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Neurotransmitter transporters as molecular targets for addictive drugs

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1. Introduction

The inhibition of biogenic amine transport can have profound behavioral and physiological consequences, and accordingly, the vesicular and plasma membrane monoamine carriers have been recognized as important targets for a wide variety of clinically important antidepressant, antihypertensive and psychostimulant drugs. Initial studies of neurotransmitter uptake systems in brain slices, synaptosomes and plasma membrane vesicles demonstrated a variety of activities that could be distinguished not only on the basis of their kinetic properties and specific ionic requirements, but also by their sensitivity to selective antagonists (reviewed by Amara and Arriza, 1993; Amara and Kuhar, 1993; Rudnick and Clark, 1993; Brownstein and Hoffman, 1994). A basic premise for the actions of psychostimulants, such as cocaine and amphetamines has been that these drugs act acutely to block transport, thus elevating extracellular concentrations of the biogenic amine neurotransmitters dopamine, norepinephrine, and serotonin, and thereby potentiating the activation of post-synaptic receptors (Fig. 1). Psychostimulants, such as amphetamines also act on a class of monoamine transporters on synaptic vesicles to promote the release of stored amines into the cytoplasm. As outlined in several of the reviews in this volume, many complex neurobiological and behavioral issues need to be considered before the biology of psychomotor stimulant addiction can be understood at a systems level. However, significant insights continue to come from investigations into the fundamental biology of the transporters themselves. This review will focus on recent developments in our understanding of the structure, mechanistic features and physiological contributions of monoamine transporters as they relate to the actions of psychostimulant drugs of abuse.

Two gene families are involved in the transport of the biogenic amines — the Na^+/Cl^--dependent plasma membrane carriers and the H^+-dependent vesicular amine carriers. Molecular cloning studies have demonstrated that the plasma membrane transporters for the monoamines, such as norepinephrine (Pacholczyk et al., 1991), dopamine (Giros et al., 1991; Kilty et al., 1991; Shimada et al., 1991; Usdin et al., 1991), and serotonin (Blakely et al., 1991; Hoffman et al., 1991) are members of a large family of Na^+/Cl^--dependent transporters which also includes the carriers for GABA, proline, glycine, creatine, betaine, taurine and other organic substrates (reviewed by Amara and Arriza, 1993; Amara and Kuhar, 1993; Rudnick and Clark, 1993; Brownstein and Hoffman, 1994). A universal feature of this class of transport proteins is that substrate entry is coupled to the inward cotransport of sodium ions, which provide the energetic driving force for accumulation within the cell. A second structurally distinct group of carriers catalyzes the movement of monoamines from the cytoplasm into secretory and synaptic vesicles. For these vesicular monoamine carriers, substrate transport is thermodynamically coupled to the outward movement of protons across the vesicle mem-

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ABR: GABA, γ-aminobutyric acid; GAT1, rat γ-aminobutyric acid transporter 1; NET, norepinephrine transporter; DAT, dopamine transporter; SERT, serotonin transporter; VMAT, vesicular monoamine transporter; VAChT, vesicular acetylcholine transporter; VGAT, vesicular γ-aminobutyric acid transporter; DA, dopamine; 5-HT, serotonin, 5-hydroxytryptamine; TM, transmembrane domain; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; CFT, (−)-2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane; DEEP, 1-[2-(diphenylmethoxy)-ethyl]-4-[2-(4-azido-3-iodophenyl)ethyl]-piperazine; RTI-82, 3β-(*p*-chlorophenyl)tropane-2β-carboxylic acid, 4′-azido-3′-iodophenylethyl ester

