Molecular Mechanism and Dynamics of S-Deoxyephedrine Moving through Molecular Channels within D$_3$R

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ABSTRACT: In this article, the trajectories of S-deoxyephedrine (SBD) along molecular channels within the complex protein structure of third dopamine receptor (D$_3$R) are analyzed via molecular dynamic techniques, including potential mean force calculations of umbrella samplings from the 4.5 version of the GROMACS program. Changes in free energy due to the movement of SBD within D$_3$R are determined, and the molecular dynamic mechanisms of SBD transmitting along molecular channels are probed. Molecular simulated results show that the change in free energy is calculated as 171.7 kJ mol$^{-1}$ for the transmission of SBD toward the outside of the cell along the $y+$ axis functional molecular channel and is 275.0 kJ mol$^{-1}$ for movement toward the intracellular structure along the $y-$ axis. Within the internal structure of D$_3$R, the changes in free energy are determined to be 103.6, 242.1, 459.7, and 127.8 kJ mol$^{-1}$ for transmission of SBD along the $x+$, $x-$, $z+$, and $z-$ axes, respectively, toward the cell bilayer membrane, which indicates that SBD leaves much more easily along the $x+$ axis through the gap between the TM5 (the fifth transmembrane helix) and TM6 (the sixth transmembrane helix) from the intracellular structure of D$_3$R. The values of free-energy changes indicate that SBD molecules can clear the protective channel within D$_3$R, which helps dopamine molecules to leave the D$_3$R internal structure along the $x+$ axis and to prevent them from exerting excessive neurotransmitter function. Therefore, our results suggest that SBD is effective for development as a drug for treating schizophrenia and its pharmacology is closely related to its dynamics and mechanisms within the molecular pathway of dopamine receptors.

INTRODUCTION

Schizophrenia is a serious mental disease with a 0.5% incidence rate of the total population (in world) and is characterized by changes in basic personality, thinking, emotion, behavior, and disharmony between mental activity and the environment. The disease does not only affect mental health but early death frequently occurs in diseased patients in 12–15 years compared with the average person. Thus, schizophrenia results in more loss of lives than that caused by most cancers and physical illnesses. Although the precise societal burden of schizophrenia is hardly known despite the wide diversity of accumulated data and employed methods, cost-of-illness data uniformly point to disquieting human and economic costs.

At present, there exist three hypotheses (theories) that physicochemically explain schizophrenia, namely, the dopaminergic hypothesis, glutamate hypothesis, and 5-hydroxytryptamine hypothesis. These three kinds of hypotheses ultimately lead to dopamine hyperfunction, in which dopamine molecules excessively overact with the neurotransmitter function. Therefore, all current medical treatments for schizophrenia work by blocking dopamine molecules overly to play a neurotransmitter function. Dopamine (DA) is an important neurotransmitter in the brain that aids the regulation of motor functions, cognitive activity, drug addiction, and other physiological and pathological processes through the expression of dopamine receptors in the central nervous system. There are five subtypes of dopamine receptor membrane proteins in the central nervous system (CNS), all of which are G protein coupled receptors and composed of seven transmembrane (TM) domains (7-GM). Studies on dopamine receptors began in the late 1970s, when the existence of D$_1$, D$_2$, two classes of dopamine subtype receptors was first confirmed. In the early 1990s, with the development of biological cloning technology, five subtypes of dopamine receptor molecules were cloned: D$_1$R, D$_2$R, and D$_3$R belonging to the D2-like receptor group and D$_4$R and D$_5$R to the D1 group.

Considering the diversity of dopamine receptor subtypes, the specificity of their distribution within brain regions, and their functional differences in the synaptic environment, the DA system in the brain is highly complex. Treatments for schizophrenia and Parkinson’s disease (PD) as well as those for drug addiction are all closely related to the complex DA system. Many findings from studies on these mental diseases have been achieved that have paved the way for more in-depth studies. For example, Arvid Carlson, Paul Greengard, and Eric Kandel won the 2000 Nobel Prize in medicine after discovering that dopamine molecules act as a signal transmission and in the regulation of motor function, cognitive...
activity, drug addiction, and other physiological and pathological processes. In 2010, the only crystal protein structure of the dopamine third receptor (D₃R) was reported after problems with membrane protein stability and crystallization had been overcome. In a previous study, we had obtained the D₃R complex protein structure based on the crystal structure to furthermore study the interaction between D₃R and dopamine and analyze how DA affects neurotransmitter function within the D₃R spatial structure, which is considered to be surrounded by seven transmembrane helices. On the basis of this complex protein, we had studied the molecular dynamics of DA moving along several directions within D₃R and obtained the trajectory of DA moving within D₃R, as shown in Figure 1.

