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Molecular dynamics of conformation-specific dopamine transporter-inhibitor complexes

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Abstract

The recreational psychostimulant cocaine inhibits dopamine reuptake from the synapse, resulting in excessive stimulation of postsynaptic dopamine receptors in brain areas associated with reward and addiction. Cocaine binds to and stabilizes the outward- (extracellular-) facing conformation of the dopamine transporter (DAT) protein, while the low abuse potential DAT inhibitor benztropine prefers the inward- (cytoplasmic-) facing conformation. A correlation has been previously postulated between psychostimulant abuse potential and preference for the outward-facing DAT conformation. The 3 β -aryltropane cocaine analogs LX10 and LX11, however, differ only in stereochemistry and share a preference for the outward-facing DAT, yet are reported to vary widely in abuse potential in an animal model. In search of the molecular basis for DAT conformation preference, complexes of cocaine, benztropine, LX10 or LX11 bound to each DAT conformation were subjected to 100 ns of all-atom molecular dynamics simulation. Results were consistent with previous findings from cysteine accessibility assays used to assess an inhibitor's DAT conformation preference. The respective 2 β - and 2 α -substituted phenyltropanes of LX10 and LX11 interacted with hydrophobic regions of the DAT S1 binding site that were inaccessible to cocaine. Solvent accessibility measurements also revealed subtle differences in inhibitor positioning within a given DAT conformation. This work serves to advance our understanding of the conformational selectivity of DAT inhibitors and suggests that MD may be useful in antipsychostimulant therapeutic design.

Graphical abstract

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Keywords

molecular dynamics; inhibitor-stabilized conformation; neurotransmitter transporter; dopamine; cocaine; psychostimulant

Introduction

The plasmalemmal monoamine transporter (MAT) proteins, one for each of the neurotransmitter substrates dopamine, norepinephrine and serotonin, are drug targets for the treatment of neuropsychiatric disorders. By clearing the synapse of substrate, these proteins quench the neurotransmission event.¹ The MATs and other members of its SLC6 transporter family move the substrate across the plasma membrane lipid bilayer by coupling its movement to downhill Na^+ and Cl^- gradients, apparently via an “alternating access” mechanism.^{2–4} Opening of an outer gate to the extracellular space allows the ions and substrate to enter a central chamber, termed the S1 binding pocket.^{5–7} Upon binding of substrate in S1, the outer gate closes and an inner gate to the cytoplasm opens, allowing ions and substrate to exit S1 and enter the cell. Offloading of ions and substrate allows the transporter protein to reset, shifting from its inward-facing (IF) conformation back to the outward- (extracellular-) facing (OF) conformation.^{8–11}

The dopamine-selective MAT, better known as the dopamine transporter (DAT), serves as a primary target of the illicit psychostimulants cocaine, methamphetamine and ecstasy. These drugs interfere with substrate occupancy of S1 and thus block DAT’s synaptic reuptake of dopamine. The neurotransmitter pools in the synapse, increasing the number of dopamine receptor activating events in the nucleus accumbens (the brain’s drug reward center), providing the characteristic euphoria and addiction.^{12–14}

Curiously, the FDA-approved drugs benzotropine (Cogentin) and methylphenidate (Ritalin) display cocaine-like DAT affinities and inhibit dopamine uptake yet do not share cocaine’s abuse potential. Attempts to explain this paradox are often pharmacokinetic in nature: of the three drugs, only cocaine reaches the brain fast enough at high concentration to trigger the phasic firing of accumbal dopamine cells needed to elicit euphoria.^{15–18} From cysteine accessibility studies, an alternative explanation arose from the finding that cocaine and benzotropine bind to different DAT conformations.^{19–21} Because cocaine recognizes the OF

conformation and benztropine recognizes the IF conformation, it was postulated that behaviorally “typical” (abusable, locomotor psychostimulant) inhibitors such as cocaine stabilize the OF DAT and “atypical” (little or no abuse potential) inhibitors favor IF DAT.^{19,21} It is unclear how conformational preference would correlate with DAT inhibitor abuse potential, as arresting the substrate translocation cycle at either conformational intermediate should abolish the DAT primary function of dopamine reuptake. Indeed, behaviorally-*atypical* cocaine analogs have now been shown to prefer the OF DAT conformation.^{19, 22}