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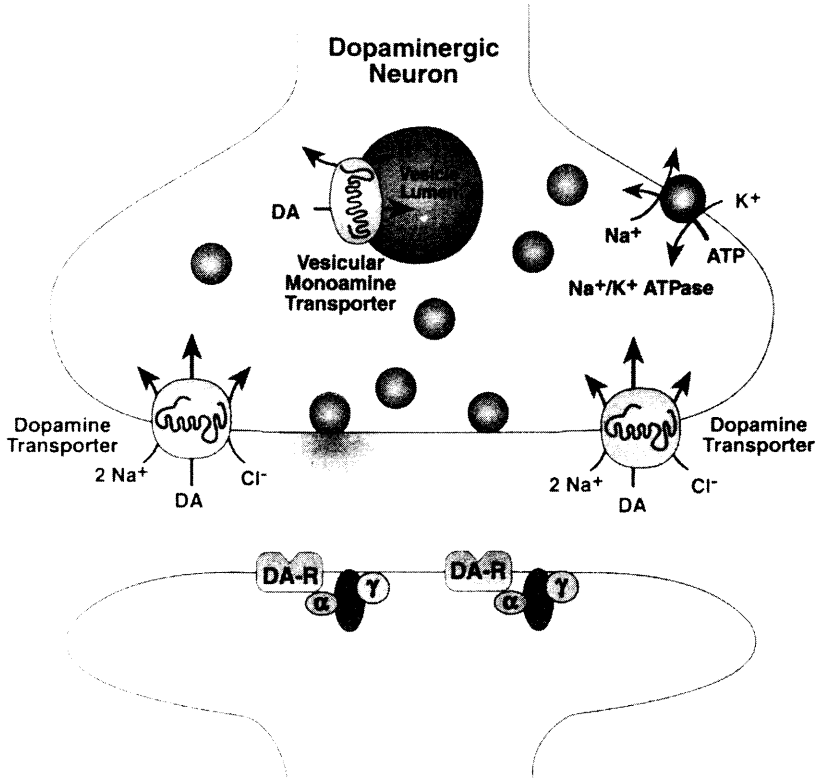

Fig. 1. Transporter proteins involved in the uptake of dopamine. Na^+- and Cl^--dependent transporters for dopamine are localized in dopaminergic neurons though not exclusively near sites of neurotransmitter release. The DAT uses the energy stored in the sodium gradient generated across the plasma membrane by the Na^+/K^+-ATPase. Neurotransmitters diffuse across the synaptic cleft to bind to their respective receptors. Both diffusion and transport act to decrease extracellular concentrations of neurotransmitters, thus limiting the stimulation of receptors. Once the neurotransmitter is transported into the cell, it can be taken up into vesicles by VMATs which can be present on both large dense core vesicles and on small synaptic vesicles. VMATs energetically couple monoamine transport to the proton electrochemical gradient across the vesicular membrane.

brane (Edwards, 1992; Schuldiner, 1994; Liu and Edwards, 1997).

The molecular characterization of plasma membrane carriers began with the purification, amino acid sequencing and cloning of a rat GABA transporter (GAT1) (Guastella et al., 1990) and was extended with the expression cloning of the human norepinephrine transporter (NET) (Pacholczyk et al., 1991). A comparison of the predicted amino acid sequence of GAT1 and NET revealed a high degree of sequence homology which became the basis for identifying cDNAs encoding the dopamine (DAT) and serotonin (SERT) transporters. Although at least four different subtypes of GABA carriers have been identified (Liu et al., 1993) the three biogenic amine transporters, NET, DAT and SERT, appear to be encoded by single genes. Sequence-based predictions have been made for the transmembrane topology and structure of the different family members, but the proposed models have not been fully verified by experiments. Based on analyses of hydrophobicity, proteins in the Na^+- and Cl^--dependent transporter family are best described by a model with 12 transmembrane domains (TMs), intracellular amino- and carboxyl-termini, and a large extracellular loop with multiple N-linked glycosylation sites between the TM3 and TM4 (Brüss et al., 1995; Vaughan and Kuhar, 1996; Clark, 1997; Hersch et al., 1997) (Fig. 2). Members of the Na^+- and Cl^--dependent transporters have no close sequence similarities with any other major carrier family, including the excitatory amino acid transporters that catalyze the transport of glutamate in the vertebrate CNS (Amara and Arriza, 1993; Attwell and Mobbs, 1994).

