Molecular dynamics simulation of α-Benzedrine transmitting through molecular channels within D₃R

Wei Xie, Ming Wang, Aijing Li and Si-Chuan Xu*

Key Laboratory of Education Ministry for Medicinal Chemistry of Natural Resource, College of Chemical Science and Technology and Pharmacy Academy, Yunnan University, Kunming 650091, China

Communicated by Ramaswamy H. Sarma
(Received 3 April 2016; accepted 14 May 2016)

Dex-Benzedrine (known as α-Benzedrine or SAT) acts in dopamine receptors of central nerve cell system. In clinic, SAT is used to treat a variety of diseases; meanwhile, it has dependence and addiction. In order to investigate the pharmacology and addiction mechanisms of SAT as a medicine, in this paper, we have studied the structure of D₃R complex protein with SAT, and based on which, using potential mean force with umbrella samplings and the simulated phospholipid bilayer membrane (or POPC bilayer membrane), the molecular dynamics simulation was performed to obtain free energy changes upon the trajectories for SAT moving along the molecular channels within D₃R. The free energy change for SAT transmitting toward the outside of cell along the functional molecular channel within D₃R is 83.5 kJ mol⁻¹. The change of free energy for SAT to permeate into the POPC bilayer membrane along the protective molecular channel within D₃R is 87.7 kJ mol⁻¹. Our previous work gave that the free energy for Levo-Benzedrine (RAT) transmitting toward the outside of cell along the functional molecular channel within D₃R is 91.4 kJ mol⁻¹, while it is 117.7 kJ mol⁻¹ for RAT to permeate into the POPC bilayer membrane along the protective molecular channel within D₃R. The values of free energy suggest that SAT relatively prefers likely to pass through the functional molecular channel within D₃R for increasing the release of dopamine molecules resulting in a variety of functional effects for SAT. The obtained results show that the pharmacology and addiction mechanisms of SAT as a drug are closely related to the molecular dynamics and mechanism for SAT transmitting along molecular channels within D₃R.

Keywords: SAT; D₃R; POPC; MD simulation; free energy

1. Introduction

Dex-Benzedrine (known as α-Benzedrine or SAT), its molecular structure shown in Figure 1, is clinically used to treat narcolepsy, fatigue, chronic alcoholism, and obesity. SAT is a kind of central stimulants, acting in dopamine receptors of central nerve cell system, which also has dependence and addiction. The pharmacological effects of SAT is considered as the same as Benzedrine, but its excitant effects in central nervous system and its inhibition of appetite both are stronger than those from Benzedrine, equivalent to two times of Benzedrine. The inhibition of appetite by SAT is equivalent to 3–4 times by Levo-Benzedrine (known as α-Benzedrine or RAT).

In central nervous cell system, there are five major DA receptor subtypes (D₁–D₅ receptors) all belonging to the members of a superfamily of proteins called G-protein-coupled receptors (GPCRs) which are all composed of seven transmembranes (7-GM, or TM). At the beginning of 1990s, with the development of biological cloning technology, five distinct DA receptors (D1–D₅ receptors) were cloned and defined as D₁R, D₂R, D₃R, D₄R, and D₅R, respectively (Bunzow et al., 1988; Dearry et al., 1990; Sokoloff, Giros, Martres, Bouthenet, & Schwartz, 1990; Sunahara et al., 1991; Van Tol et al., 1991). Among them, D₂R, D₃R, and D₄R belong to D₂-like receptor, while D₁-like receptor includes D₁R and D₃R. D₄R and D₂R are abundant in the brain, relatively more studied, and with more understanding the significance of their physiological function (Kebabian & Calne, 1979). The contents of D₁R, D₃R, and D₄R in brain are much lower than those of D₂R and D₂R, and currently it is a focus to study their structure, function, and application to finding drugs (Bontempi & Sharp, 1997; Carlsson, Waters, Waters, & Carlsson, 2000; Gandhimathi & Sowdhamini, 2016; Kakarala & Jamila, 2016; Li, Shu, & Bao, 2003; Nowroozi & Shahlaei, 2016; Plante-Bordeneuve et al., 1997; Salum, Roque da Silva, & Pickering, 1999; Suri, Bargas, & Arbib, 2001; Xiao et al., 2015; Xu et al., 1997).

In the human brain, there are hundreds of billions of nerve cells. SAT molecules arriving into nerve cells through cell membranes only transmit through the directional action in order to play their functions like dopamine molecules produced in the nerve cells.
Because there is a cliff gap between nerve cells and nerve cells, like a crack between two cliffs, SAT molecules skip the crack through the nerve cells at a prominent small cliff called synapses for passing through the gap in order to play a functional role. The main ingredients of synapses include all kinds of dopamine subtype receptors. Within the dopamine receptor structures there are molecular channels (Bian, Shi, & Xu, 2012; Xu, Shi, & Chi, 2012; Zhang, Bian, Shi, & Xu, 2014). Molecular channels are divided into two categories defined, respectively, as (1) functional molecular channel: to transfer molecules and to play effects of function; (2) protective molecular channel: to prevent excess molecular function from molecular damage and then to play a function of protective body. Since SAT acts in dopamine receptors of the central nervous system, it should pass through molecular functional channel within dopamine receptors to play its functional effects, or pass through protective molecular channel within dopamine receptors to prevent excess molecular function from molecular damage. Therefore, to study SAT transmitting through molecular channels within D3R would help to understand the role of SAT’s pharmacological mechanisms and kinetics in nerve cell systems.

In this paper, the movement of SAT is characterized by free energy changes for SAT transmitting along molecular channels within dopamine receptors. The free energies for SAT moving along the molecular channels can be experimentally measured or obtained by molecular dynamics simulations. Based on the protein structure of D3R and the simulated phospholipid bilayer membrane (or POPC bilayer membrane), we will study and obtain the change of free energy for SAT moving along the molecular channels within D3R using potential mean force (PMF) with umbrella samplings to probe the mechanism and molecular dynamics of SAT moving along the molecular channels.

