ELSEVIER

European Journal of Pharmacology 405 (2000) 329–339

ejp

www.elsevier.nl/locate/ejphar

# Structure and function of the dopamine transporter

Nianhang Chen [a,b], Maarten E.A. Reith [a,*]

[a] *Department of Biomedical and Therapeutic Sciences, College of Medicine, University of Illinois, Box 1649, Peoria, IL 61656, USA*
[b] *Department of Pharmacology, School of Basic Medical Sciences, Nanjing Medical University, 140 Han Zhong Road, Nanjing, Jiangsu 210029, People's Republic of China*

Accepted 28 June 2000

**Abstract**

The dopamine transporter mediates uptake of dopamine into neurons and is a major target for various pharmacologically active drugs and environmental toxins. Since its cloning, much information has been obtained regarding its structure and function. Binding domains for dopamine and various blocking drugs including cocaine are likely formed by interactions with multiple amino acid residues, some of which are separate in the primary structure but lie close together in the still unknown tertiary structure. Chimera and site-directed mutagenesis studies suggest the involvement of both overlapping and separate domains in the interaction with substrates and blockers, whereas recent findings with sulfhydryl reagents selectively targeting cysteine residues support a role for conformational changes in the binding of blockers such as cocaine. The dopamine transporter can also operate in reverse, i.e. in an efflux mode, and recent mutagenesis experiments show different structural requirements for inward and outward transport. Strong evidence for dopamine transporter domains selectively influencing binding of dopamine or cocaine analogs has not yet emerged, although the development of a cocaine antagonist at the level of the transporter remains a possibility. 

*Keywords:* Dopamine transporter; Cocaine; Cocaine analog; Phosphorylation; Chimera; Site-directed mutagenesis

## 1. Introduction

The present article is linked to Professor De Wied and the Rudolf Magnus Institute in two ways. First, one of the present authors (M.E.A.R) is fortunate to have experienced the inspiring leadership of Professor De Wied while studying for the Masters degree in the Rudolf Magnus Institute, and he is indebted to De Wied's mentorship while preparing for the PhD degree. De Wied's role model of scholarship, discipline, and creativity has been crucial in developing the experiments in this laboratory that are part of the present review of the dopamine transporter area. Second, De Wied's pioneering work on the role of neuropeptides in learning and memory has important ramifications for our understanding of mechanisms involved in the action of drugs of abuse, which clearly involve components of learning and memory. One class of drugs of abuse, the psychostimulants, has the dopamine transporter as a major target, and it is this protein that is the subject of the present mini-review.

The dopamine transporter or carrier, located on the plasma membrane of nerve terminals, transports dopamine across the membrane. By taking up synaptic dopamine into neurons, it plays a critical role in terminating dopamine neurotransmission and in maintaining dopamine homeostasis in the central nervous system (Giros et al., 1996; Jones et al., 1998a,b). Many substances, such as the psychostimulant amphetamine, the dopaminergic neurotoxin 1-methyl-4-phenylpyridinium ($MPP^+$) and various sympathomimetic amines, structurally resemble dopamine. They are thus substrates for the dopamine carrier and can be transported (Jones et al., 1998a,b; Miller et al., 1999b). The dopamine transporter is also a major molecular target for the addictive drug cocaine and, to a lesser extent, antidepressants (Tatsumi et al., 1997; Amara and Sonders, 1998). These drugs cannot be transported but can bind to the dopamine carrier to block dopamine transport. Therefore, interactions with the dopamine transporter protein can have profound neurobiological, pathophysiological, and pharmacological consequences. In the last decade, since the cloning of the dopamine transporter (Giros et al., 1991;

* Corresponding author. Tel.: +1-309-671-8545; fax: +1-309-671-8403.
*E-mail address:* maartenr@uic.edu (M.E.A. Reith).

0014-2999/00/$ - see front matter 
PII: S0014-2999(00)00563-X

Kilty et al., 1991; Shimada et al., 1991; Usdin et al., 1991), major advances have been made in the characterization of dopamine carriers. The focus of this review is on the recent progress in elucidating structure–function relationships for the dopamine transporter.