2. Vesicular monoamine carrier family

A novel expression strategy was utilized for the cloning of a monoamine carrier (Liu et al., 1992b). The expression of a vesicular monoamine carrier confers resistance to MPP^+, the toxic metabolite of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) by sequestering MPP^+ into vesicles, thereby limiting the toxic actions of the compound on mitochondrial function. This property became the basis for selection of cells expressing a cDNA encoding a vesicular

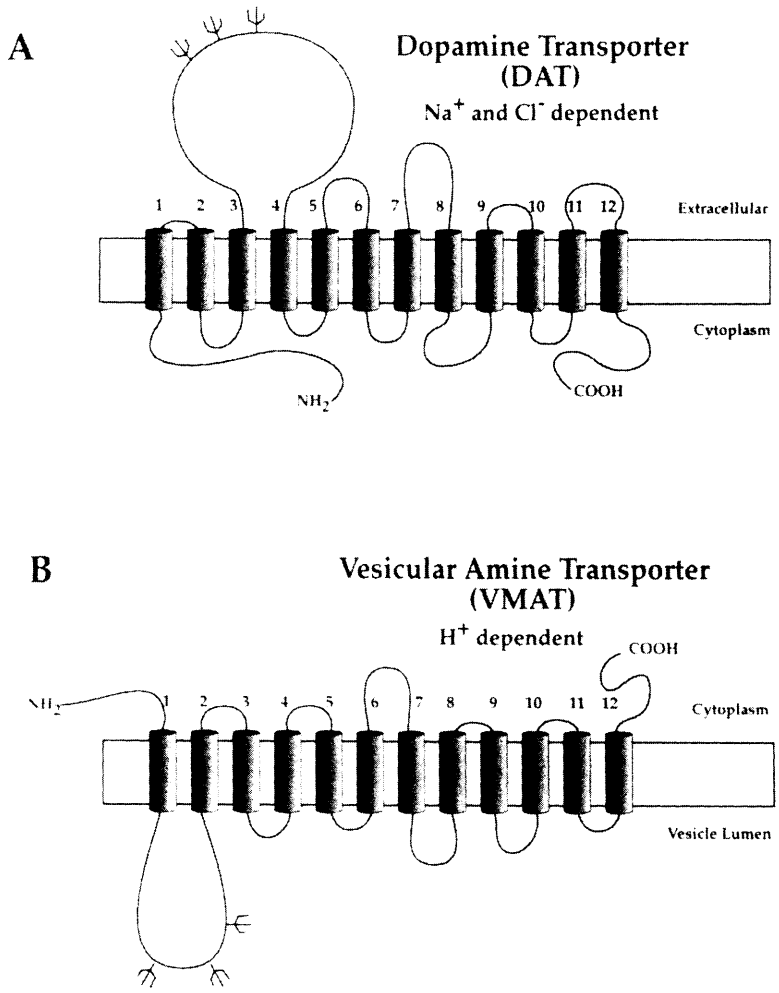

Fig. 2. Proposed topology and structural features of (A) the dopamine transporter (DAT) and (B) a vesicular monoamine transporter (VMAT). As predicted by hydrophobicity analyses of the predicted protein sequences, the both carrier families have 12 TMs with N- and C-termini within the cytoplasm. Large glycosylated extracellular loops are present between TM 3 and 4 and TM 1 and 2 in DAT and VMATs, respectively.

monoamine transport activity exhibiting high affinity for multiple monoamine neurotransmitters, a dependence on the proton electrochemical gradient and a characteristic sensitivity to pharmacological agents, such as reserpine (Liu et al., 1992a, Lin et al., 1996). This family has since been expanded to include two distinct vesicular monoamine transporters (VMAT1 and VMAT2) (Erickson et al., 1992; Liu et al., 1992a,b; Erickson and Eiden, 1993) and a cDNA encoding a vesicular transporter for acetylcholine (VAChT) (Roghani et al., 1994; Varoqui et al., 1994). Hydrophobicity analyses of the predicted vesicular monoamine carrier proteins suggest a topology of 12-membrane spanning domains and a large luminal loop between the first and second transmembrane segments (Fig. 2). VMAT1 and VMAT2 differ in their substrate selectivity and inhibitor sensitivity. The neuronal VMAT2 exhibits a higher affinity for monoamine substrates, particularly histamine, and has a greater sensitivity to the inhibitor tetrabenazine than the non-neuronal VMAT1.