2. Materials and methods

Due to difficulty of crystallization and problem of stability for dopamine receptor proteins, among them only a crystal protein structure of D3R currently was reported with a protein code of 3PBL (Chien et al., 2010; De Paulis, Hall, & Ogren, 1985; Griffon, Pilon, Sautel, Schwartz, & Sokoloff, 1996; Rosenbaum et al., 2007; Roth, Hanson, & Stevens, 2008). In fact, the 3PBL crystal protein can be considered as a mutated crystal protein, because the existence of multiple point mutations in this protein is designed in order to obtain a stable D3R protein crystal. In addition, a series of hydrophilic residue groups designed with T4-lysozyme for feasible crystallization is outreached to supersede most of the third cytoplasmic loop (ICL3) (Arg222–Arg318). Further its stabilization is carried out with the antagonist eticlopride, a potent D2R/D3R antagonist introduced into the purified protein.

In our previous work, based on the 3PBL protein structure, we had studied and built an original human D3R complex structure with dopamine (DA) by docking technology and molecular dynamics simulation (Jin et al., 2011). Because five subtype dopamine receptors have high homology, D3R protein structure can be used as a representative of dopamine receptors to investigate free energy for SAT moving along the molecular channels within dopamine receptor structures. Therefore, the built D3R–DA complex structure reported in our previous work is exploited as a material of D3R protein to work in this paper (Jin et al., 2011).

Bio-membrane is an important external cause of stability for membrane protein structure. Similar to our previous study (Jin et al., 2011), this work is taking a phosphatidyl-choline molecule: 1-palmitoyl-2-oleoylsn-glycerol-3-phospha-tidyl-cho-line (POPC) to build a lipid bilayer membrane. It contains an unsaturated double C=C bond in the end of POPC, compared with the saturated phospholipid molecules at the end, which is preferred to simulate the biological cell membrane (Dunkin, Pokorny, Almeida, & Lee, 2011; Hoff, Strandberg, Ulrich, Tieleman, & Clemens, 2005; Janosi & Gorfe, 2010; Su & Wang, 2011). A pre-equilibrated and well-characterized structure of the POPC membrane model reported in the literature (Hoff et al., 2005) we use in this paper contains 128 POPC molecules and SPC model water (Marrink, Lindahl, Edholm, & Mark, 2001; Miyamoto & Kollman, 1992).

2.1. SAT molecule docked with D3R

For docking work, we used similar methods and steps reported in literatures (Chi, Xie, Zhang, & Xu, 2015; Shi et al., 2012; Wang et al., 2011; Xu et al., 2012). Using MP2/6-31G (d,p) method to optimize the molecular structure of SAT with no imaginary frequency as the optimization standard (Frisch et al., 2003), SAT was then docked into D3R to replace DA molecule by the Dock6 program (Lang et al., 2006).

2.2. Molecular dynamics simulation for D3R–SAT complex protein

For the D3R–SAT protein obtained by docking, we used the same steps reported in the literature...
(Jin et al., 2011) to inlay it into the POPC membrane model. Using the Gromacs 4.5.3 software package, the MD simulation was performed with visual molecular dynamics (VMD) program for analyzing simulated results (Berendsen, Van der Spoel, & Van Drunen, 1995; Hub, de Groot, Grubmüller, & Groenhof, 2014; Humphrey, Dalke, & Schulten, 1996; Van der Spoel et al., 2005). The steps of MD simulation are similar to those in our previous work (Chi et al., 2015; Xu et al., 2009). The force field parameters of POPC originated from the reported work were rewritten to be in consistence with the format of gmx force fields (Hoff et al., 2005). The force field parameters of SAT were individually defined using gmx force fields, while the parameters of residues for D3R were taken from the Gromacs package. The initial structure of SAT was optimized at the level of MP2/6-31G(d,p) with no imaginary frequency. The gmx-united atom types with Mulliken atomic charges were applied to define the parameters of SAT (Daura, Mark, & Van Gunsteren, 1998; Van Gunsteren et al., 1996), where the CH, CH2, and CH3 groups were used as single atoms. The parameters of SAT are available in the Supplementary Material with a proof obtained in a box of 3 × 3 × 3 nm for SAT with H2O molecules performed by 50 ns of MD simulation. The complex protein was put into a box (+y axis corresponding to extra-cellular orientation), where a buffer of 2.0 nm was given between protein atoms and the box edge. Using Na+ ion to neutralize the system, therefore, in the system there are two Na+ ions, 8207 water, 84 POPC, 279 residues, and one SAT, total 31,743 atoms. The complex protein geometry was minimized to meet a standard of 1000 kJ mol⁻¹ nm⁻¹ using the steepest descent method to remove bad van der Waals contacts between atoms, and then the position-restrained MD simulation was run for 200 ps. Water molecules move freely, while the LINCS algorithm was applied to restrain the equilibrium positions of solute bonds, in which at the condition of one standard atmosphere pressure, the temperature rose slowly from 0 to 310 K. Next, using 2 fs of time step, 50 ns of MD simulation was carried out and output into a file of coordinates written in every 2000 steps (4 ps) for calculating various energies and analyzing system structures. Non-bonding interactions in every 10 steps for a pair list of neighbors were calculated. Along the simulation box, periodic boundary conditions were applied with a cut-off distance (1.0 nm) both for short-range electrostatic interactions and for Lennard-Jones non-bonding interactions. A PME procedure was used to treat the long-range electrostatic effect with default parameters. The Berendsen thermostat for 310 K temperature was coupled with the time constant .1 ps. The Berendsen exponential relaxation pressures were coupled with the time constant of .5 ps.