The recent availability of ligand-bound MAT or homolog crystal structures in the OF, IF or occluded (both gates closed) conformation has provided credible templates for construction of homology models.^{23–29} These computational structures serve as powerful tools to elucidate MAT structure and function. Beyond ligand docking and mapping ligand-transporter interactions, MAT computational models provide an atomistic perspective on the intramolecular interactions key to the transport mechanism.^{6, 7, 11, 22, 30–36} Molecular dynamics (MD) simulations may reveal ligand-based conformational preferences as well as subtle intermediate conformations not detected with high-resolution crystallography.

The 3 β -aryltropane cocaine analogs LX10 and LX11 possess certain functional groups common to cocaine and benztropine (Fig. 1A). Both LX compounds stabilize OF DAT. LX10 has 10-fold greater affinity for DAT than LX11, and only LX11 elicits the atypical, benztropine-like behavioral pattern.²² The study described herein evaluates the binding and DAT conformational preferences of LX10 and LX11 as well as cocaine and benztropine using molecular dynamics, toward detecting minute or transient differences in the disposition of the inhibitors relative to the protein.^{3, 37}

Materials and Methods

System preparation for MD

The DAT-inhibitor complexes previously described²² are used in this study. Using cocaine as the example, MOE (version 2013.0802) was used to generate docked poses of cocaine within the S1 (primary) substrate binding site of outward- and inward-facing DAT. The docking site was defined with the placement of dummy atoms using the Site Finder tool of MOE. An induced fit docking protocol was implemented using the Triangle Matcher placement method and force field refinement with the Amber10:Eht force field. The resulting poses were London dG-scored (MOE) and the top scoring poses were evaluated for intermolecular interactions with DAT residues similar to those seen in the 4M48 crystal structure with dDAT bound to nortriptyline. The protein-ligand complexes include the IF and OF DAT conformations bound to cocaine, BZT, LX10 or LX11. Sodium and chloride ions (2 Na⁺, 1 Cl[–]) were included in DAT models based on ion coordinates within the crystal structures of LeuT and dDAT.^{26, 32} The protein-ligand complexes were inserted into a 110 Å × 110 Å 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) membrane using CHARMM-GUI Membrane Builder and solvated with the TIP3P water model.^{38–43} The final system size was 123 Å × 123 Å × 105 Å, containing approximately 110,000 atoms. The CHARMM36 force field with the TIP3P water model was used for parameterizing the protein, lipid, and water molecules in all MD simulations.^{40,43–45} Force field parameters

previously developed for cocaine were used, and force field parameters for BZT, LX10 and LX11 were obtained from the ParamChem web server (v0.9.7.1) with the CHARMM General Force Field (CGenFF) v2b8 force field, which is compatible with the CHARMM36 force field.^{46, 47}

MD simulations

Eight inhibitor-bound systems were constructed with each inhibitor bound in the OF and IF DAT models along with two DAT apoprotein (APO) systems. First, a 50 ps energy minimization was performed on the OF and IF DAT APO models to eliminate steric clashes. A 10 ns equilibration was then performed with the protein backbone atoms (C α , N) remaining fixed, with a 1.0 kcal/mol/Å force constant for the atom's restraining potential.^{40, 43} MD was performed with the constraints removed for an additional 10 ns of all-atom classical MD. Cocaine was initially docked in the OF DAT, and the selected pose was based on similar interactions found in the dDAT co- crystal structure.^{23, 27} Cocaine-OF DAT was simulated for 5 ns, after which BZT, LX10 or LX11 replaced cocaine in the OF DAT S1 binding site. The same was carried out for BZT in IF DAT, where BZT was first simulated for 5 ns and then replaced by cocaine, LX10 or LX11 in the IF DAT S1 binding site.