Serotonin, norepinephrine, dopamine and histamine can all serve as substrates for the vesicular monoamine transporters. These carriers are the sites of action of reserpine and tetrabenazine and have long been implicated in the depletion of vesicular monoamines caused by amphetamines. Amphetamines have complex actions that interfere with both vesicular and plasma membrane monoamine transporters. Amphetamines enter the cell as substrates of the plasma membrane carriers but they can also cross the plasma membrane by lipophilic diffusion. Once in the cell, amphetamines and other related β-phenethylamines act directly on the vesicular monoamine carriers, exchanging with the vesicular pool of amines (Fischer and Cho, 1979). Several reports suggest that amphetamine-like compounds may also act indirectly by a weak base mechanism to

dissipate the vesicular pH gradient and further exacerbate vesicle depletion (Sulzer and Rayport, 1990; Sulzer et al., 1993) though amphetamines are also substrates of the VMATs (Schuldiner et al., 1993; Peter et al., 1994). The resulting elevation in cytoplasmic monoamine concentrations lead to conditions that favor an enhanced efflux of cytoplasmic neurotransmitter resulting from a plasma membrane transporter-mediated exchange as alternative substrates (e.g. amphetamines) are imported into the cells.

Recently, a vesicular GABA transporter has been identified as the product of the nematode *Caenorhabditis elegans unc*-47 gene (McIntire et al., 1997). Characterization of the protein and its rat homologue (rVGAT) reveal a new family of vesicular neurotransmitter transporters which has only 10 putative TMs and, in contrast to the VMATs, a relatively greater dependence on the electrical ($\Delta\Psi$) than on the chemical ($\Delta[H^+]$) component of the vesicular proton gradient.

3. Lessons from transporter knockouts

Cocaine is a moderately potent antagonist of all three biogenic amine transporters, but the addictive and euphoric properties of the drug have been attributed to inhibition of dopamine reuptake in the nucleus accumbens and other targets of the mesolimbic dopamine system. This focus on the dopamine transporter as the primary target for the effects of cocaine has been supported by self-administration studies, in which reinforcing properties of cocaine and related drugs correlate with their potencies at inhibiting [^{3}H]mazindol binding to dopamine, but not serotonin or norepinephrine transporters (Ritz et al., 1987). However, additional physiological actions of cocaine may also be determined by their ability to block other monoamine transporters. For example, the elevations of blood pressure are largely the consequences of increased sympathetic tone caused by reuptake blockade at both peripheral noradrenergic synapses and noradrenergic terminals within critical brainstem nuclei. As will be discussed below, amphetamines are somewhat different in their mechanism of action as they interact with both the vesicular and plasma membrane amine transport systems.

Cocaine raises extracellular concentrations of dopamine which, in turn, can activate receptors over wider distances and longer durations than are normal. These studies have formed the basis for the dopamine hypothesis of psychomotor stimulant action and support the notion that inhibition of dopamine reuptake is the primary mechanism mediating self-administration of cocaine and other psychomotor stimulants (Ritz et al., 1987; Di Chiara, 1995; Wise, 1996). A formal demonstration of the significance of the DAT to the action of cocaine and amphetamines has come from the generation of a transgenic mouse line in which the DAT gene has been deleted by targeted disruption (Giros et al., 1996). The striking hyperactivity associated with this mouse strain mimics some of the most salient behavioral actions of psychostimulants. Furthermore, the locomotor behavior and rate of dopamine clearance observed in homozygotes are not altered by cocaine or amphetamine administration, providing additional support for the importance of the DAT as an obligate target for these stimulant drugs of abuse. In addition to a markedly enhanced persistence of dopamine in the extracellular space, homozygotes display several profound neurochemical alterations in the nigrostriatal dopaminergic system, including a decrease in the releasable pool of dopamine, a decrease in the number of D_2 autoreceptors and a decrease in the content of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis. Detailed examination of dopamine release and uptake in the nucleus accumbens has not been completed in homozygote mice, however, such studies are likely to provide further insight on the contribution of DAT to processes associated with addiction. Interestingly, deletion of the DAT also leads to anterior pituitary hypoplasia, dwarfism, and an inability to lactate, all apparent consequences of the elevated dopamine concentrations which reduce the hypothalamic content of growth hormone-releasing hormone and down-regulate the dopamine receptors on pituitary lactotrophs (Bossé et al., 1997).