2.3. Free energy surfaces for SAT moving along the trajectories within D3R

Based on the simulated D3R–SAT–POPC–H2O system, using PMF with umbrella samplings (Hess, Kutzner, Van der Spoel, & Lindahl, 2008), the molecular dynamics simulation was performed to obtain free energy changes upon the trajectories for SAT moving along the molecular channels within D3R. The principle of PMF is based on the formula: \( \Delta G = -RT \ln K \), in which \( \Delta G \) is free energy and \( K \) is equilibrium constant obtained by molecular simulation (Bian, Zhang, Wang, & Xu, 2014; Van der Spoel et al., 2013; Zhang et al., 2014). The initial structure of trajectory comes from the file of the simulated structure, and the topology necessary for the trajectory simulation is similar to its structural simulation. Along x, z, and y axes with positive and negative directions, six trajectories were obtained by simulating 500,000 steps in 2 fs every step at the simulated temperature of 310 K. SAT molecule was set up as a moving group, on which is exerted an external force of smaller than 2000 kJ mol⁻¹, with less than 10 nm ns⁻¹ moving speed. A residue in the very opposite direction of movement was selected as a reference group. After the simulation of trajectory, the appropriate umbrella samplings were selected from the data file of trajectory by a general rule of .05-nm distance. To generate the trajectories for SAT moving along the molecular channels, it is necessary that external forces are provided to make SAT move, which results in the molecular configuration and the D3R system to be deviated from their equilibrium. Therefore, it is necessary to perform the simulations of umbrella samplings, i.e. the distances between the moving and reference groups are limited to their original mass central distance, and then perform MD simulations to let the molecular configuration and the D3R system rebalanced. For the simulation of umbrella samplings, there are three files including the selected umbrella sampling files, the topology file and a new pointing parameter file. The parameters for simulations of umbrella samplings are almost the same as those of trajectory simulations, just only the velocity set to 0 nm ns⁻¹ indicating that SAT molecule do not move along the trajectory, but is limited within the given original mass central distance of two groups. The simulations were set to 400,000 steps (800 ps) in 2 fs every step for umbrella samplings rebalanced, which is determined by root mean square deviation (RMSD). After the simulations of umbrella samplings were completed, using Weighted Histogram Analysis Method (WHAM) (Hub, de Groot, & Van der Spoel, 2010) of the Gromacs 4.5 programs, the biased sampling results were converted to unbiased sampling of statistical results, and the PMF is drawn out from a series of umbrella samplings of statistical results. PMF surface is the free energy surface, on the basis of
which the changes of binding free energy (ΔGbind) can be calculated. The other files output from the g_wham calculations are the data files of histograms for umbrella samplings. The results of umbrella histograms show the overlapping degree of umbrella samplings, and the better overlapping implies the reliability of ΔG obtained by the calculation through WHAM.

3. Results and discussion

3.1. SAT molecule docked with D3R

In Figure 2 is the docking result. The main and top views both display that SAT molecule is docked into the cavity surrounded by seven transmembrane helices near middle but close to the extra-cellular position. This position for SAT is similar as dopamine is docked within D3R (Jin et al., 2011). The docking energy is −29.1 kJ mol⁻¹ for SAT molecule docked with D3R. If the positive-ion state of SAT is used to dock with D3R, its docking energy is 1.04 × 10⁴ kJ mol⁻¹, a positive energy to mean a repulsive energy, which indicates that molecular channels are not suitable for ion-state molecule, they are molecular channels, but not ion molecule channels. Therefore, in this paper, the neutral molecular state of SAT is used, while an ionic state of SAT is used in the system without protein to permeate through the bilayer membrane (Wang, 2015).

3.2. Molecular dynamics simulation of D3R–SAT complex protein

Figure 3 is a plot of RMSD vs. 50 ns MD simulation for the D3R–SAT complex protein within the bilayer POPC–H₂O membrane. The values of RMSD demonstrate that after 25 ns of MD simulation, the D3R–SAT structure reaches a balance of stability with the background values of ±0.01 nm located at 0.46 nm for D3R and 4.66 nm for the whole system. On the view of molecular dynamics simulation, the values of RMSD show that the system achieves a balance that can be used as the initial structure to study the trajectories of SAT moving within D3R.

3.3. Free energy potential surfaces for SAT moving along the trajectories within D3R

3.3.1. Free energy potential surfaces for SAT moving along the trajectories of functional molecular channels within D3R

For exogenous molecules, it is well known that they usually depend on their permeability through cell membrane...
arriving inside cell, and under normal conditions, any molecules with a strong ability to penetrate through the cell membrane would have the potentials as medicines. SAT has a strong ability to penetrate through cell membranes (Wang, 2015) corresponding to its functional role in clinic. On the basis of the refined D3R complex protein with SAT, we have studied six trajectories for SAT moving along molecular channels within D3R shown in Figure 4. The $+y$ axis is supposed for SAT moving from the interior of D3R toward the extra-cellular orientation, while the $-y$ axis is designed for SAT moving from the interior of D3R toward the intra-cellular direction. The $+x$, $-x$, $+z$, and $-z$ axes are considered for SAT moving from the interior of D3R toward the middle space of bilayer membranes of cell. Considering four directions, it may cover the most likely path for SAT entering the space of bilayer membrane.

Along the $+y$ axis, Leu129 is selected as a reference group for SAT moving. The selection of the reference group is upon the reverse direction as near as possible close to SAT. Because water and phospholipid molecules have relatively large movability in the system, they are not suitable to be used as a reference. Along the $-y$ axis, Ser242 is selected as the reference group for SAT. Their trajectories are shown in Figure 4, which are generated by a suitable external force of less than 2000 kJ mol$^{-1}$ allotted from the simulation program.

Figure 4. Six orientations for SAT moving within D3R, and the tracks toward the outside of cell along the $+y$ axis (down and left panel) and toward the inside of cell along the $-y$ axis.