Figure 1. Molecular channels for dopamine to transmit from the space among cell gaps arriving inside the space region of D₃R to play a function of neurotransmitter and then leaving from the internal structure to keep a normal physiological balance of dopamine.

In Figure 1, the transmembrane helix region of the receptor protein can form a functional space but it also constitutes a molecular pathway for the transport of dopamine molecules to move within the seven transmembrane helices. Molecular channels are divided into two categories, namely: (1) a functional molecular channel for transferring molecules to play an important role of function as neurotransmitter; and (2) a protective molecular channel to prevent excess molecular function from molecular damage and to play a role in body mental protection. Considering the role of these channels, it is reasonable to concur that all current pharmacological treatments for schizophrenia work by blocking dopamine receptors by means of blocking these functional molecular channels. Furthermore, schizophrenia may also occur if the free energy needed by DA to move along a protective channel increases or as a result of a protective channel blocked by the receptor protein conformational variation. Therefore, it is essential to maintain the smoothness of the DA protective channel to avoid dopamine hyperfunction or to prevent DA from excessive function as a neurotransmitter, which is a plausible new method for controlling and treating schizophrenia.

Because it is known that S-deoxyephedrine (SBD) acts on dopamine receptors in the CNS cells, it may also play a functional role by passing through the functional channel of a dopamine receptor or may prevent excessive molecular function by transmitting through the protective channel. In this article, we studied the trajectories and movement of SBD along the channels within D₃R and changes in free energy along the trajectories to investigate the kinetics and mechanism of SBD and its CNS pharmacological effects within dopamine receptors. Our results show SBD to have a very unique character preferably moving through a protective channel unlike some molecules preferably moving through the functional channel. SBD moves along the functional molecular channel within D₃R toward the outside of the cell, with a free-energy change of 171.7 kJ·mol⁻¹, whereas movement toward intracellular orientation induces a free-energy change of 275 kJ·mol⁻¹. Furthermore, SBD trajectories along the protective molecular channels within D₃R in the x+ , x− , z+, and z− axes toward spaces in the cellular membrane result in free-energy changes of 103.6 , 242.1 , 459.7 , and 127.8 kJ·mol⁻¹ respectively. In this article, the results suggest that SBD is more likely to leave the internal structure of D₃R between TM5 and TM6 along the protective channels.

■ RESULTS AND DISCUSSION

Research Systems of D₃R with SBD. Like some molecules reported in our previous articles, SBD is not a large molecule because large molecules are not accessible within dopamine molecular channels. In addition, SBD is a molecular state because the charged residues along the molecular channels have a very strong interaction with the ion state of molecules. If molecules are the ion states, they cannot move along the channels. On the basis of the D₃R–DA complex protein structure, using dopamine as a pointer molecule, the SBD molecule is docked into D₃R to form the SBD–D₃R complex protein, which is performed for 20 ns of molecular dynamics simulation, whose results are shown in Figure 2 (right). Because the D₃R protein structure originates from the crystal structural data, for modifying the crystal protein structure, 20 ns of MD simulation may be available. In 2011, we had demonstrated that the 300 ns MD simulation of D₃R protein structure is not better than it for 20 ns of MD simulation to modify the crystal protein structure of D₃R by comparing the binding free energy between the dopamine molecule and D₃R protein. Generally, it is known that the crystal protein structure without MD modification of simulation does have structural problems but if the MD
simulation modification time is too long, it will create new problems; at the same time, it will make the protein structure lose a lot of important protein crystal data. Therefore, the basic characteristics of the binding free energy between SBD and D3R protein should be maintained for both the 20 ns simulated protein and the protein in the nature. SBD is a chiral molecule with S chiral configuration, whose molecular structure is also shown in Figure 2.