A more detailed examination of amphetamine effects in the DAT knockout mice has provided additional support for the importance of DAT for the releasing action of amphetamine in dopaminergic neurons (Jones et al., 1998). Extracellular dopamine concentrations, as determined by microdialysis in freely moving animals, do not change after amphetamine administration in mice lacking the DAT, but increase 10-fold in wild-type mice. Similarly, voltammetric measurements of extracellular dopamine in striatal slices show amphetamine-induced increases only in slices from mice that have an intact DAT gene. However, elevation of the cytoplasmic dopamine concentration alone does not appear sufficient to cause efflux: when a fast acting reserpine-like compound is used to displace dopamine from the vesicles in wild-type animals, efflux is not observed unless amphetamine is added. In the absence of the plasma membrane carrier amphetamine can still diminish stimulated release, consistent with the drugs entering the cell by lipophilic diffusion across the plasma membrane and subsequently causing vesicular depletion. Nevertheless, DAT is required for amphetamine to promote dopamine efflux.

4. VMAT-2 knockouts — supersensitivity and quantal release

The targeted disruption of the gene encoding VMAT2, the predominant vesicular monoamine carrier in brainstem and midbrain regions, not only confirms the importance of VMAT for behavior and monoamine content but also reveals the tight linkage between transporter expression and the amount of neurotransmitter stored within each synaptic vesicle (Fon et al., 1997; Takahashi et al., 1997; Wang et al., 1997). Although their brains appear grossly normal and monoaminergic cell group and pathways appear indistinguishable from wild-type littermates, newborn homozygotes show decreased spontaneous motor activity, feed poorly and die within a few days after birth. Both monoamine storage and vesicular release are severely impaired, but amphetamine promotes dopamine release and, in vivo, increases movement, improves feeding and allows the mice to survive for longer periods of time (Fon et al., 1997). These vivid experiments suggest a 'neurohumoral' contribution of dopamine in motor control that is consistent with therapies for Parkinson's disease which elevate the availability of dopaminergic agonists in a diffuse fashion. Furthermore, the indication that amphetamine can stimulate dopamine efflux in the absence of vesicular stores affirms the importance of the plasma membrane DAT in the drug's action.

In heterozygous adult mice, extracellular striatal dopamine levels, as well as K^+- and amphetamine-stimulated dopamine release are diminished, but the mice show a dramatic adaptive supersensitivity to the locomotor effects of apomorphine, cocaine, amphetamine and ethanol (Wang et al., 1997). In addition the VMAT2 heterozygotes do not develop additional sensitization to repeated administration of cocaine. Another group observed that in the heterozygous VMAT2 knockout, amphetamine enhanced locomotor behavior but diminished behavioral reward as measured in a conditioned place preference test (Takahashi et al., 1997). When compared with the wild-type strain, VMAT2 heterozygotes have half the VMAT2 protein, half the monomine content, and half the VMAT activity. Cultures and slice preparations from midbrain regions of these mice release approximately half as much dopamine as their normal littermates, suggesting that the rate of vesicle recycling exceeds the rate of vesicle filling, or alternatively, that a reduced number of vesicles have VMAT present (Fon et al., 1997). Because neural activity determines the rate of vesicle recycling, the most suprising implication of these findings is that the system operates near a set point where neuronal activity and transporter expression can influence quantal size.