Figure 5. PMF for SAT moving toward the outside of cell along the $+y$ axis and toward the inside of cell along the $-y$ axis with the histograms of umbrella samplings obtained by WHAM algorithm.
Along the +y axis, 66 samplings were selected from the trajectory data file by a rule of .05-nm mass central distance and used to simulate the free energy of SAT. In the trajectory file, there are 1001 trajectory points preserved along each direction, without necessity for all used to perform simulation. The 1001 trajectory data points correspond to 1000 ps time of MD simulation, i.e. every 1 ps keeping a trajectory point of system conformation data. In Supplementary Tables S1 and S2, besides the sampling numbers indicating the scope to calculate PMF, they also mean the simulation time length (in ps unit). Using the trajectory point systems, we calculate the mass central distances between the reference and moving groups for umbrella sampling. Due to the change of external force in different regions of system, SAT has not only rotational motion but also translational motion, leading to the mass central distance changes to be larger, so that even if two neighboring samplings, the changing value of their mass central distance is larger than .05 nm. It is a general rule using .5 nm to select umbrella samplings listed in Supplementary Tables S1 and S2.

Along the −y axis, 48 samplings were selected. These samplings, limited to their original mass central distance, are simulated to achieve both SAT molecule and D3R structural system rebalanced, which are determined by RMSD. For each axis, we chose four samplings as the representative to analyze the values of RMSD. The four representative sampling systems are shown in Figure 4 and labeled in o, a, b, c four trajectory points, also listed in Supplementary Tables S1 and S2 in bold type of letter. In Supplementary Figure S1, along the +y and −y axes, from 700 to 800 ps, the RMSD values of four representative samplings are maintained at the background values of ±.01 nm. The values of RMSD demonstrate that after 400,000 steps of MD simulation, the sampling systems have been rebalanced. For other samplings, the values of RMSD are similar, but their figures and data are not given in this paper. On the basis of rebalanced sampling systems, using WHAM, the biased sampling results are converted to unbiased sampling of statistical results, and PMF is drawn out from the statistical results.

Along the +x axis within D3R into the POPC bilayer membrane, PMF of the track, and the histograms of umbrella samplings.

Figure 6. The figures of tracks from main view (upper left panel) and top view (upper right panel) for SAT moving along the +x axis within D3R into the POPC bilayer membrane, PMF of the track, and the histograms of umbrella samplings.
The VMD program is used to display and analyze the SAT trajectories, as well as to identify and distinguish the various positions of SAT situating within D3R. For the sake of analysis, the images of four key positions for SAT moving along each channel are shown in Figure 5. Their labels in bold line and the distances of center mass are listed in Supplementary Tables S1 and S2. The distances of center mass shown in Figure 5 gained by WHAM have some deviations from the values calculated by the GROMACS tool. For example, along the +y axis, the minimum distance of 0.86 nm is obtained by WHAM in Figure 5, corresponding to the minimum distance of 1.02 nm from sampling 2 calculated by the GROMACS tool. In the trajectory of Figure 4, along the +y axis, SAT starts from the O point (0.86 nm shown in Figure 5), through the a point (2.43 nm) and then passing through the b point (3.29 nm) arriving at the c point (3.56 nm), where it leaves out of the D3R interior structure. The result shown in Figure 5 gives that the free energy change is 83.5 kJ mol⁻¹ for SAT moving along the +y axis. Along the +y axis, SAT starts from the O point (1.76 nm shown in Figure 5), through the a point (2.81 nm) and then the b point (3.38 nm) arriving at the c point (3.84 nm), where it leaves out of the D3R interior structure. The change of free energy in Figure 5 is 261.8 kJ mol⁻¹ for SAT moving along the −y axis trajectory.

3.3.2. Free energy potential surfaces for SAT moving along the trajectories of protective molecular channel within D3R

For the free energy surfaces for SAT moving along protective molecular channels within D3R, we have studied four trajectories along the +x, −x, +z, and −z axes all possible for SAT transferring from the interior structure of D3R into the space of bilayer membranes.

Figure 6 is the trajectory of SAT moving along the +x axis into the space of bilayer membrane, where Trp24 acts as a reference group. Figure 7 is the trajectory of SAT moving along the −x axis into the space of bilayer membrane, where Ala137 acts as a reference group. Figure 8 is the trajectory of SAT moving along the +z axis into the space of bilayer membrane, where Cys172 acts as a reference group. Figure 9 is the trajectory of SAT moving along the −z axis into the space of bilayer membrane, where Val56 acts as a reference group.

From the trajectory along the +x axis, 64 samplings are picked up and listed in Supplementary Table S3. From the trajectory along the −x axis, 56 samplings are selected...
up and listed in Supplementary Table S4. From the trajectory along the $+z$ axis, 67 samplings are chosen and listed in Supplementary Table S5. From the trajectory along the $-z$ axis, 55 samplings are picked up and listed in Supplementary Table S6. For all the selected samplings, the distances between the SAT moving and reference groups are limited to their original mass central distance, and using MD simulation they are rebalanced, which are characterized by the system’s RMSDs from four representative samplings. The respective samplings are shown in Figures 6–9 and labeled in o, a, b, c four trajectory points, or listed in Supplementary Tables S3–S6 in bold type of letter. These numerical results of RMSD are available in the Supplementary Material, see Figures S2–S5. In Figures S2–S5, from 700 to 800 ps, all the RMSD values from respective samplings are kept at the background values within the vibrating value of ±0.01 nm to demonstrate after through 400,000 steps of MD simulation the sampling systems all are rebalanced. For other samplings, the similar results are not given in the paper.

Also shown in Figures 6–9, respectively, are PMFs drawn out from the statistical results using WHAM, and the corresponding weighted histograms of umbrella samplings to respective SAT reaction coordinates with good overlapping of umbrella samplings to show the reliability of free energy.

In Figure 6, SAT moves along the $+x$ axis, from the o point (.60 nm), through the a point (1.12 nm), and then the b point (1.72 nm) arriving at the c point (2.57 nm), where it seems to be left out of the D$_3$R interior structure. On the views of free energy curve with the main and top views for SAT positioning on the system, from the gap between TM2 and the TM3, SAT leaves out of D$_3$R. It can be judged that at the b point (1.72 nm) SAT actually and basically breaks away from the control of D$_3$R with $\Delta G$ of 87.7 kJ mol$^{-1}$. From the b point to the c point, actually SAT is moving within the POPC bilayer membrane, during which $\Delta G$ is 33.1 kJ mol$^{-1}$ in agreement with 30.3 kJ mol$^{-1}$ obtained by MD simulation for SAT in ion molecule state moving within pure POPC bilayer membrane (Wang, 2015).

