Distinctions between Dopamine Transporter Antagonists Could be Just around the Bend

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ABSTRACT

Abuse of psychostimulants such as cocaine and amphetamines has a tremendous social and economic impact. Although replacement therapies are offered for addiction to opioids, nicotine, and alcohol, there is no approved replacement treatment for psychostimulant addiction. Recent studies on an emerging group of benztropine- and rimcazole-based compounds provide hope that replacement therapies for cocaine and amphetamine addiction may come in the near future. A new study (p. 813) now investigates the molecular interaction of the benztropine and rimcazole compounds with their target, the dopamine transporter, and provides an intriguing explanation as to why use of these compounds, unlike cocaine, do not lead to locomotor stimulation and drug discrimination behaviors in animal models.

Drug use typically begins as a voluntary decision that, with repeated exposure, is superseded by involuntary craving and pursuit of illicit agents despite adverse physical and social outcomes (Volkow and Li, 2005). Overall, the social and economic impact of drug addiction is enormous. Estimates indicate the presence of nearly 30 million drug addicts in the United States and Western Europe alone (Pouletty, 2002). Cocaine and amphetamine addicts face distinct challenges in kicking their habits because, unlike those seeking treatment for alcoholism or opiate addiction, no clinically proven substitution therapies are available for psychostimulant abuse. The psychostimulant properties of cocaine are considered to be mediated primarily through the blockade of the dopamine (DA) transporter (DAT), which results in increased levels of extracellular DA. This concept has gained perhaps its strongest recent support from the work of Chen et al. (2006) using transgenic mice bearing engineered mutations in DAT that reduce cocaine potency in vitro, where cocaine becomes a motor depressant and loses the ability to support conditioned place preference behavior. One often-discussed but currently impractical concept for reducing the grip of cocaine on the DAT in human addicts is the use of a nonreinforcing blocker of cocaine binding that does not itself perturb DA transport (Lin and Madras, 2006). This ambitious goal first requires the identification of molecules that can bind to DAT in a mode distinct from that of cocaine and most other DAT antagonists. In a new study, Loland et al. (2008) find that benztropine- and rimcazole-based compounds, recently reported as potential medications for cocaine addiction (Dutta et al., 2003), promote non–cocaine-like conformations of DAT and could be a pivotal step toward realizing psychostimulant substitution therapy.

We have long known that cocaine and other psychostimulants can potentiate DA signaling by binding to DATs and preventing DA clearance from the synapse. The study by Loland et al. (2008) supports a more nuanced view that the physiological result of DAT blockade depends heavily upon the temporal profile of the drug’s access to the transporter and on the conformational changes produced after binding. This new work stems from a recent report of benztropine (BZT) and rimcazole-based DAT inhibitors that yield behavioral outcomes in animal studies quite distinct from tests...

**ABBREVIATIONS:** DA, dopamine; DAT, dopamine transporter; BZT, benztropine; MTS, methanethiosulfonate; NET, norepinephrine transporter.
with cocaine (Katz et al., 1999, 2003). These differences include lack of locomotor stimulation and an inability to discriminate the drug from saline. Given that blockade of DAT by cocaine is thought to be the primary mechanism whereby locomotor stimulation and psychostimulant discrimination are thought to arise, the absence of these behaviors in mice treated with these analogs, despite exhibiting high affinity for DAT, seems paradoxical. Loland et al. (2008) tackle this issue with molecular approaches that can define conformational states in DAT before and after antagonist binding.

The adoption of distinct conformational changes in DAT upon antagonist binding compared with DA-bound DAT has already been suggested using protease sensitivity assays (Gaffaney and Vaughan, 2004). Now, Loland et al. (2008) probe conformational changes in DAT, both before and after binding of the BZTs, by monitoring accessibility of an introduced cysteine at residue Ile159 to cysteine-reactive methanethiosulfonates (MTS). Reaction of DAT I159C with MTS reagents has been used by others to infer changes in protein conformation, such as one might envision with the rotation of a helix or the shift from open to closed status of an extracellular gate (Chen and Rudnick, 2000; Loland et al., 2004). Accessibility and modification of I159C by MTS reagents results in loss of DA transport, which the authors believe reflects a state in which an outer gate limiting access to the DA permeation pathway is open; inability of MTS reagents to react with I159C and inactivate transport reports that this gate is closed. Results from the Loland et al. (2008) MTS studies suggests that several BZT analogs bind to a conformation of DAT with the outer gate closed. This is in direct contrast with cocaine, which seems to bind to, and stabilize, the "open" state of the transporter.

But is there any evidence for structural parallels to these "gates"? Before the pioneering studies of Yamashita et al. (2005) on the high-resolution structure of a bacterial homolog of DAT, LeuTα, this was just speculation. In the LeuTα structure, charged residues have been identified that can serve as either extra- or intracellular gates (Fig. 1). The breaking and stabilization of salt bridges between these charged residues is postulated to promote either the entry or exit of substrate (e.g., DA) from the transporter. So the prediction is that whereas a DAT/cocaine complex binds to a conformation with the outer pair of residues separated ("gate open"), binding of some BZT-based compounds promotes a DAT conformation in which the salt bridge is stabilized ("gate closed"). Of course, these gates are expected to open and close across the course of the transport cycle, although structures that validate this concept have yet to be reported. In addition, although candidate residues for the DAT gates are easy to identify, proof of their use in such a mechanism awaits a high-resolution structure of DAT in the absence and presence of antagonists.

Loland et al. (2008) provide additional evidence that cocaine and the BZT compounds bind different forms of DAT using a clever pharmacological strategy that takes advantage of the unique properties of a previously studied Y335A mutant (Loland et al., 2002, 2004). The Y335A mutant has been shown to promote a change in conformation of DAT, consistent with a shift of the protein to an "inward facing" conformation. Using competition binding analyses, Loland et al. (2008) demonstrate that several BZT compounds exhibit an apparent increase in binding affinity to this "inward facing" state, whereas cocaine's affinity is actually decreased. Although there may be other explanations for these effects, the data strongly suggest that BZT-based compounds bind a form of DAT conformationally distinct from that bound by cocaine. Mason et al. (2007) recently reported that novel norepinephrine transporter (NET) antagonists, typified by desvenlafaxine sulfate, exhibit sensitivity to membrane disruption that classic NET antagonists such as desipramine and nisoxetine do not. Perhaps the latter observations can also be explained by distinct conformations stabilized by different classes of NET antagonists, only some of which are allowed after membrane disruption.

This differential binding between BZTs and cocaine at DAT is interesting, but perhaps of greater significance is the link discovered by the authors between binding characteristics of the compound and the compound's impact on animal behavior. When mice or rats are used to assess locomotor behavior or drug discrimination, the propensity of a BZT analog to favor the Y335A-induced "inward-facing" DAT conformation seems predictive of a concomitant loss of cocaine-like behaviors. The unique characteristics of these compounds assure their further study as prospective leads for psychostimulant substitution therapy. The potential generality of these findings to those interested in other transporters, such as the antidepressant-sensitive NET or serotonin transporter, are easy to see and may ultimately be of larger impact given the high lifetime incidence of mood disorders commonly treated with NET or serotonin transporter block...
ers. Besides the opportunities highlighted by Loland et al. (2008) for DAT-targeted therapies, perhaps novel antidepressants that act faster, have better efficacy, and permit safer discontinuation are also just around the bend.

References

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