The isobaric-isothermal (NPT) ensemble in the Nanoscale Molecular Dynamics (NAMD) program (v2.10b1) was used to perform all classical MD calculations with periodic boundary conditions.⁴⁸ Langevin dynamics and the damping Langevin piston were utilized to maintain 310 K temperature and 1 atm pressure, respectively, with a damping coefficient of 10.0.⁴⁸ The Langevin piston had a target pressure of 1 atm, an oscillation period of 100 fs, and a damping timescale of 50 fs. A cutoff distance of 12.0 Å and switch distance of 8.0 Å were used for short-range electrostatic and van der Waals (vdW) interactions. The PME algorithm was used to estimate the long-range electrostatic interactions with a grid spacing of 1.0 Å.⁴⁹ A time step of 2 fs was used for the integration of motion for short- and long-range interactions. All eight DAT-inhibitor systems and the two APO DAT were subjected to a total of 100 ns of MD. Analysis of trajectories was performed in VMD.⁵⁰

Solvent accessibility surface area (SASA) analysis

SASA was measured using VMD.⁵⁰ Selected atoms were determined to be solvent-accessible with a 1.4 Å probe radius. Residues for the external cavity are defined by DAT residues lining the extracellular pathway above the externally accessible gating residue pairs in the trans-membrane domains surrounding the binding site: Y88 (TM1), W84 (TM1), R85 (TM1), F155 (TM3), I311 (TM6), D312 (TM6), T315 (TM6), D475 (TM10), T481 (TM10).^{2, 24} Residues for the internally open cavity was defined selected residues that line the cytoplasmic pathway: F69 (TM1), G75 (TM1), G257 (TM5), S261 (TM5), V265 (TM5), T268 (TM5), F331 (TM6), G424 (TM8), E427 (TM8) and T431 (TM8).^{30, 36, 51, 52}

Results and Discussion

All-atom MD simulations were performed to evaluate the binding of four DAT inhibitors that have been shown through cysteine accessibility assays to stabilize particular DAT

conformations.²² Previous studies of DAT conformations after 35 ns MD suggested that cocaine, LX10 and LX11 prefer the OF DAT conformation and BZT prefers the IF DAT conformation.²² The present study examines differences in the OF DAT conformations of the four inhibitors after 100 ns MD.

This work began prior to the availability of the cocaine-*Drosophila* DAT (dDAT) co-crystal 4XP4;²⁴ thus, the cocaine-bound OF rat DAT (rDAT) homology model was superposed with the 4XP4 coordinates (Fig. 1B) to ensure that the present system was experimentally relevant.²⁴ The RMSD was measured for the backbone atoms of DAT after 100 ns of MD to assess the stability of the system (Fig. S1). Additionally, the key residues that are in the binding site were inspected for comparison. For the cocaine-bound OF DAT, a cocaine-D79 (TM1) residue ion pair was observed, as was a shift in the TM3 – TM8 extracellular gate residue pair Y156-F319 characteristic of the outward-occluded conformation.⁵³ Although the D79-Y156 distance is reported to be too large for a hydrogen bond interaction,³¹ 100 ns of MD indicated a stable H-bond (Fig. S2). In the dDAT co-crystal, cocaine was found to have a face-to-edge interaction with F325 (TM6); however, after 100 ns of MD the F325 side chain had disengaged from cocaine (Fig. 1B). Overall, the rDAT homology model was sufficient to reproduce the experimentally determined binding orientation of cocaine in OF dDAT.²⁴

Access to the S1 binding site is dependent on the ability of the ligand to travel from the extracellular space and reach the site.^{1,5} In the OF DAT conformation, the “outer cavity” includes the extracellular vestibule and S1 pocket, and is larger than the “inner cavity” delineated by the internal gate residues. The two external gate residue pairs R85-D475 and Y156-F319, when disrupted, allow accessibility to the S1 pocket from the extracellular space. In contrast, the IF DAT intact external gate interactions and disruption of the internal gate residue pair R60-D435 yield an internally open cavity that reaches into S1.^{2, 54, 55} Thus, the hydration of the outer and inner cavities differs for the outward- and inward-facing conformations (Fig. 2).