## 2. Molecular and pharmacological characteristics of the dopamine transporter

The dopamine transporter has been identified from brains of various species and from *Caenorhabditis elegans* (for references see Table 1). A transporter with neuronal dopamine transporter properties and a partial cDNA clone has also been characterized from the African green monkey kidney (COS) cell line (Sugamori et al., 1999). The mammalian dopamine transporters exhibit high sequence identity (Table 1). The reported longer C-terminus of the bovine dopamine transporter was due to an error of the authors in the translation of the cDNA sequence from the corresponding mRNA, introducing a frame shift. The correct translation of the bovine dopamine transporter C-terminal sequence results in a C-terminus of 40 amino acids, not longer than the C-termini of other dopamine transporters (Brüss et al., 1999). Among other members of the neurotransmitter transporter gene family, the mammalian dopamine transporters display highest amino acid homology with the norepinephrine transporter (~67%), serotonin transporter (~49%), and γ-amino butyric acid (GABA) transporter (~45%). Dopamine uptake by all the identified dopamine transporters has a similar pharmacological profile including a highly comparable sensitivity to cocaine analogs (Table 1). It should be noted that high-affinity binding of the phenyltropane analog of cocaine, 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (CFT = WIN 35,428), is not known for the mouse (Brüss et al., 1999; Wu and Gu, 1999) and *C. elegans* (Jayanthi et al., 1998) dopamine transporter, appears to be poor in bovine dopamine transporter (Lee et al., 1996a,b, however, see above), or is not found in COS dopamine transporter (Sugamori et al., 1999). Additionally, *C. elegans* dopamine transporter has a relatively lower amino acid identity and appears highly sensitive to the serotonin transporter inhibitor imipramine (Jayanthi et al., 1998), which distinguishes it from mammalian dopamine carriers. All of the identified dopamine transporters are functionally dependent on the presence of external sodium and chloride (see references in Table 1). Recent transport, binding, and electrophysiological investigations reveal that the interactions of ions with the dopamine carrier are considerably more complex than the simple picture of two $Na^+$ ions and one $Cl^-$ ion being co-transported with one dopamine molecule (Sonders et al., 1997; Chen et al., 1999; Earles and Schenk, 1999; Li and Reith, 1999, 2000).

## 3. Structure of dopamine transporter

Up to date, no X-ray crystallographic or high-resolution structural information is available for the topological assignments of the transporters. Hydropathy analysis of the primary sequences of mammalian monoamine transporters

Table 1
Comparison of cloned dopamine transporters

| Species | Name | Size (amino acid) | Homology to hDAT (%) | DA $K_m$[a] (nM) | Sensitivity to inhibitors ($K_i$ ratio)[b] GBR:MAZ:COC:BUP[c] | Reference |
|---|---|---|---|---|---|---|
| Human | hDAT | 620 | 100 | 1220 (Ltk-, 37°C)<br>2400 (COS, 22°C)<br>1500 (COS, 25°C)<br>2540 (COS, 37°C) | 1:0.65:3.4:19<br>1:4.3:53:56 | Vandenbergh et al., 1992<br>Giros et al., 1992, 1994<br>Pristupa et al., 1994<br>Eshleman et al., 1995 |
| Rat | rDAT | 619 | 92 | 885 (Hela, 37°C)<br>300 (COS, 37°C)<br>1320–2090 (COS, 37°C) | 1:1.4:19:31 | Shimada et al., 1991<br>Kilty et al., 1991<br>Giros et al., 1991, 1992, Kitayama et al., 1993, 1998 |
| Bovine | bDAT | 617 | 88 | 31500 (CV-1, 37°C)<br>990–1910 (COS, 37°C) | 1:0.98:4.7:53 | Usdin et al., 1991<br>Lee et al., 1996, 1998 |
| Mouse | mDAT | 619 | 93.4 | 930 (HEK293, 35°C)<br>2000 (MDCK, 22°C) | 1:–:56:288 | Brüss et al., 1999<br>Wu and Gu, 1999 |
| *C. elegans* | CeDAT | 615 | 43 | 1200 (HeLa, 37°C) | 1:0.05:29:– | Jayanthi et al., 1998 |
| COS cell | COS-DAT | | 96 (TM[d]1–6) | 130 (high, 22°C)<br>8300 (low, 22°C) | 1:0.05:0.41:1.08 | Sugamori et al., 1999 |