Figure 2 also shows that SBD and ETQ (eticlopride) have similar binding sites within D3R. In a previous study, we determined the active site residues via quantum chemical calculations.20 In the D3R structure, the seven residues that act as active sites for SBD include Asp75, Asp110, Cys114, Trp342, Phe346, Gly372, and Tyr373. The remaining six residues except Asp75 are located about 6 Å around the ETQ molecule (Table 1), whereas similar various amino acids act as the active sites for SBD and ETQ. However, the SBD molecule is located in the inner cavity of D3R. ETQ is an antischizophrenia drug whose active cavity is located within D3R, as shown in Figure 2. This target of the cavity also should be the active site of SBD for binding to D3R.

Figure 3 displays the system structure, which contains a total of 23,484 atoms. In addition to D3R and SBD, there are 84 1-palmitoyl-2-oleoyls-glycero-3-phosphatidylcholine (POPC) phospholipid molecules, 5438 water molecules, and 8 Cl− ions that neutralize the positive charge of the system.

Table 1. Residues within 6 Å (0.6 nm) of the D3R Structure around ETQ and SBD, the Red Letters Indicating the Active Residues for SBD

| SBD  | ETQ  | SBD  | ETQ  | SBD  | ETQ  | SBD  | ETQ  |
|------|------|------|------|------|------|------|------|
| Ile43| Asp10| Asp10| Phe188| Phe346| Phe346| Asp110| Val111|
| Leu71| Met112| Met112| Ser192| Ser193| Thr353| Met113| Cys114|
| Ala78| Asp75| Asp75| Val194| Val194| Tyr365| Met114| Cys114|
| Asp75| Met115| Asp75| Val194| Val194| Tyr365| Leu76| Cys115|
| Cys114| Thr115| Tyr373| Ser196| Ser196| Thr368| Cys114| Thr115|
| Val78| Ala116| Val78| Ala116| Val78| Ala116| Ala116| Val78|
| Ala79| Ser117| Ser117| Tyr198| Tyr198| Thr369| Ser117| Ser117|
| Val82| Val82| Val82| Ala337| Ala337| Phe371| Val82| Val82|
| Met83| Met83| Met83| Val86| Val86| Val86| Met83| Met83|
| Val86| Leu168| Leu168| Trp342| Trp342| Tyr373| Leu168| Leu168|
| Leu89| Cys181| Cys181| Trp342| Trp342| Tyr373| Cys181| Cys181|
| Phe106| Ser182| Ser182| Ile343| Ile343| Asn375| Ser182| Ser182|
| Val107| Ile183| Ile183| Pro344| Pro344| Ser376| Ile183| Ile183|
| Val107| Ile183| Ile183| Phe345| Phe345| Ser376| Val107| Val107|

Figure 3 (right panel) shows the plot of RMSD versus 20 ns MD simulation for the D3R complex system, with SBD within the double-layer POPC-H2O membrane, D3R, and SBD. The values of RMSD demonstrate that after 2.5 ns of MD simulation, the D3R–SBD system reached stable equilibrium with background values of ±0.01 nm located at 4.11 nm, ±0.01 nm located at 0.29 nm for D3R, and ±0.01 nm located at 0.14 nm for SBD. On the basis of the molecular dynamics simulation, these results show that the system achieved a balanced equilibrium and can be used as the initial structure to study the trajectories of SBD moving within D3R.