The hydration of the two cavities was evaluated for the final 50 ns of MD. Outer cavity hydration was greater for all OF DAT simulations than for IF DAT (Fig. 2C). The number of water molecules in the inner cavity was less for OF DAT than IF DAT in the APO, cocaine and LX10 systems. Interestingly, the accumulation of water molecules in the inner cavity for the BZT- and LX11-bound systems was greater in OF DAT than in IF DAT (Fig. 2D), which would suggest the binding of these ligands stabilizes a DAT conformation that allows for increased solvation of the inner cavity.

Salt bridge side chain interactions for the external and internal gates were evaluated to determine the accessibility of the binding site from the extracellular and intracellular spaces (Fig. 3A, 3B).⁵⁵ The distance was measured between the residues comprising the extracellular (R85-D475) gate salt bridge (Fig. 3A) as well as the intracellular (R60-D435) gate salt bridge (Fig. 3B). Interactions were evaluated using the shortest distance measured between an arginine nitrogen atom and an aspartic acid carboxylate oxygen. Distances greater than 3.5 Å indicated loss of interaction between the gating residues. Thus, for the IF DAT, one would expect to see distances that are > 3.5 Å. The distance measured between the

R85 and D475 side chains in the OF DAT-cocaine complex indicated no direct bridging during the final 75 ns of the MD simulation. In contrast, the R85-D475 separation in the OF DAT-BZT complex after 45 ns indicated a stable salt bridge (Fig. 3A). The distance measurements between the extracellular gate residues R85 and D475 further support the preference of BZT for IF DAT, reducing extracellular accessibility to the S1 binding site, whereas the binding of cocaine to outward-facing DAT allows for access to the binding site from the extracellular side of the protein, consistent with cysteine accessibility assays.²²

The solvent accessible surface area (SASA) was monitored for the residues in the outer and inner cavities. The SASA values measured from the trajectories for OF and IF DAT conformations differed substantially and revealed details of which conformation was being sampled with inhibitors bound (Fig. 3C, 3D).^{30,36} SASA measured in the defined outer cavity in the OF DAT conformation (Fig. 3C, blue lines) should offer a larger outer cavity, allowing for greater water penetration compared to that of the IF DAT (Fig. 3C, red lines). The SASAs of the inner cavity of OF and IF DAT were measured to determine the extent of IF DAT and the intracellular accessibility. The inner cavity is defined here by accessibility to the cytoplasm-proximate residues previously determined to line the cytoplasmic half of the substrate permeation pathway, plus the S1 pocket in the case of IF DAT (Fig. 3D). A larger inner cavity is expected for the IF DAT conformation (Fig. 3D, red lines) relative to that of the OF DAT conformation (Fig. 3D, blue lines).

In the BZT-bound system, the distance between R85 and D475 in OF DAT decreased from approximately 5 Å to 3.1 Å during the last 50 ns of the simulation (Fig. 3A, blue line). There was also a decrease in SASA to an average value of 195 Å² at t=45 ns (Fig. 3C, blue line). A similar SASA value was found for IF DAT. This is an indication of reduced accessibility from the extracellular space and is characteristic of the inward-facing conformation. The measured values in both OF and IF DAT further support the expected binding of BZT to reduce external accessibility. The distance measured between internal gate residues R60 and D435 in IF DAT was greater than 10 Å, indicating no salt bridge; however, in OF DAT the salt bridge remains intact throughout the entire 100 ns.