In Figure 7, SAT moves along the $-x$ axis, from the o point (.33 nm), through the a point (1.98 nm), and then the b point (2.34 nm) arriving at the c point (3.48 nm), where it completely leaves out of the D$_3$R interior structure. On the views of free energy curve with the main
and top views for SAT positioning in the system, from the gap between TM6 and the TM7 SAT leaves out of D3R, and furthermore it can be judged that at the a point (2.34 nm) SAT basically breaks away from the control of D3R, during which $\Delta G$ is 309.4 kJ mol$^{-1}$. From the b point to the c point, SAT is moving within the POPC bilayer membrane, in which its $\Delta G$ is 42.0 kJ mol$^{-1}$ to agree with the result obtained for SAT in ion state moving within POPC bilayer membrane.

In Figure 8, SAT moves along the +z axis, from the o point (.42 nm), through the a point (1.62 nm), and then the b point (2.57 nm) arriving at the c point (3.48 nm), where it completely leaves out of D3R interior structure. On the views of free energy curve with the main and top views for SAT positioning in the system, from the gap between TM1 and the TM2 SAT leaves out of D3R. From the view of free energy curve, the free energy is rising in uniform, until the b point, the free energy curve reaches a platform with the value of 224.3 kJ mol$^{-1}$. On the main view for SAT in the system, the b point just is ready for SAT to leave out of D3R from the gap between TM1 and TM2. After that, from the point of b to reach the c point it is actually to move within the POPC bilayer space with the free energy of 29 kJ mol$^{-1}$, also in agreement with 30.3 kJ mol$^{-1}$ obtained by MD simulation for SAT within POPC bilayer membrane.

In Figure 9, SAT moves along the −z axis, from the o point (.63 nm), through the a point (1.93 nm), and then the b point (2.36 nm) arriving at the c point (3.49 nm) where it completely leaves out of D3R. On the main and top views for SAT in the system, from the gap between TM3 and the TM5, SAT leaves out of D3R. On the views of free energy curve for SAT in the system, the free energy is basically rising in uniform, until the c point, the free energy curve reaches a platform with $\Delta G$ of 221.7 kJ mol$^{-1}$.

From the trajectory pictures, it is seen that SAT moves along the molecular channels within D3R with a certain degree of flexibility. SAT does not keep moving along a single axis direction, but starts to move along an axis as the initial direction, and transfers always along the direction of the minimum energy, which are verified by four trajectory figures, if necessary, sometimes it performs slanted movements. When SAT is blocked by front of a transmembrane helix, it will slightly change the direction of movement, then from the transmembrane helix column gap move toward the lowest energy direction. If SAT moves along the Y axis, the most likely
direction for SAT moving is not to transfer through the column gap of transmembrane helices. Now we have studied four directions for SAT moving along +x, −x, +z, −z axes, it should cover the most probable trajectory for SAT to transfer into the space of POPC bilayer membrane. For SAT moving along the protective molecular channels within D3R, the most probable trajectory of channel is to leave through the gap between TM2 and TM3 from D3R into the space of POPC bilayer membrane with ΔG of 87.7 kJ mol\(^{-1}\).

By MD simulation, we can even see SAT moving along different trajectories through the different TM gaps within D3R. If using the experimental method, we cannot peer the real-time trajectories of SAT moving along the molecular channels within D3R. Furthermore, the changes of free energy obtained by MD simulation for SAT moving along the molecular channels within D3R should be similar to those determined with the experimental method, although there is no direct evidence to support it, we can compare the related experimental data to acquire part of support for our results.

According to reports in the literature, using the PMF method, the free energy barrier obtained by molecular simulation for water molecules to permeate through phospholipid bilayer membrane is 16.8–29.4 kJ mol\(^{-1}\) (Bemporad & Essex, 2004; Marrink & Berendsen, 1994; Marrink, Jähnig, & Berendsen, 1996; Shinoda, Mikami, Baba, & Hato, 2004; Zahn & Brickmann, 2002), and the free energy barrier determined by experiments for water molecules to permeate through the cell membrane is 16.8–37.8 kJ mol\(^{-1}\) (Andrasko & Forsén, 1974; Benga, Pop, Popescu, & Borza, 1990; Graziani & Livne, 1972; Jansen & Blume, 1995; Nichols & Deamer, 1980), from which we notice that they are very well consistent. Recently reported in the literature, using the same PMF method, the free energy barriers obtained by molecular simulation are 115.0, 99.1, and 92.0 kJ mol\(^{-1}\) for Na\(^+\)-Cl\(^-\) ion, and Na\(^+\) ion to permeate through phospholipid bilayer membrane, respectively (Khavrutskii, Gorfe, Lu, & McCammon, 2009), while they are (98.7 ± 11.3) and (87.4 ± 1.7) kJ mol\(^{-1}\) determined by experimental measure for Cl\(^-\) ion and Na\(^+\) ion to permeate through phosphatidylycerine membrane, respectively (Papahadjopoulos, Nir, & Oki, 1972), which also show a very good consistency. Since in pure phospholipid bio-membrane system, molecular simulated results are very well compared with experimental measurements, for now only the pure protein system mixed with phospholipid bio-membrane, molecular simulated results should also have good consistency with experimental measurements.