Trajectory and Free-Energy Potential Surface of SBD Moving along the Functional Molecular Channel within D3R. On the basis of the D3R–SBD complex optimized structure obtained by molecular dynamics simulation, we study six trajectories of SBD moving within D3R (Figure 4). The direction of the y+ axis is defined as the direction of SBD moving from the internal to external structure of D3R, and the y− axis refers to the movement of SBD from the internal to intracellular structure of D3R. The directions along the x+, x−, z+, and z− axes are the directions of SBD moving from D3R into the middle of the cell membrane. The most probable direction of SBD movement into the cellular membrane space can be determined by taking these four directions into consideration.
In the y+ axis direction, Leu95 is selected as the reference group because it is near the SBD molecule in the reverse direction of motion. Because there is a relatively large space for the movement of water molecules and phospholipids in the system, such substances are not suitable to be used as the reference group. A maximum external force is set to 2000 kJ·mol⁻¹, which forces SBD to move along the y+ axis direction and permeate out of the cell. However, in the actual process, procedures are based on a system that will provide a suitable external force of less than 2000 kJ·mol⁻¹, prompting SBD to move and generate the trajectories of the coordinates. Figure 4 shows the structural figures of SBD’s four-point systems on the trajectory, where o point is the starting point system. After the a and b point systems reach the c point trajectory system, the SBD molecule moves out of D₃R through a molecular pathway. Along the y+ axis direction, from the SBD trajectory coordinate file, 50 samples are selected to simulate rebalance of the sample system; these samples are listed in Table 2. In the SBD trajectory file, data of 1001 points are to be preserved in different directions but are not necessary to use to simulate the umbrella samples. The 1001 trajectory points of the conformational data correspond to the length of 1000 ps molecular simulation, in which every 1 ps extracts a point of trajectory conformation data. Therefore, the sample number corresponds to the length of the trajectory simulation (ps unit). Using trajectory point system conformation data, centroid distances between the reference and motion groups can be calculated in different numbers of samples. According to the center distance of mass between SBD and the reference system, the selection of samples increases by about 0.05 nm. In different regions, because of the change of a given force, SBD displays rotational motion in addition to translational motion, resulting in a large change in the distance between the reference and moving groups, even if the centroid distance variations in adjacent umbrella samples are greater than 0.05 nm. During molecular motion, the reference frame of the system and centroid position of the moving group both change, restricting the actual distance in accordance with the 0.05 nm integer times; or in other words, the sample interval is not fully consistent with the 0.05 nm interval. For the selected 50 samples, the centroid distance between the motion and reference groups is respectively fixed; then, after 800 ps of molecular dynamic simulation, the SBD molecule and whole system are rebalanced, which are characterized using RMSD values. The results of this study are similar to those of previous studies, which also reported that the RMSD values of each sample system were maintained between ±0.02 and the ground value from 600 to 800 ps to show the achievement of a balanced sample system after 400 000 steps of molecular dynamics simulation (the data is not given in this article). Although the external force needs to form the trajectory caused the system to deviate slightly from the equilibrium, this deviation is not much different from that of the original system, meaning the system can be quickly rebalanced.

Although many samples are selected along the two trajectories and the molecular dynamics simulation is used to realize the rebalance, the simulations still belong to the biased results from the limited samplings. Therefore, the weighted histogram analysis method (WHAM) in Gromacs 4.5 program is adopted to convert the results of biased sampling into unbiased sampling. Then, the mean force potential (PMF) is calculated from the results of a series of umbrella samples to obtain the free-energy change diagram in Figure 4.

The VMD program is used to demonstrate and analyze the trajectory of SBD. Using the distance between the sample and reference in the trajectory listed in Table 2 and considering the

Figure 3. (Left panel) Research materials: the complex structure of D₃R with SBD, including the double-layer phospholipid POPC-H₂O membrane; (right panel) the figure of root-mean-square deviation (RMSD) vs 20 ns MD simulation for the total system, D₃R and SBD.
free-energy change of SBD on the trajectory coordinates, the locations of SBD within D3R can be determined. For the sake of simplicity, in each trajectory, only four key positions of SBD’s image are given. Along the $y^+$ axis direction in Figure 4, the SBD trajectory starts at $o$ after point (1.38 nm) and $b$ point (2.16 nm) to reach $c$ point (3.49 nm) position, where it leaves out of the D$_3$R internal structure. The figure shows that the free energy of SBD on the trajectory is 171.7 kJ mol$^{-1}$.

In Figure 5, Ser156 is selected as the reference system and the 44 samples are listed in Table 3. Along the $y^-$ axis direction, SBD begins at starting point $o$, after point (2.47 nm) and $b$ point (3.06 nm) to reach $c$ (3.54 nm) position, and arriving at point $c$; (right below panel): the potential of the mean force (PMF) of the track along the $y^+$ axis for SBD to move.

Table 2. Umbrella Samplings Obtained by SBD Moving within D$_3$R along the $+y$ Axis$^a$

| $n$ | $D$/nm | $n$ | $D$/nm | $n$ | $D$/nm | $n$ | $D$/nm |
|-----|--------|-----|--------|-----|--------|-----|--------|
| 3   | 1.01   | 114 | 1.74   | 157 | 2.48   | 200 | 3.05   |
| 10  | 1.06   | 116 | 1.80   | 158 | 2.58   | 205 | 3.11   |
| 28  | 1.11   | 118 | 1.85   | 165 | 2.63   | 210 | 3.16   |
| 49  | 1.16   | 125 | 1.90   | 169 | 2.68   | 221 | 3.21   |
| 64  | 1.21   | 139 | 1.93   | 175 | 2.73   | 228 | 3.26   |
| 77  | 1.26   | 147 | 2.01   | 180 | 2.79   | 231 | 3.39   |
| 93  | 1.31   | 149 | 2.05   | 182 | 2.85   | 233 | 3.40   |
| 104 | 1.36   | 151 | 2.27   | 187 | 2.90   | 236 | 3.51   |
| 107 | 1.41   | 153 | 2.36   | 195 | 2.95   | 237 | 3.56   |
| 111 | 1.54   | 155 | 2.43   | 198 | 2.99   | 250 | 3.60   |