5. Structure–function relationships and drug action

The sequence conservation among the transporters for dopamine and norepinephrine has guided the study of transporter functions. Studies of site-specific transporter mutants and chimeric proteins have pointed to amino acids and domains involved in substrate specificity and psychostimulant interactions. The analysis of chimeras has provided correlative information on the domains that contribute to the unique properties of each carrier, such as its specificity for different substrates or its sensitivity to selective inhibitors. For the monoamine transporters, chimeric proteins have been constructed from domains of DAT and NET (Buck and Amara, 1994, 1995; Giros et al., 1994) and from human and rat SERT (Barker et al., 1994; Barker and Blakely, 1996). Although the functional domains are neither fully defined nor spatially determined, the evidence collectively indicates the importance of amino acids in transmembrane regions 1–3 and transmembrane regions 9–11 for substrate affinity, while sequences in transmembrane regions 5–8 contribute to substrate translocation efficiency and inhibitor interactions (Buck and Amara, 1994, 1995; Giros et al., 1994). Other results using SERT chimeras between human and rat homologs indicated that the comparatively higher affinity of the human transporter for tricyclic antidepressants could be attributed to a region which is C-terminal to TM7, and that for imipramine itself, may be ascribed to any of five divergent amino acid residues in TM12 (Barker et al., 1994). Further pursuit led to identification of a single amino acid, 586 (phenylalanine in hSERT, valine in rSERT) as being a principal determinant for this affinity difference (Barker and Blakely, 1996).

Mutagenesis of sites conserved among monoamine transporters has suggested the differential importance of specific residues in TM1 (D79) and in TM7 (S356, S359) in the rat DAT for uptake function and for binding the cocaine analogue CFT. Replacement of an aspartate residue within TM1 (D79) with alanine or glycine lowered the binding affinity of [^{3}H]CFT by seven- to 10-fold without affecting its B_{max}. These mutations also reduced the apparent affinity for [^{3}H]DA uptake and strongly reduced its V_{max}, but their more striking effect was to lower the potency of dopamine for inhibiting [^{3}H]CFT binding by >20-fold. In contrast, mutations of serine residues in TM7 mainly affected substrate translocation and had little effect on binding of [^{3}H]CFT; replacement of serines 356 and 359 with alanine or glycine substantially reduced the V_{max} for [^{3}H]DA transport (Kitayama et al., 1992) whereas alanine substitutions for residues 350 and 353 preferentially elevated the V_{max} of [^{3}H]MPP$^+$ transport (Kitayama et al., 1993). These observations suggest that it is possible to partially

dissociate some transporter domains affecting translocation from others influencing both cocaine analog binding and substrate movement. The data which have been most sought after — but not yet found — is the evidence for structures which strongly modulate cocaine analog binding but affect neither dopamine recognition nor translocation. If such results were found, they would lend credence to the idea that a cocaine 'antagonist' might be identified which would not itself elevate extracellular dopamine levels.

Interestingly, even though the D79A and D79G mutants strongly affected the binding affinity of both CFT and dopamine (Kitayama et al., 1992), recent chemical structure-activity studies suggest that what is in common is not a direct ionic interaction of the wild-type aspartate with positively charged amine groups in both dopamine and cocaine. Analogs of cocaine lacking a protonated nitrogen either due to N-sulfonyl substitution (Kozikowski et al., 1994) or N replacement (Meltzer et al., 1997) still potently block both dopamine uptake and transport-inhibitor binding by DAT.

In a few regions, site-directed mutations in residues conserved between biogenic amine transporters have revealed comparable changes in function. A pair of conserved cysteine residues in the large extracellular loop between putative TMs 3 and 4 appears to form disulfide bonds in both rDAT (Wang et al., 1995) and rSERT (Chen et al., 1997; Sur et al., 1997). Mutation of either of the two residues results in a diminution of expression due to problems in trafficking of the transporter to the membrane, but replacement of both cysteines with serines appears to alleviate this deficit. In rSERT, the double mutant C200S–C209S can be biotinylated at the cell surface to a similar extent as the wild-type transporter, however, it also displays deficiencies in cocaine analog (β-CIT) binding and 5-HT transport that may be attributable in both cases to decreased affinities for sodium ions (Chen et al., 1997). This is the first report suggesting that the large extracellular loop contributes to ligand or ion binding or substrate translocation beyond regulating the level of expression. Earlier studies had demonstrated that mutations in the large extracellular loop of hNET influenced expression, but these mutations prevented glycosylation of the loop and inhibited trafficking of transporters to the cell surface (Melikian et al., 1996; Nguyen and Amara, 1996).