Conversely, cocaine, LX10 and LX11 are expected to stabilize the outward-facing conformation.²² In the OF DAT cocaine-bound system, there was a significant decrease from 10 Å to 3 Å in the first 10 ns of the simulation, followed by an increase in the distance between the residues during the last 50 ns. The distance averaged 5.3 Å, suggesting breakage of the external gate R85-D475 bridge (Fig. 3A), whereas the internal R60-D435 bridge was intact for OF DAT (Fig. 3B).

The measured distance between R85-D475 in the LX10- and LX11-bound in OF DAT fluctuated mildly but fairly constantly. Notably, the LX10-DAT complex appeared to allow the salt bridge to form starting at t=25 ns, which correlates with the loss of the intracellular gate bridge R60-D435 (Fig. 3A, 3B, blue lines). The inter-residue distance then fluctuates wildly for LX10 starting at 25 ns, forming intermittent interactions for the remainder of the simulation, whereas the distance measured in IF DAT between R60-D435 had stabilized. There are also measured fluctuations for LX11-DAT; however, the interaction is eventually regained. Fluctuations measured for this internal gate in OF DAT are likely due to the size of

the LX ligands within the S1 binding site, and in increased interaction with TM8, causing a displacement of D435 (Fig. 4A and Supplementary Figure S3).

SASAs measured for the inner and outer cavities for cocaine- and BZT-DAT complexes were as expected for OF DAT and IF DAT, respectively (Fig. 3C, 3D). Moreover, the number of water molecules measured in the outer DAT cavity for the DAT-cocaine and DAT-benztrapine complexes supported the reduced accessibility of the outer cavity by solvent molecules when benztrapine is bound (Fig. S4, Panel A). LX10 binding to IF DAT enlarged the inner cavity from 75 Å² to approximately 270 Å². Unexpectedly, the binding of LX11 to the OF DAT resulted in a larger inner cavity than for IF DAT. Comparison with the values seen for the outer cavity suggests that the binding of LX11 to OF DAT results in an opening of both the outer and inner cavities (Fig. 3C, 3D). Though the increase in the measured SASA of the outer cavity supports findings from cysteine accessibility assays,²² the values measured for the inner cavity with LX11 bound suggest that the inhibitor could stabilize a conformation of the transporter that is simultaneously open to some extent to the external and internal spaces.

Differences in inner cavity hydration were observed for OF DAT bound to LX10 and LX11 (Fig. 4). The LX10 interaction with TM1 and TM6 resulted in breakage of the internal R60-D435 gate (Fig. 4A, blue), in agreement with the distance measured for this gate pair (Fig. 3B). Unlike LX10, DAT binding of LX11 caused a TM8 shift that increased inner cavity space, enhancing hydration of the inner cavity (Fig. 4B, 4C). The binding of LX11 in OF DAT sustained a shift within the binding site (Fig. S1, Panel C.) Relative to LX10-DAT, there are approximately 15 more water molecules in the LX11-DAT inner cavity that approach the cytoplasmic interface (Fig. 2D).

A network of ionic interactions at the cytoplasmic interface maintains a DAT conformation closed to the cytoplasm.⁵⁵ The distances between the charged residues in the inner cavity were measured to provide quantitative differences between LX10 and LX11 DAT complexes (Fig 5). Residue pairs included R60 (TM1) – E427 (TM8), D435 (TM8) – R444 (TM9), E61 (TM1) – R444 (TM9), and D435 (TM8) – K133 (IL2, connecting TM2 and TM3). The interactions were measured at the shortest distance possible between the arginine/lysine side chain nitrogen atoms and the aspartate/glutamate carboxylate oxygen atoms.^{2, 55}

For LX10-bound DAT (Fig. 5A), D435 is coordinated with both R60 (orange) and R444 (pink) until 18 ns into the simulation. R60 – D435 is disrupted at that point, followed by breakage of the D435 – R444 interaction at 30 ns and alignment of D435 with K133 (green). R60 trades its D435 interaction for a brief interaction with E427 (blue), lasting about 20 ns, before this bond distance fluctuates. Upon loss of the D435 interaction (pink), R444 forms a bridge with E61 (red) at approximately 40 ns, lasting until the 75 ns mark. During this time frame D435 makes brief contact with R60, but never regains a stable interaction with either R60 or R444. For LX11-bound DAT (Fig. 5B), the distance between R60 and D435 (orange) remains fairly constant until the 50 ns mark. The bond breaks for 30 ns before reforming at t = 80 ns. At that time, E61 is within proximity of R444 (red), but unlike LX10-DAT, the E61 – R444 interaction is never formed.