$^a$Umbrella sampling chosen according to the distance of 0.05 nm between the sampling and reference groups from the track file recorded for SBD to move along the $+y$ axis, $n$: the serial number recorded in the track file; $D$: the distance between the sampling and reference groups. The reference group is Leu95.
which basically leaves out of the internal structure of D3R. In Figure 5, data show that the free-energy change required for SBD to leave out of the internal structure of D3R toward the intracellular position is 275.0 kJ·mol\(^{-1}\).

One reason for the difference in free energy between the \(y^+\) and \(y^-\) axes may be derived from the initial structural conformation of the D3R crystal protein. Because the outer end of the protein is combined with an antagonist, it creates an open protein structure. Another reason is that the molecular channel itself is not used to transport SBD molecules from the external environment into the cell. SBD itself needs to permeate through the cell membrane for playing a functional role, unlike dopamine through the synaptic transfer mainly through the dopamine transporter to complete transport through the cell membrane back to the inside cell, consistent with the results observed in some experiments.26–28

**Figure 5.** (Up panels): Four pictures of the tracks along the \(y^-\) axis for SBD to move from point \(o\) passing \(a\) and \(b\) and arriving at point \(c\) (below panel): the potential of mean force (PMF) of the track along the \(y^-\) axis for SBD to move.

**Table 3. Umbrella Samplings Obtained by SBD Moving within D3R along the \(y^-\) axis**

| \(n\) | \(D/\text{nm}\) | \(n\) | \(D/\text{nm}\) | \(n\) | \(D/\text{nm}\) | \(n\) | \(D/\text{nm}\) | \(n\) | \(D/\text{nm}\) |
|---|---|---|---|---|---|---|---|---|---|
| 2  | 1.51 | 76  | 1.99 | 124 | 2.45 | 180 | 2.95 | 214 | 3.40 |
| 10 | 1.58 | 81  | 2.05 | 140 | 2.49 | 184 | 3.01 | 221 | 3.45 |
| 21 | 1.64 | 93  | 2.10 | 144 | 2.53 | 187 | 3.05 | 226 | 3.49 |
| 30 | 1.69 | 95  | 2.15 | 152 | 2.60 | 195 | 3.10 | 230 | 3.54 |
| 41 | 1.74 | 101 | 2.20 | 161 | 2.65 | 197 | 3.15 | 236 | 3.60 |
| 54 | 1.79 | 105 | 2.26 | 166 | 2.70 | 200 | 3.20 | 238 | 3.65 |
| 57 | 1.85 | 111 | 2.30 | 169 | 2.76 | 201 | 3.24 | 238 | 3.69 |
| 61 | 1.89 | 115 | 2.35 | 178 | 2.81 | 205 | 3.30 | 289 | 3.72 |
| 65 | 1.94 | 117 | 2.40 | 179 | 2.91 | 207 | 3.36 |        |        |

*The reference group is Ser156.*
Figure 6 shows that SBD moves along the x+ axis into the space trajectory of the bilayer membrane, where the reference group is a Leu15 residue, and the selected 39 sample systems are listed in Table 4. Combining the main and top views in Figure 6, it can be seen that SBD starts from the start point of o along the x+ axis, after a point (1.88 nm) and b point (2.23 nm) to reach the c point (3.34 nm) position. From the slit of TM5−TM6 between the two transmembrane helices, SBD exits the internal structure of D3R. Along the x+ axis trajectory, the free-energy change of SBD movement in this process is determined to be 103.6 kJ·mol⁻¹.

Table 5 lists 35 samples of SBD selected along the x− axis, where the reference group is a Val173 residue. Figure 7 displays the trajectory of SBD moving along the x− axis into the cell membrane. The main view and top views in Figure 7 both show that SBD starts from the start point of o along the x− axis direction, after a point (2.20 nm) and b point (2.78 nm) to reach the c point (3.21 nm) position. From the slit of TM1−TM7 between the two transmembrane helices, SBD leaves the D3R internal structure. Along the x− axis trajectory, the free-energy change of the process is determined to be 242.1 kJ·mol⁻¹ for SBD movement.