4. Conclusions

The change of free energy is 83.5 kJ mol\(^{-1}\) for SAT moving along the functional molecular channel within D3R toward the extra-cellular direction of trajectory, and it is 261.8 kJ mol\(^{-1}\) for SAT toward the intra-cellular direction of trajectory. The changes of free energy are 87.7, 309.4, 224.3, and 221.7 kJ mol\(^{-1}\), respectively, for SAT moving along the different protective molecular channels within D3R into the space of cell bilayer membrane. It is likely that through the column gap between TM2 and TM3, SAT moves with ΔG of 87.7 kJ mol\(^{-1}\) along the protective molecular channel into the space of POPC bilayer membrane. Our previous work presented that the free energy for Levo-Benzedrine (RAT) to transmit toward the outside of cell along the functional molecular channel within D3R is 91.4 kJ mol\(^{-1}\), while it is 117.7 kJ mol\(^{-1}\) for RAT to transmit into the space of POPC bilayer membrane along the protective molecular channel within D3R (Xie, Xu, Wang, & Xu, 2016). The values of free energy imply that SAT relatively easily passes through the functional molecular channel within D3R for exerting its molecular functions and to increase the release of functional dopamine molecules resulting in a variety of functional effects from SAT. Therefore, the obtained results show that the pharmacology and addiction mechanisms of SAT as a medicine are closely related to the molecular dynamics and mechanism for SAT to transmit along molecular channels within dopamine receptors.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 21163024], [grant number 21563032].

Supplementary material

The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2016.1190947.

References

Andrasko, J., & Forsén, S. (1974). NMR study of rapid water diffusion across lipid bilayers in dipalmitoyl lecithin vesicles. Biochemical & Biophysical Research Communications, 60, 813–819. doi:10.1016/0006-291X(74)90313-1

Bemporad, D., & Essex, J. W. (2004). Permeation of small molecules through a lipid bilayer: A computer simulation study. The Journal of Physical Chemistry B, 108, 4875–4884. doi:10.1021/jp035260n

Benga, G., Pop, V. I., Popescu, O., & Borza, V. (1990). On measuring the diffusional water permeability of human red blood cells and ghosts by nuclear magnetic resonance. Journal of Biochemical & Biophysical Methods, 21, 87–102. doi:10.1016/0165-022X(90)90057-3
Berendsen, H. J. C., Van der Spoel, D., & Van Drunen, R. (1995). Gromacs – A message-passing parallel molecular-dynamics implementation. *Computer Physics Communications, 91*, 43–56. doi:10.1016/0010-4655(95)00042-E

Bian, F. Y., Shi, G. J., Chi, S. M., & Xu, S. C. (2012, October 15–18). The perspective insight into the pathology of parkinsonism using the molecular channel theory of dopamine inside its receptor membrane protein. Chinese Chemical Society at the Second National Conference on Bio-physical Chemistry (NCBPC2) and the International Forum on Development of Chinese Bio-physical Chemistry, Wuhan University, Wuhan, China.

Bian, F. Y., Zhang, J. W., Wang, D., & Xu, S. C. (2014). Molecular dynamics simulation of the permeation of methyldopa through POPC phospholipid bilayer membrane. *Acta Physico-Chimica Sinica, 30*, 1947–1956. doi:10.3866/PKU.WHXB201408271

Bontempi, B., & Sharp, F. R. (1997). Systemic morphine-induced fos protein in the rat striatum and nucleus accumbens is regulated by mu opioid receptors in the substantia nigra and ventral tegmental area. *Journal of Neuroscience, 17*, 8596–8612. doi:10.1007/BF00384706

Bunzow, J. R., Van Tol, H. M. G., Grandy, D. K., Albert, P., Bontempi, B., & Sharp, F. R. (1995). Methylation of the dopamine D3 receptor in complex with a POPC lipid bilayer. *European Journal of Medicinal Chemistry, 30*, 237–242. doi:10.1016/0223-5234(95)80029-0

Chien, E. Y. T., Liu, W., Zhao, Q., Katritch, V., Won Han, G., Hanson, M. A., … Stevens, R. C. (2010). Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science, 330*, 1091–1095. doi:10.1126/science.1197410

Chi, S. M., Xie, W., Zhang, J. W., & Xu, S. C. (2015). Theoretical insight into the structural mechanism for the binding of vinblastine with tubulin. *Journal of Biomolecular Structure & Dynamics, 33*, 1–21. doi:10.1080/07391102.2014.999256

Daura, X., Mark, A. E., & Van Gunsteren, W. F. (1998). Parametrization of aliphatic CHn united atoms of GROMOS96 force field. *Journal of Computational Chemistry, 19*, 535–547. doi:10.1002/(SICI)1096-987X(19980415)19:5<535::AID-JCFC3.0.CO;2-N

De Paulis, T., Hall, H., & Ogren, S. O. (1985). Synthesis, crystal structure and antidiopaminergic properties of eticlopride (FLB 131). *European Journal of Medicinal Chemistry, 20*, 273–276. Retrieved from https://www.researchgate.net/publication/279715996_Synthesis_crystal_structure_and_anti dopaminergic_properties_of_eticlopride_FLB_131

Dearry, A., Gingrich, J. A., Falardeau, P., Fremeau, R. T., Jr., Bates, M. D., & Caron, M. G. (1990). Molecular cloning and expression of the gene for a human D1 dopamine receptor. *Nature, 347*, 72–76. doi:10.1038/347072a0

Dunkin, C. M., Pokorny, A., Almeida, P. F., & Lee, H. S. (2011). Molecular dynamics studies of transportan 10 (Tp10) interacting with a POPC lipid bilayer. *The Journal of Physical Chemistry B, 115*, 1188–1198. doi:10.1021/jp107763b

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., … Pople, J. A. (2003). Gaussian 03, Rev B.01. Pittsburgh, PA: Gaussian.

