or an embarrassment of riches? *Neuroscientist 6*, 252–262 (2000).

26. Kimura, K. T. et al. Structures of the 5-HT(3) receptor in complex with the antiulcers phoscodeine and zotepine. *Nat. Struct. Mol. Biol. 26*, 121–128 (2019).

27. Peng, Y. et al. 5-HT_(3A) receptor structures reveal the structural basis of GPCR polypharmacology. *Cell 172*, 719 (2018).

28. Chon, E. et al. Fusion partner toolset for the stabilization and crystallization of G protein-coupled receptors. *Structure 20*, 967–976 (2012).

29. Chu, E. et al. Redesign of a four-helix bundle protein by phage display coupled with proteolysis and structural characterization by NMR and X-ray crystallography. *J. Mol. Biol. 323*, 253–262 (2002).
9. Barty, A. et al. Cheetah: software for high-throughput reduction and analysis of serial femtosecond X-ray diffraction data. J. Appl. Crystallogr. 47, 1118–1131 (2014).
10. White, T. A. et al. CrystFEL: a software suite for snapshot serial crystallography. J. Appl. Crystallogr. 45, 335–341 (2012).
11. White, T. A. et al. Recent developments in CrystFEL. J. Appl. Crystallogr. 49, 680–689 (2016).
12. McCoy, A. J. et al. Phaser crystallographic software. J. Appl. Crystallogr. 40, 658–674 (2007).
13. Adams, P. D. et al. PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D 66, 213–221 (2010).
14. Liebschner, D. et al. Polder maps: improving OMIT maps by excluding bulk solvent. Acta Crystallogr. D 73, 148–157 (2017).
15. Davis, I. W. et al. MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic Acids Res. 35, W375–W383 (2007).
16. Cheng, H. C. The power issue: determination of $K_0$ or $K_I$ from $IC_{50}$: a closer look at the Cheng-Prusoff equation, the Schild plot and related power equations. J. Pharmacol. Toxicol. Methods 46, 61–71 (2001).

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Author contributions

D.I. designed constructs, expressed, purified, and crystallized the receptor. D.I. and T.U. generated the antibody. D.I., T.F., and C.M. prepared microcrystals. D.I., T.F., Y.Y., T.T., A.Y., and E.N. collected data at SACLA. T.N. processed the data. K.T. contributed to the beamline operation at SACLA. Y.S. and N.N. prepared mutants. A.I. and F.M.N.K. designed, performed, and analyzed the functional assay under J.A.’s supervision. H.A. and K.T.K. performed the binding assay. T.S. designed the constructs and solved and refined the structure as well as supervised the project. D.I., S.I., and T.S. wrote the manuscript. S.I. advised T.S. All authors discussed the results and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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