A more classical photoaffinity labeling strategy has also been used to study the structure and function of the DAT from rat striatal preparations. Two radiolabeled photoaffinity ligands react covalently with different regions of rDAT, presumably within transmembrane domains. The reagent [^{125}I]DEEP (based on the flexible GBR series of DAT ligands) incorporates into the N-terminal half of DAT whereas the phenyltropane derivative [^{125}I]RTI-82 (which more closely resembles cocaine) labels a distinct, more C-terminal region (Vaughan and Kuhar, 1996). Based on peptide-directed antibodies, trypsin proteolysis and carbohydrate digestion data, the authors show that [^{125}I]DEEP incorporates into a peptide that terminates before TM 3 whereas [^{125}I]RTI-82 labels a region comprising TMs 4–7. Although there are several caveats to the interpretation of the results of photoaffinity labeling, these data support the possibility that different classes of DAT inhibitors bind to distinct regions of DAT. In light of data showing that several potent inhibitors of DAT have quite different potential for abuse, it is intriguing to entertain the possibility that such differences in addictiveness could result from interactions with distinct domains of DAT (Rothman and Glowa, 1995).

Structure–function relations of the VMATs have been explored extensively by chimeric and site-directed mutagenesis strategies. After the recognition that TMs 5–8 and 9–12 from VMAT2 are both required to confer VMAT2-like pharmacology to chimeric constructs (Peter et al., 1996), a hunt was made to identify key residues contributing to serotonin, histamine, dopamine, tryptamine, and tetrabenazine sensitivity by substituting VMAT1 residues into a VMAT2 background (Finn and Edwards, 1997, 1998). Though it was found that some single amino acid substitutions could switch the affinities for particular ligands, these studies reveal that the relation between transporter structure and function is highly complex. Not only was it found that multiple residues could contribute to individual functions, but also that particular residues were determinants in multiple processes. Furthermore, work on VMATs also demonstrate that the interactions between transmembrane domains are important for function (Merickel et al., 1997).

6. Transporter-mediated currents and drug actions

Monoamine transport across the plasma membrane involves the cotransport of additional ions along with neurotransmitter. Depending on the net charge of substrates and ions translocated, the process could be expected to be electrogenic. Estimates of the ionic stoichiometry of monoamine carriers have come predominantly from experiments measuring the ionic dependence of initial rates of substrate transport. Earlier work examining the ionic dependence of activation of [^{3}H]substrate transport provided data consistent with ion stoichiometries in which norepinephrine and serotonin are cotransported with one sodium and one chloride ion, whereas dopamine is cotransported with two sodium ions and one chloride ion (Rudnick and Clark, 1993). In mammals, serotonin transport

also appears to involve a countertransported potassium ion which, in principle, would make the transport cycle electroneutral. However, electrophysiological investigations of neurotransmitter transporters reveal that all are electrogenic and that their electrical behaviors are considerably more complex than would be expected from a simple picture of cotransport of Na^+, Cl^-, and substrate (reviewed by Sonders and Amara, 1996; Lester et al., 1994).

Cloned neurotransmitter transporters have been studied directly using voltage clamp techniques to assess macroscopic currents in heterologous expression systems including *Xenopus laevis* oocytes and mammalian cells. Substrates were seen to elicit transmembrane electrical currents, referred to as 'transport-associated currents' that are dependent on the presence of extracellular ions Na^+ and Cl^-. Current measurements with the cloned biogenic amine transporters demonstrated that more charge moves during transport than would be expected for stoichiometric coupling of driving ions and substrate molecules — in some cases only slightly more though in other cases, hundreds of fold greater charge than substrate flux. Using *Xenopus* oocytes to express rSERT, Mager et al. (1994) found that measurements of [^{3}H]serotonin uptake velocity and serotonin-elicited currents in parallel oocytes yielded ratios of 5–12 net charges per serotonin molecule translocated. Concurrent measurements of dopamine uptake and of dopamine-elicited currents in single hDAT-expressing oocytes also exhibit net charge/substrate flux ratios in excess of those predicted by its putative coupling stoichiometry of $DA^+/2Na^+/1Cl^-$ (Sonders et al., 1997). Galli et al. (1997) have examined the *Drosophila* SERT using similar techniques and report large discrepancies between charge and serotonin flux. Large disparities between charge and substrate fluxes have also been observed in HEK-293 cells stably-transfected with hNET (Galli et al., 1995) and with hSERT (Qian et al., 1997). These studies indicate the existence of currents that are activated by substrate, but are not accounted for by the apparent stoichiometry of substrates and zcosubstrate ions during translocation.