Gandhimathi, A., & Sowdhamini, R. (2016). Molecular modelling of human 5-hydroxytryptamine receptor (5-HT2A) and virtual screening studies towards the identification of agonist and antagonist molecules. *Journal of Biomolecular Structure & Dynamics, 34*, 952–970. doi:10.1080/07391102.2015.1062802

Graziani, Y., & Livne, A. (1972). Water permeability of bilayer lipid membranes: Sterol-lipid interaction. *The Journal of Membrane Biology, 7*, 275–284. doi:10.1007/BF01867920

Griffon, N., Pilon, C., Sautel, F., Schwartz, J. C., & Sokoloff, P. (1996). Antipsychotics with inverse agonist activity at the dopamine D3 receptor. *Journal of Neural Transmission, 103*, 1163–1175. doi:10.1007/BF01271201

Hess, B., Kutzner, C., Van der Spoel, D., & Lindahl, E. (2008). GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation. *Journal of Chemical Theory & Computation, 4*, 435–447. doi:10.1021/ct070301q

Hoff, B., Strandberg, E., Ulrich, A. S., Tieleman, D. P., & Clemens, P. (2005). 2H-NMR study and molecular dynamics simulation of the location, alignment, and mobility of pyrene in POPC bilayers. *Biophysical Journal, 88*, 1818–1827. doi:10.1529/biophysj.104.052399

Hub, J. S., de Groot, B. L., Grubmüller, H., & Groenhof, G. (2014). Quantifying artifacts in Ewald simulations of inhomogeneous systems with a net charge. *Journal of Chemical Theory & Computation, 10*, 381–390. doi:10.1021/ct400626b

Hub, J. S., de Groot, B. L., & Van der Spoel, D. (2010). G_wham – A free weighted histogram analysis implementation including robust error and autocorrelation estimates. *Journal of Chemical Theory and Computation, 6*, 3713–3720. doi:10.1021/ct100494z

Humphrey, W., Dalke, A., & Schulten, K. (1996). VMD: Visual molecular dynamics. *Journal of Molecular Graphics, 14*, 33–38. doi:10.1016/0263-7855(96)00018-5

Janosi, L., & Gorfe, A. A. (2010). Simulating POPC and POPC/POPG bilayers: Conserved packing and altered surface reactivity. *Journal of Chemical Theory & Computation, 6*, 3267–3273. doi:10.1021/ct100381g

Jansen, M., & Blume, A. (1995). A comparative study of diffusive and osmotic water permeation across bilayers composed of phospholipids with different head groups and fatty acyl chains. *Biophysical Journal, 68*, 997–1008. doi:10.1016/S0006-3495(95)80275-4

Jin, Y., Wang, Y., Bian, F. Y., Shi, Q., Ge, M. F., Wang, S., … Xu, S. C. (2011). Three-dimensional structure of dopamine 3-subtype receptor with the active site residues for the binding of dopamine. *Acta Physico-Chimica Sinica, 27*, 2432–2446(15). doi:10.3866/PKU.WHXB201111001

Kakarala, K. K., & Jamila, K. (2016). Biased signaling: Potential agonist and antagonist of PAR2. *Journal of Biomolecular Structure & Dynamics, 34*, 1363–1376. doi:10.1080/07391102.2015.1079556

Keabian, J. W., & Calne, D. B. (1979). Multiple receptors for dopamine. *Nature, 277*, 93–96. doi:

Khavrutskii, I. V., Gorfe, A. A., Lu, B., & McCammon, J. A. (2009). Free energy for the permeation of Na(+) and Cl(−) ions and their ion-pair through a zwitterionic dimyristoyl phosphatidylethanolamine lipid bilayer by umbrella integration with harmonic fourier beads. *Journal of the American Chemical Society, 131*, 1706–1716. doi:10.1021/ja8081704

Lang, P. T., Moustakas, D., Brozell, S., Carrascal, N., Mukherjee, S., Pegg, S., & Kuntz, I. (2006). DOCK 6.1. San Francisco: University of California.
Shinoda, W., Mikami, M., Baba, T., & Hato, M. (2004). Molecular dynamics study on the effects of chain branching on the physical properties of lipid bilayers: 2. Permeability. The Journal of Physical Chemistry B, 108, 9346–9356. doi:10.1021/jp035998j

Sokoloff, P., Giros, B., Martres, M. P., Bouthenet, M. L., & Schwartz, J. C. (1990). Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature, 347, 146–151. doi:10.1038/347146a0

Sunahara, R. K., Guan, H. C., O’Dowd, B. F., Seeman, P., Laurier, L. G., Ng, G., … Niznik, H. B. (1991). Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. Nature, 350, 614–619. doi:10.1038/350614a0

Su, Z. Y., & Wang, Y. T. (2011). Coarse-grained molecular dynamics simulations of cobra cytotoxin A3 interactions with a lipid bilayer: Penetration of loops into membranes. The Journal of Physical Chemistry B, 115, 796–802. doi:10.1021/jp107599v

Suri, R. E., Bargas, J., & Arbib, M. A. (2001). Modeling functions of striatal dopamine modulation in learning and planning. Neuroscience, 103, 65–85. doi:10.1016/S0306-4522(00)00554-6

Van der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A. E., & Berendsen, H. J. (2005). Gromacs: Fast, flexible, and free. Journal of Computational Chemistry, 26, 1701–1718. doi:10.1002/jcc.20291

Van der Spoel, D., Lindahl, E., Hess, B., Van Buuren, A. R., Apol, E., Meulenhoff, P. J., & Berendsen, H. J. (2013). Gromacs user manual version 4.5. Retrieved from www.gromacs.org

Van Gunsteren, W., Billetter, S., Eising, A., Hunenberger, P., Kruger, P., Mark, A., & Tironi, I. (1996). Biomolecular simulation: The Gromos 96 manual and user guide (1st ed.). Zurich, Switzerland: Hochschulverlag AG an der ETH Zurich.

Van Tol, H. H. M., Bunzow, J. R., Guan, H. C., Sunahara, R. K., Seeman, P., Niznik, H. B., & Civelli, O. (1991). Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature, 350, 610–614. doi:10.1038/350610a0

Wang, D. (2015). The molecular dynamics simulations for benzphetamine to move through the phospholipid bilayer membrane (MS dissertation). Yunnan University, Kunming City.