Figure 8 shows that SBD moved along the z+ axis and entered the space trajectory of the bilayer membrane, in which the reference group is a Val251 residue and the 50 sample systems are listed in Table 6. In the main and top views in...
Table 4. Umbrella Samplings Obtained by SBD Moving within D3R along the x+ Axis

| n  | D/nm | n  | D/nm | n  | D/nm | n  | D/nm |
|----|------|----|------|----|------|----|------|
| 2  | 1.64 | 85 | 2.21 | 114 | 3.10 | 185 | 3.75 |
| 10 | 1.69 | 88 | 2.35 | 128 | 3.16 | 195 | 3.80 |
| 15 | 1.74 | 90 | 2.41 | 133 | 3.22 | 201 | 3.85 |
| 21 | 1.79 | 96 | 2.46 | 138 | 3.28 | 315 | 3.90 |
| 32 | 1.84 | 99 | 2.52 | 143 | 3.35 | 355 | 3.95 |
| 46 | 1.89 | 101| 2.70 | 143 | 3.47 | 442 | 4.00 |
| 61 | 1.94 | 104| 2.81 | 145 | 3.52 | 544 | 4.05 |
| 67 | 1.99 | 107| 2.83 | 151 | 3.56 | 602 | 4.10 |
| 79 | 2.04 | 109| 2.89 | 171 | 3.62 | 720 | 4.14 |
| 80 | 2.12 | 110| 2.95 | 179 | 3.68 |      |      |

“The reference group is Leu15.

Table 5. Umbrella Samplings Obtained by SBD Moving within D3R along the x− Axis

| n  | D/nm | n  | D/nm | n  | D/nm | n  | D/nm |
|----|------|----|------|----|------|----|------|
| 3  | 1.62 | 84 | 2.06 | 139 | 2.57 | 183 | 3.21 |
| 10 | 1.65 | 95 | 2.11 | 157 | 2.62 | 184 | 3.24 |
| 18 | 1.70 | 98 | 2.18 | 162 | 2.67 | 185 | 3.30 |
| 30 | 1.75 | 99 | 2.23 | 164 | 2.72 | 186 | 3.35 |
| 46 | 1.80 | 101| 2.34 | 167 | 2.79 | 192 | 3.37 |
| 56 | 1.85 | 105| 2.38 | 169 | 2.93 | 281 | 3.40 |
| 64 | 1.91 | 113| 2.41 | 174 | 3.03 | 927 | 4.14 |
| 69 | 1.97 | 116| 2.47 | 179 | 3.07 | 979 | 3.46 |
| 71 | 2.01 | 128| 2.52 | 182 | 3.11 |      |      |

“The reference group is Val173.

Figure 8, it can be seen that along the z+ axis, SBD started at the start point of o, after a point (1.14 nm) and b point (1.69 nm), to reach the c point (3.02 nm) position. From the slit of TM1−TM2 between the two transmembrane helices, SBD exits the D3R internal structure. Along the z+ axis trajectory, the free-energy change of the process is 459.7 kJ·mol⁻¹ for SBD movement.

In Figure 9, it can be seen that SBD move along the z− axis and entered the space trajectory of the bilayer membrane. For this axis, we select a Leu51 residue as the reference group and 45 samples, which are listed in Table 7. Combining the main and top views in Figure 9 reveals that SBD begins at start point o along the z− axis direction, after a point (1.51 nm) and b point (1.97 nm), to reach the c point (3.16 nm) position. SBD then exits the internal structure of D3R from the slit of TM6−TM7 between the two transmembrane helices. Along the z− axis trajectory, the free-energy change of the process is 127.8 kJ·mol⁻¹ for SBD movement.

When SBD moves along the molecular channels within D3R, there is a certain degree of flexibility. It is also determined that within a certain range, the SBD molecules are always moving along the direction of less energy. When a transmembrane helix is in front of the SBD trajectory, the direction of SBD changes slightly and moves from the transmembrane helix to the gap to exit the cell. The trajectories along the y axis direction are farthest from the membrane’s helix column gap and move toward the internal structure, unless subjected to the ring structure. By studying the four directions through x+, x−, y+, and z− axes, it should be possible to cover the most likely trajectories of SBD movement into the space within cell bilayer membrane. Therefore, for SBD movement along the protective molecular channels within D3R, the most probable trajectory of SBD is to leave the D3R internal structure between the TM5 and TM6 gap along the x+ axis, with a free-energy change of 103.6 kJ·mol⁻¹.