Comparison of the two DAT complexes indicates the interaction between R60 and D435 is more stable in the LX11-bound DAT. The distance between D435 and R444 (Fig. 5B, red) was stable at 5 Å for LX11-DAT before decreasing to allow bond formation, which may have stabilized the R60 – D435 bond at 80 ns. It should be noted that the DAT model lacks the N-terminal cytoplasmic tail (residues 1 – 59). Omitting this is fairly standard for integral membrane proteins considering that such regions have too many degrees of freedom to represent accurately; nevertheless, the DAT N-tail could interact with regions at the cytoplasmic interface including R60 and D435. For LX10-DAT, the distance between R60 and D435 increases and continues to fluctuate, and the distance between D435 and R444 remains constant at 10 Å. Unlike LX11-DAT (Fig. 5B, blue), an interaction forms between R60 and E437 in LX10-DAT (Fig. 5A, blue). Despite the fluctuation measured in the distance between R60 and D435 in the LX10-bound systems in OF DAT (Fig 3, blue lines), the SASA of the inner cavity and the number of water molecules remained constant (Fig. 2). In the LX10-bound IF DAT system, the distance between R60 and D435 remained constant (Fig. 3, red lines), but there was increased hydration of the inner cavity (Fig. 2).

The stability of the DAT backbone atoms was evaluated using root-mean-square fluctuation (RMSF; Fig. 6A). RMSF of all DAT C α atoms was measured over 100 ns to track the movement and stability of DAT residues upon the binding of inhibitors. RMSF comparison of cocaine-DAT with LX10- and LX11-DAT showed that LX10 (blue) is similar to cocaine (orange) in measured fluctuations, with a variation seen in EL4. In contrast, LX11-DAT (green) displayed a greater RMSF difference relative to cocaine-DAT in the extracellular and intracellular loops. RMSF traces for LX10- and LX11-OF DAT complexes were superposed for comparison. The fluctuations were very similar for these two systems except in TM6, TM7, EL1, EL4, and IL5.

The RMSD was measured for the backbone atoms in TM1, TM3, TM6, TM8 and TM10 to assess the effect of inhibitor binding on the transmembrane helices surrounding the binding site (Fig. 6B, 6C). OF DAT bound to cocaine (orange) was used as the reference structure to compare the outward-facing DAT complexes with LX10 (blue) and LX11 (green). Comparison of the RMSD values indicates LX10 interaction with atoms in TM1, TM3, TM6, TM8 and TM10 is similar to cocaine, with a measured deviation less than 2 Å. However, comparison of LX11 to cocaine showed deviation in TM8 and TM10, with values greater than 2 Å (Fig. 6B). Specifically, the binding of LX11 resulted in RMSDs of 2.4 Å and 2.1 Å in TM8 and TM10, respectively, whereas with LX10 there was an RMSD of 1.7 Å for both TM8 and TM10 (Fig 6B). The OF DAT apoprotein was also compared to cocaine, LX10 and LX11 (Fig. S5). Comparison of the RMSD with reference to OF DAT apoprotein (purple) indicates that binding of cocaine (orange), LX10 (blue) or LX11 (green) resulted in a RMSD greater than 2 Å in TM10; binding of LX11 also created a RMSD greater than 2 Å in TM8. It should be noted that the RMSD measured between cocaine and LX11 in TM8 (Fig. 6B) represents the deviation measured for the entire 100 ns, whereas Figure 6B represents a snapshot of the final coordinates of the systems after 100 ns of MD.

