The partial occlusion of the central site observed in the dDAT:DCP complex is mediated by an inward movement of TM1b, 2, and 6a. The movement is probably mediated by a movement of three conserved phenylalanine residues: Phe318, Phe319, and Phe325. Phe319 rotates to occlude the binding pocket, completely preventing solvent access and, thus, dehydrating the pocket. Also, Phe325 moves toward the catechol ring of DCP and stabilizes its binding. Phe318 rotates away from the binding site and participates in the inward movement of TM6b. To accommodate these side chain shifts, TM11 undergoes an outward movement and TM2 moves in. Interestingly, TM2, 7, and 11 form a binding site for cholesterol which has shown to be important for ligand binding.

14.3 DRUGS TARGETING BIOGENIC AMINE TRANSPORTERS: SPECIFICITY, USE, AND MOLECULAR MECHANISM OF ACTION

The biogenic amine transporters, DAT, NET, and SERT, are targets for a wide variety of drugs. Overall, these drugs can be classified as either pure inhibitors that block substrate binding and transport, or as substrates that in addition to compete with the endogenous substrate also are transported themselves.

The binding mode of inhibitors to the biogenic amine transporters has been derived from the crystal structures of dDAT and LeuT solved in complex with antidepressants and illicit drugs such as cocaine and amphetamine. Common for all the drugs is that they bind competitively with the substrate. The binding site can be divided into subsites A, B, and C. In the dDAT, subsite A consists of residues Phe43 and Asp46 from TM1 and Gly322 and Ser421 from TM6 and 8, respectively. Subsite B consists of Ala117, Val120, Asp121, and Tyr124 from TM3, Phe325 in TM6 and Gly425 from TM8. Subsite C consists of Phe319 from TM6 and Asp475 and Ala479 from TM10 (Figure 14.3). In general, subsite A accommodates the polar amine moiety of the inhibitors as it does for substrates. This is mainly mediated by Asp43, and also from the main chain carbonyl oxygen and nitrogen from the unwound regions of TM1 and 6 as potential hydrogen bonding partners. Ser421 does also contribute to the polarity in subsite A. Subsite B defines a nonpolar ridge (Phe325 and Val120) that accommodates the hydrophobic groups of the drugs. It also forms a groove (Ala117, Asp121, Ser421, and Gly425) that accommodates the polar groups in the drugs' rings, such as the chloro-, dichloro-, trifluoromethyl-, and benzodioxol groups. Subsite C is distal to the substrate binding site and located to the extracellular vestibule. This subsite interacts with bulky drugs that probably enhance affinity and specificity.

14.3.1 Cocaine, Benztropine, and Other Tropane Class Inhibitors

The most thoroughly studied class of inhibitors at the biogenic amine transporters is the tropane class with cocaine as the most well-known member (Figure 14.3). Cocaine is a moderately potent antagonist inhibiting the function of all three transporters nonselectively. However, correlative studies, as well as studies on genetically modified mice suggest the presynaptic DAT as the primary target for cocaine's stimulatory action. DAT knock-out mice are insensitive to the administration of cocaine and, moreover, knock-in mice expressing a functional DAT mutant incapable of binding cocaine shows insensitivity to cocaine administration. It is, therefore, the current view that the rapid increase in extracellular dopamine concentration elicited by cocaine inhibition of DAT produces the psychomotor stimulant and reinforcing effect that underlie cocaine abuse.

Some closely related cocaine analogs possess higher potency toward the biogenic amine transporters and, thus, have been more suitable than cocaine itself in experimental set-ups (e.g., radioligand binding assays) directed toward understanding the pharmacological properties of the transporters. Important examples include CFT (2β-carbomethoxy-3β-(4-fluorophenyl)tropane or WIN 35,428) and RTI-55 ((−)-2β-carbomethoxy-3β-(4-iodophenyl)tropane or β-CIT) (Figure 14.3). Both compounds display nanomolar affinity for the biogenic amine transporters; however, while CFT shows selectivity for DAT over NET and SERT, RTI-55 shows selectivity for SERT and DAT over NET.