When free dopamine molecules are located at cell synaptic clefts, they bind with different subtypes of dopamine receptors on the posterior membranes to initiate signal transmission and play the role of a neurotransmitter to control functions of movement, cognition, emotion, etc. During dopamine transport, dopamine molecules are easily combined with dopamine receptors along the functional molecular channels to act as neurotransmitters. The protective molecular channel provides a pathway for the dopamine molecules to remove themselves from the receptors after functioning as neurotransmitters. If there is no way for dopamine molecules to leave or if the free energy of the passage for dopamine becomes larger under certain conditions, dopamine molecules continually combine with their receptors and maintain as neurotransmitters. To prevent this from occurring, dopamine molecules need to move along a trajectory of less free energy. Our previous researches show that in the D3R structure, dopamine molecules are most likely to leave out of the D3R internal structure between the TM5 and TM6 gaps along the x+ axis, with a free-energy change of 65.8 kJ·mol⁻¹. From this presented study, our results reveal that 103.6 kJ·mol⁻¹ is the minimum value of free-energy change for SBD to move along the x+ axis in the molecular channels and exit the internal structure of D3R from the gap of TM5−TM6 between two transmembrane helices. Before then, SBD first permeates into the inner cells and from the inner cells moves toward the active pocket along the reverse direction of y− axis, which is a spontaneous process with free energy of −275 kJ·mol⁻¹. In the structure, the space of the gap between TM5 and TM6 between is much wider than other gaps, which is viewed from the complex D3R protein with SBD. It has been demonstrated that it is easier for SBD to leave the D3R interior from the TM5 and TM6 gap along the protective molecular channel because the movement of SBD molecules along the functional channel requires a high free-energy change of 171.7 kJ·mol⁻¹. Considering that the exit path for SBD molecules out of D3R is the same as that for dopamine out of the D3R internal structure, SBD can “dredge” the protective molecular channel to increase the movement of dopamine out of the internal structure of D3R because SBD as a drug is of a high concentration relative to dopamine and has a selective movement along the protective molecular channel. This will also help to reduce the presence of dopamine within D3R and prevent dopamine molecules from excessively functioning as neurotransmitters, which contributes to the severity of schizophrenia symptoms.

Besides D3R, D1R, D2R, D4R, and D5R are all important systems for researches of schizophrenia and Parkinson’s disease. D3R has strong homology with them and much common characteristics with them. At the first stage, because D3R is only one crystal protein structure of dopamine receptors, it is important for D3R used as a representative of them to study the free-energy changes for SBD moving along the molecular channels within D3R. In the crystal structure of D3R protein, for obtaining the stable protein crystal, the researchers used ETQ, an antagonist that is designed to stabilize the protein structure and facilitate protein crystallization. However, ETQ does not prevent dopamine molecules from excessively functioning as neurotransmitters, which contributes to the severity of schizophrenia symptoms.

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energy for SBD moving along the channels within the simulated D3R, D2R, D4R, and D5R protein structures to investigate the different characteristics for SBD moving in these channels.

It is noted that our study idea is different from the traditional blocking method of representing antipsychotic activity. Our strategy to study schizophrenia is through dredging rather than blocking dopamine molecular channels. This result is characterized by SBD moving along the protective channel in the change of free energy being lower at 68.1 kJ·mol⁻¹ than it is along the functional channel, and animal experiments have demonstrated its efficacy for the treatment of schizophrenia.

Similarly, we found three molecules preferentially moving through the functional channel because the free-energy barrier for them moving along the functional channel is lower at 60 kJ·mol⁻¹ than it is for them moving along the protective channel, and animal experiments proved them to be effective for the treatment of Parkinson’s disease.

In addition, the method of umbrella sampling is widely used to study changes of free energy for some molecules moving along the molecular passages, which is a typical method, by which a lot of work has been published. For the interaction energies between SBD and the active sites of D3R, we use

Figure 7. (Up panels): Four pictures of the tracks along the x-axis for SBD to move from point o, passing a and b, arriving at point c from within D3R into the POPC phospholipid bilayer membrane on the main view and top view, respectively; (below panel): the potential of mean force (PMF) of the track along the x-axis for SBD to move.
quantum chemical calculation, an effective and useful method, by which many articles have also been published. More details in consistence with some corresponding experiments are described in the section of Methods and some references.