Inspection of the binding revealed that in all cases, the inhibitors were found to interact favorably with the charged aspartate D79 residue of the S1 pocket (Fig. S6). In the OF DAT systems, all inhibitors were similarly wedged between TM6a and TM6b (Fig. 6D–F), and

the respective 2 β - and 2 α -substituted phenyltropanes of LX10 and LX11 interacted with hydrophobic regions of the binding site that were inaccessible to cocaine (Fig. 6D, 6E).

The chlorophenyl groups of LX10 and LX11 interacted with TM8. For LX10, these substituents share the same DAT region as the benzene ring of cocaine, although the chlorophenyl group penetrated deeper into the S1 pocket, situated between Phe319 (TM6a) and Phe325 (TM6b) (Fig. 6D). However, the interaction of LX11 with TM8 occurred on the other side of the helix (Fig. 6F), with the chlorophenyl ring penetrating farther toward the intracellular side of the protein and causing a displacement of E427 (TM8) (Fig. 4A). This results in increased solvation of the inner cavity compared to LX10 (Fig. 4B, 4C). The LX11 diphenyl moiety also reaches deeper into the pocket with another portion of the substituent, between Phe319 (TM6a) and Phe325 (TM6b), similar to LX10 (Fig. 6F). A portion of the diphenyl moiety of LX10 interacted moreover with TM6. The difference in TM6 interaction between LX10 and LX11 is supported by the RMSF measured for the OF DAT C α residues (Fig. 6A), where the TM6 residues are more stable in LX11-DAT than in LX10-DAT or COC-DAT.

Conclusions

In the present study, the intermolecular interactions between the DAT and inhibitors of nonidentical DAT conformation preference were evaluated using molecular dynamics (MD). The MD differences observed for DAT-inhibitor complexes were consistent with reported preferences for the outward- or inward-facing (OF or IF) conformation as determined with accessible cysteine alkylation assays. A concern in interpreting such cysteine accessibility findings is that the assay requires several minutes and non-physiologic temperatures, and the cysteine alkylation itself may stabilize the DAT conformation. In contrast, MD analysis of binding of cocaine, benztropine or their analogs to the DAT allows for a true equilibrium between conformational states of the protein, and minute differences in inhibitor positioning can be detected. The distinctions in DAT interactions with LX10 and LX11 are supported by RMSF values measured for the DAT residues and changes measured in distances between salt bridge residues within OF DAT. These results further reinforce the hypothesized similarity in DAT binding interactions for cocaine and LX10,²² and provide molecular insight into possible differences in LX10 and LX11 positioning in the OF DAT conformation.

While the need for an inhibitor to stabilize the OF DAT conformation in order to be an abusable drug appears unlikely, it is possible that certain DAT-inhibitor complexes adopt a 3D structure that is conducive to eliciting (or blocking) euphoria. This might be somewhat analogous to the phenomenon of “biased agonism”, in which the signal transduction pathway employed by G protein-coupled receptors is dictated by the agonist.⁵⁶ For example, subtle 3-D differences between mu opioid receptor complexes with morphine versus TRV130 afford the latter drug powerful analgesic properties without sharing morphine’s abuse potential and other adverse effects.⁵⁷ Similarly, changes in DAT conformation may promote interactions with selected intracellular macromolecules that provoke discrete signaling mechanisms, manifested as behavioral differences such as seen with cocaine and benztropine. A better understanding of the intricacies of DAT-inhibitor interactions may

provide the next lead compounds in the elusive search for an effective therapeutic for psychostimulant abuse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Molecular dynamics (MD) simulations addressed the previously-hypothesized correlation between psychostimulant abuse potential and the dopamine transporter (DAT) conformation recognized by the drug.
- Cocaine analogs of varying abuse potential were tested for DAT conformation preference via MD.
- MD may be useful in antipsychostimulant therapeutic design.