This dissociation between charge and substrate movement suggests that transporters share some of the characteristics of ligand-gated ion channels (see also Lin et al., 1996; Galli et al., 1997). The analogy between transporters and channels is further strengthened by the observation that several transporters manifest macroscopic currents that are thermodynamically 'uncoupled' from substrate translocation *per se*. Striking examples of transporters which give rise to uncoupled currents can be found in the excitatory amino acid transporter (EAAT) family which display anion fluxes that are gated by substrates, such as glutamate and aspartate, yet the anions that comprise the current are unrelated to the cotransported ions required for substrate translocation (Fairman et al., 1995).

Additional currents that have been described for biogenic amine transporters include 'leak' and 'transient' currents. Leak currents are also uncoupled from substrate translocation since they can be observed in the absence of any substrate and yet are blocked by transporter ligands, such as cocaine for DAT, fluoxetine for SERT, and desipramine for NET. Transient currents are thought to arise from Na^+ ion binding, conformational change, and/or ion flux in response to step changes in membrane potential (Mager et al., 1993, 1996; Lu et al., 1995). Some studies have revealed that the ionic and pharmacologic sensitivities of the transient (Mager et al., 1994) and leak currents (Mager et al., 1994; Galli et al., 1995; Lin et al., 1996; Cao et al., 1997; Sonders et al., 1997) are distinguishable from those of the Na/Cl-dependent transport-associated currents. For instance, leak currents of hDAT and rSERT appear to be predominantly carried by protons while the rSERT is also somewhat unique in that part of its transport-associated current is carried by protons (Cao et al., 1997). Though it is not yet known whether transporter-regulated ion fluxes have physiological consequences, electrophysiological studies have revealed unanticipated complexity in the function of neurotransmitter transporters. Such tools allow transporters to be investigated with higher resolution than other methods presently used, and they may facilitate the development of therapeutic agents
tailored to modulate very particular transporter actions as our appreciation of carrier mechanisms improves.

What other functions could additional conductances associated with transporters have? Two intriguing papers suggest that the transport-associated currents mediated by electrogenic GABA and glutamate transporters may themselves trigger intracellular signaling. One group has reported that GABA-evoked transport currents in isolated skate retinal horizontal cells are of sufficient magnitude to depolarize the cells and cause the opening of voltage-sensitive calcium channels (Haugh-Scheidt et al., 1995). In the GH3 pituitary cell line, others have demonstrated that entry of glutamate through a sodium-dependent transporter was also capable of causing an influx of calcium (Villalobos and García-Sancho, 1995). In both studies, the investigators demonstrate that the pharmacological and ionic-dependence of dihydropyridine-sensitive calcium entry was consistent with the actions of substrate acting at transporters rather than at other receptors. Taken together these studies support the hypothesis that transporter-mediated cur-

rents, generated either by cotransport or by the existence of thermodynamically uncoupled conductances, can alter membrane potential and suggest a greater diversity of roles for neurotransmitter transporter in regulating the action of neurotransmitters in the CNS.

7. Summary

The neurotransmitter dopamine lies at or near the center of current theories of drug abuse and dependence. Multiple lines of evidence indicate that dopaminergic cells play key roles in a variety of motivated behaviors. Accordingly, it is not surprising that cocaine and amphetamines — some of the most widely used illicit drugs — elevate extraneuronal dopamine concentrations through their actions on the plasma membrane dopamine transporter. From the point of view of developing novel pharmacological interventions for the treatment or prevention of psychostimulant abuse, practical benefits may arise from an improved understanding of how neurotransmitter transporters operate and how drugs interact with them.

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