[a] Except for MDCK, $K_m$ data were taken from references using transiently transfected cells. For comparison purpose, the host cell and the assay temperature are included in parentheses.
[b] Except for mDAT, the $K_i$ ratios ($K_i$ inhibitor/$K_i$ GBR) for inhibiting DA uptake are calculated according to data reported in the cited reference.
[c] GBR, GBR 12909; MAZ, mazindol; COC, cocaine; BUP, bupropion.
[d] TM, transmembrane domain.

predicts a topology with 12 transmembrane segments connected by alternating extracellular and intracellular loops with the N- and C-termini located in the cytosol. A study using cysteine/lysine-modifying reagents and biotinylated probe scanning has agreed with the proposed topology on predicting extracellular domains of the serotonin transporter (Chen et al., 1998). For dopamine carriers, some aspects of the proposed topology have also been experimentally verified. Electron microscopic immunocytochemical evidence confirms the cytoplasmic location of N-terminal and the extracellular location of the sequence between transmembrane domain 3 and 4 (Nirenberg et al., 1996: Hersch et al., 1997). Proteolytic mapping and epitope-specific immunoprecipitation of photolabeled dopamine transporters demonstrates the presence of at least one transmembrane helix or other membrane-anchoring structure in two different regions of the protein predicted to contain transmembrane domains and again identifies the extracellular orientation of the region between putative transmembrane 3 and 4 (Vaughan and Kuhar, 1996). Determination of the accessibility of endogenous cysteines, located on the putative intracellular or extracellular loops of the human dopamine transporter, for membrane-permeable and membrane-impermeable sulfhydryl reagents also supports the originally proposed topology: $Cys^{90}$ and $Cys^{306}$ appear to be extracellular, and $Cys^{135}$ and $Cys^{342}$ appear to be intracellular (Ferrer and Javitch, 1998). Additionally, three residues on the extracellular face of the human dopamine transporter, $His^{193}$, $His^{375}$, and $Glu^{396}$, have been characterized to form three coordinates in the $Zn^{2+}$-binding site. The common participation of these residues in binding the small $Zn^{2+}$ ion suggests a close proximity between extracellular loop 2, transmembrane domain 7, and transmembrane domain 8 in the tertiary structure of the human dopamine transporter (Norregaard et al., 1998; Loland et al., 1999).

Photoaffinity labelling studies show that dopamine carriers are glycoproteins with a molecular mass of ~80 kDa on polyacrylamide gels. Prevention of transporter glycosylation by glycosidase reduces the molecular mass to 50 kDa (Vaughan et al., 1996; Patel, 1997). All of the dopamine transporters cloned to date have at least three consensus sites for N-linked glycosylation in the second extracellular loop located between transmembrane segments 3 and 4. Preliminary data indicates that removal of more than two of the glycosylation sites dramatically reduces plasma membrane expression (Lin et al., 1999b). It has been shown that the stability and trafficking/targeting, not ligand recognition, of the human norepinephrine transporter to the membrane greatly depend on glycosylation of the consensus sites (Melikian et al., 1996; Nguyen and Amara, 1996). Whether such an effect also applies to the dopamine carrier remains to be explored. Two cysteines in the second extracellular loop are also conserved completely within monoamine transporters, which have been presumed to form a disulfide bridge and to be involved in the cell surface expression in the case of the rat dopamine (Wang et al., 1995) and serotonin (Chen et al., 1997a,b; Sur et al., 1997) transporter.