Wang, Y., Bian, F. Y., Deng, S. R., Shi, Q., Ge, M. F., Wang, S., … Xu, S. C. (2011). The key residues of active sites on the catalytic fragment for paclitaxel interacting with poly(ADP-ribose) polymerase. Journal of Biomolecular Structure & Dynamics, 28, 881–893. doi:10.1080/07391102.2011.10508615

Xiao, X., Min, J. L., Lin, W. Z., Liu, Z., Cheng, X., & Chou, K. C. (2015). iDrug-Target: Predicting the interactions between drug compounds and target proteins in cellular networking via benchmark dataset optimization approach. Journal of Biomolecular Structure & Dynamics, 33, 2221–2233. doi:10.1080/07391102.2014.998718

Xie, W., Xu, Z. R., Wang, M., & Xu, S. C. (2016). Molecular dynamics simulation for levo-benzphetamine to transmit through molecular channels within D3R. Acta Physico-Chimica Sinica, 32, 907–920. doi:10.3866/PKU.WHXB201601141

Li, F., Shu, S. Y., & Bao, X. M. (2003). Structure and function of dopamine receptors. Chinese Journal of Neuroscience, 19, 405–410. Retrieved from http://d.wanfangdata.com.cn/Periodical/zgjjkxx200306014

Marrink, S. J., & Berendsen, H. J. C. (1994). Simulation of water transport through a lipid membrane. The Journal of Physical Chemistry, 98, 4155–4168. doi:10.1021/j100066a040

Marrink, S. J., Jähnig, F., & Berendsen, H. J. (1996). Proton transport across transient single-file water pores in a lipid membrane studied by molecular dynamics simulations. Biophysical Journal, 71, 632–647. doi:10.1016/S0006-3495(96)79264-0

Marrink, S. J., Lindahl, E., Edholm, O., & Mark, A. E. (2001). Simulation of the spontaneous aggregation of phospholipid into bilayers. Journal of the American Chemical Society, 123, 8638–8639. doi:10.1021/ja0159618

Miyamoto, S., & Kollman, P. A. (1992). Settle: An analytical version of the shake and rattle algorithm for rigid water models. Journal of Computational Chemistry, 13, 952–962. doi:10.1002/jcc.540130805

Nichols, J. W., & Deamer, D. W. (1980). Net-proton-hydroxyl permeability of large unilamellar liposomes measured by an acid-base titration technique. Proceedings of the National Academy of Sciences, 77, 2038–2042. doi:10.1073/pnas.77.4.2038

Nowroozi, A., & Shahlaei, M. (2016). A coupling of homology modeling with multiple molecular dynamics simulation for identifying representative conformation of GPCR structures: A case study on human bombesin receptor subtype-3. Journal of Biomolecular Structure & Dynamics. doi:10.1080/07391102.2016.1140593

Papahadjopoulos, D., Nir, S., & Oki, S. (1972). Permeability properties of phospholipid membranes: Effect of cholesterol and temperature. Biochimica et Biophysica Acta (BBA) – Biomembranes, 266, 561–583. doi:10.1016/0006-3002(72)90001-7

Plante-Bordeneuve, V., Taussig, D., Thomas, F., Said, G., Wood, N. W., Marsden, C. D., & Harding, A. E. (1997). Evaluation of four candidate genes encoding proteins of the catalytic fragment for paclitaxel interacting with poly(ADP-ribose) polymerase. Journal of Biomolecular Structure & Dynamics, 15, 962. doi:10.1021/jp107599v

Rothenberg, D. M., Cherezov, V., Hanson, M. A., Rasmussen, S. G., Thian, F. S., Kobikka, T. S., … Kobikka, B. K. (2007). GPCR engineering yields high-resolution structural insights into beta2-adrenergic receptor function. Science, 318, 1266–1273. doi:10.1126/science.1150609

Roth, C. B., Hanson, M. A., & Stevens, R. C. (2008). Stabilization of the beta2-adrenergic receptor TM4–TM5 helix interface by mutation of Glu223.41, a critical residue in GPCR structure. Journal of Molecular Biology, 376, 1305–1319. doi:10.1016/j.jmb.2007.12.028

Salum, C., Roque da Silva, A., & Pickering, A. (1999). Striatal dopamine in attentional learning: A computational model. Neurocomputing, 26–27, 845–854. doi:10.1016/S0925-2312(98)00129-5

Shi, G. J., Wang, Y., Jin, Y., Chi, S. M., Shi, Q., Ge, M. F., … Xu, S. C. (2012). Structural insight into the mechanism of epothilone A bound to beta-tubulin and its mutants at Arg282Gln and Thr274Ile. Journal of Biomolecular Structure & Dynamics, 30, 559–573. doi:10.1080/07391102.2012.687522.
Xu, M., Koeltzow, T. E., Santiago, G. T., Moratalla, R., Cooper, D. C., Hu, X. T., … Tonegawa, S. (1997). Dopamine D3 receptor mutant mice exhibit increased behavioral sensitivity to concurrent stimulation of D1 and D2 receptors. *Neuron, 19*, 837–848. doi:10.1016/S0896-6273(00)80965-4

Xu, S. C., Chi, S. M., Jin, Y., Shi, Q., Ge, M. F., Wang, S., & Zhang, X. K. (2012). Molecular dynamics simulation and density functional theory studies on the active pocket for the binding of paclitaxel to tubulin. *Journal of Molecular Modeling, 18*, 377–391. doi:10.1007/s00894-011-1083-7

Xu, S. C., Deng, S. R., Ma, L. Y., Shi, Q., Ge, M. F., & Zhang, X. K. (2009). Active sites for retinal binding to bovine rhodopsin. *Acta Physico-Chimica Sinica, 25*, 1290–1296(7). doi:10.3866/PKU.WHXB20090701

Zahn, D., & Brickmann, J. (2002). Molecular dynamics study of water pores in a phospholipid bilayer. *Chemical Physics Letters, 352*(s 5–6), 441–446. doi:10.1016/S0009-2614(01)01437-3

Zhang, J. W., Bian, F. Y., Shi, G. J., & Xu, S. C. (2014). Molecular dynamics simulation of dopamine diffusion within and permeation through popc phospholipid bilayer membrane. *Acta Physico-Chimica Sinica, 30*, 183–193. doi:10.3866/PKU.WHXB201311281