Figure 1.

A) DAT inhibitors cocaine (COC), benztropine (BZT), and 3β-aryltropanes with 2-substituted phenyltropane analogs LX10 and LX11. The asterisk denotes the tropane C-2 position at which the analogs stereochemically differ. B) PDB coordinates of the *Drosophila* DAT co-crystallized with cocaine (cyan), superposed with the rat DAT homology model bound to cocaine (red). Key S1 pocket side chains are annotated and color-coordinated with the ligand for a given model.

Figure 2.

Representation of the water molecules (red spheres) within the outer and inner cavities in the A) OF and B) IF DAT. C, D) Number of water molecules within 5 Å of the residues in the outer (residues D79 (TM1), W84 (TM1), R85 (TM1), Y88 (TM1), F155 (TM3), Y156 (TM3), I311 (TM6), D312 (TM6), T315 (TM6), F319 (TM6), D475 (TM10), T481 (TM10)) and inner (residues F69 (TM1), G75 (TM1), F76 (TM1), G257 (TM5), S261 (TM5), V265 (TM5), T268 (TM5), F325 (TM6), F331 (TM6), G424 (TM8), E427 (TM8) and T431 (TM8)) cavities. The average number of water molecules was analyzed between each pair of OF (green) and IF (violet) systems by two-tailed t-test. * $P < 0.05$; error bars (red) represent the standard error of the mean.

Figure 3.

Distances measured between amino acid side chains for A) the external gate residue pair R85-D475 and B) the internal gate residue pair R60-D435 in inhibitor-bound OF (blue) and IF (red) DAT after 100 ns MD. SASA measurements for OF DAT (blue) or IF DAT (red) after 100 ns simulation for C) the outer cavity and D) the inner cavity.

Figure 4.

A) LX10 (blue) and LX11 (green) in OF DAT are superposed for comparison of the inhibitors in the S1 binding pocket. The ligands (vdW spheres) and DAT residues are color-coded. The tropane C3 chlorophenyl ring and a ring portion of the C2 diphenyl ether moiety of LX11 are oriented toward the intracellular space after 100 ns of MD. The red arrows indicate regions of structural differences seen after 100 ns. B) LX10 and C) LX11 bound in IF DAT, with water molecules solvating the inner cavity. Surfaces (gray) surrounding the water molecules show regions of solvation. The snapshots represent the last frame of the simulation. Average values are given in Figure 2D.

Figure 5.

For the A, B) LX10 or C, D) LX11 complex with OF DAT, distances between amino acid residue side chains at the TM-cytoplasm interface: R60-D435 (orange), R60-E427 (blue), D435-R444 (pink), E61-R444 (red) and D435-K133 (green). Values represent the shortest possible distance between the nitrogen atoms of arginine/lysine side chains and carboxylate oxygen atoms of aspartate/glutamate side chains.

Figure 6.

A) RMSF calculations for the C α atoms of OF DAT residues. RMSF values are plotted against residue number. RMSF traces are superposed to better compare the inhibitor-bound systems: Cocaine (orange) and LX10 (blue), cocaine (orange) and LX11 (green), and LX10 (blue) and LX11 (green). B, C) The final snapshot of the 100 ns simulation is represented and the OF DAT bound to cocaine (orange) was superposed with the OF DAT bound to LX10 (blue) or LX11 (green). The final structure of OF DAT bound to cocaine (orange) after 100 ns MD was used as the reference structure in RMSD measurements for the C α atoms of residues in TM1, TM3, TM6, TM8, and TM10 for the 100 ns trajectory of LX10 and LX11 bound in OF DAT. D–F) Binding site interactions in OF DAT superposed after 100 ns simulation. Ligands are represented as ball-and-stick; colored side chains corresponding to the inhibitor-bound systems are represented as lines. D) COC (orange) and LX10 (blue); E) COC (orange) and LX11 (green); F) LX10 (blue) and LX11 (green).