**CONCLUSIONS**

The free-energy change of 103.6 kJ·mol⁻¹ shows that SBD molecules are more easily accessible along the protective channels within D₃R. The access of SBD molecules is significant because this can help clear protective channels within the D₃R structure and aid removal of dopamine molecules out of the D₃R internal structure along the z⁺-axis, which prevents excessive neurotransmitter functioning of dopamine. Therefore, SBD has good potential as a drug for schizophrenia treatment and its pharmacology is closely related to its transmission dynamics along molecular channels and mechanism of transmission channels within dopamine receptors.

**METHODS**

Details of the D₃R complex protein structure used in this study are based on results of our previous study. This D₃R complex protein with DA was obtained using docking technology and molecular dynamics based on the crystal structure of mutated D₃R (crystal number: 3PBL), in which the original protein crystal structure for point mutations of
Table 6. Umbrella Samplings Obtained by SBD Moving within D3R along the z+ Axisa

| n  | D/nm | n  | D/nm | n  | D/nm | n  | D/nm |
|----|------|----|------|----|------|----|------|
| 3  | 0.92 | 147| 1.42 | 210| 1.97 | 246| 2.54 | 287| 3.12 |
| 19 | 0.97 | 151| 1.47 | 214| 2.01 | 248| 2.58 | 296| 3.16 |
| 37 | 1.02 | 156| 1.52 | 216| 2.07 | 250| 2.63 | 318| 3.21 |
| 91 | 1.07 | 164| 1.57 | 217| 2.12 | 253| 2.77 | 328| 3.25 |
| 111| 1.12 | 173| 1.61 | 218| 2.24 | 257| 2.82 | 667| 3.30 |
| 119| 1.17 | 181| 1.66 | 220| 2.31 | 258| 2.85 | 673| 3.35 |
| 126| 1.22 | 187| 1.71 | 221| 2.34 | 264| 2.90 | 699| 3.40 |
| 135| 1.27 | 198| 1.76 | 224| 2.40 | 274| 2.95 | 836| 3.45 |
| 140| 1.32 | 203| 1.81 | 230| 2.45 | 278| 3.00 | 839| 3.50 |
| 143| 1.38 | 204| 1.83 | 231| 2.50 | 285| 3.05 | 846| 3.52 |

aThe reference group is Val251.

Table 6. Umbrella Samplings Obtained by SBD Moving within D3R along the z+ Axis

The reference group is Val251.

Table 6. Umbrella Samplings Obtained by SBD Moving within D3R along the z+ Axis

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groups, so that the SBD molecule and total system were rebalanced. The input files for simulation of the umbrella samples included the sample, topological, and pointer files with the parameter file. The parameters for the simulation of the umbrella samples were the same as those for the simulation of trajectories but with a speed set to 0 nm·ns⁻¹, indicating that the SBD molecule does not move along the trajectory; considering the distance between the two groups, rebalancing of the molecular dynamics simulations of the samples were determined using RMSD values, where simulations steps were set to 400 000 (800 ps) with a step length of 2 fs. Finally, from the biased sampling results of partial sampling into no statistical results and from a series of umbrella sample simulations, the weighted histogram analysis method from Gromacs 4.5 was used to extract the potential of mean force (PMF) with a calculation deviation of 10⁻⁶ kJ·mol⁻¹ (convergence calculation

Figure 9. (Up panels): Four pictures of the tracks along the z− axis for SBD to move from point o passing a and b arriving at point c from within D3R into the POPC phospholipid bilayer membrane on the main view and top view respectively; (below panel): the potential of mean force (PMF) of the track along the z− axis for SBD to move.
which means good overlap of the histogram no occurrence of a zigzag type PMF potential energy surface, of PMF. On the contrary, our research results repeatedly show not good, it will produce a zigzag type potential energy surface. The free energy barriers were determined to be 115.0, 99.1, and 92.0 kJ·mol⁻¹. Another PMF method can be used to simulate the permeation of water molecules through the modeled POPC biological membranes, their interaction in schizophrenia - therapeutic implications. The authors declare no competing financial interest.

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