Dopamine transporters themselves undergo in vivo phosphorylation in stably expressing cells and striatal synaptosomes (Vrindavanam et al., 1996; Huff et al., 1997; Vaughan et al., 1997). Protein kinase C-mediated dopamine transporter phosphorylation (Huff et al., 1997; Vaughan et al., 1997) results in sequestration of the transport protein (Zhu et al., 1997; Pristupa et al., 1998; Melikian and Buckley, 1999) and down-regulation of transport activity (Kitayama et al., 1994; Huff et al., 1997; Vaughan et al., 1997; Zhang et al., 1997). The primary sequence of the dopamine carrier contains multiple phosphorylation sites in the putative intracellular domains. Although preliminary studies with antibodies reveal that serine residues are involved in the phosphorylation of the protein (Vrindavanam et al., 1996), dopamine transporters do not appear to be directly phosphorylated at their consensus phosphorylation sites. Removing all the potential protein kinase C sites failed to affect the ability of protein kinase C activators or inhibitors to regulate dopamine transport (Lee et al., 1998), whereas mutation of the CaM kinase site, $Thr^{613}$ in the human dopamine transporter, has shown contradictory results on the effect of CaM kinase inactivation (Quan et al., 1998). Canonical protein kinase C sites also do not seem to be responsible for protein kinase C-mediated changes in the norepinephrine (Bönisch et al., 1998) or serotonin (Qian et al., 1997) transporter. Thus, direct dopamine transporter phosphorylation might occur at non-consensus phosphorylation sites. It is also likely that the phosphorylation of the dopamine carrier is substantially impacted by ligand occupancy as shown for the serotonin transporter (Vrindavanam et al., 1996; Ramamoorthy and Blakely, 1999).

## 4. Substrate structure / active form and substrate recognition

The structural requirements for the interaction of substrates with the dopamine transporter have been examined by comparing the transport of phenethylamine derivatives in striatal slice preparations (Meiergerd and Schenk, 1994). These studies indicate that the dopamine transporter requires molecules that possess a phenyl ring with a primary ethylamine side chain for optimal activity, and the β rotamer of the extended conformation of catecholamines is transported preferentially (Meiergerd and Schenk, 1994). It is proposed that the catechol appears to mediate the recognition of the substrate, whereas the amine side chain apparently facilitates the conformational change of the transporter that results in movement of dopamine across the membrane. Recent transport studies on the cloned human dopamine transporter suggest that, although β- or phenolic ring hydroxylation of a substrate results in change

in the $K_m$ over a wide range, the presence of a phenolic hydroxyl group is not a perquisite for optimal function of the transporter. Compounds without a phenolic hydroxyl group such as β-phenethylamine, amphetamines, and $MPP^+$, can bind to the carrier and be transported with the same $V_{max}$ as dopamine (Chen and Justice, 2000). Therefore, phenethylamine seems the most important structural element accommodated by the dopamine transporter.

Dopamine can exist in the anionic, neutral, cationic, or zwitterionic form depending on the ambient pH. The question of which is the active form of dopamine for the cloned human dopamine transporter has been addressed (Berfield et al., 1999). The data are in favor of the cationic forms of dopamine, perhaps including the zwitterion, being the more likely substrate. Moreover, the $K_i$ for dopamine in inhibiting the binding of the cocaine analog [$^3$H]CFT displays a pH dependence identical to that of the $K_m$ for [$^3$H]dopamine uptake, suggesting that changes in uptake result from changes in dopamine recognition. pH-induced changes in the interaction between dopamine and its transporter could result from changes at either the ligand level or the transporter level. At the ligand level, introduction of positive charges on the ligand by protonation may result in conformational changes in the ligand itself. At the transporter level, the protonated amine group of dopamine could interact with a negatively charged residue, such as $Asp^{79}$, as suggested by site-directed mutagenesis experiments (Kitayama et al., 1992) and molecular modeling techniques (Edvardsen and Dahl, 1994), or with a binding domain that is susceptible to pH.

In evaluating the effect of a mutation on binding affinity of a substrate, it should be noted that substrate binding to the dopamine transporter cannot be measured directly because of its rapid dissociation within the time scale of the separation of bound from free ligand in binding assays. Furthermore, the half-saturation substrate concentration ($K_m$) for transport is not identical to the dissociation constant ($K_d$) for the binding of the substrate to the transporter. $K_m$ also depends in a complex manner on rate constants for transporter orientation (Bönisch 1998). Thus, a change in a step beyond substrate recognition but associated with dopamine translocation can influence the $K_m$ value, confounding the interpretation for substrate affinity. Indeed, it is not uncommon that mutations showing lower $V_{max}$ values display lower $K_m$ values. In this scenario, the change in $K_m$ cannot be simply interpreted as a change in substrate binding affinity. For better evaluation of dopamine affinity for a mutant dopamine transporter, the ability of dopamine to compete for a non-transportable ligand binding site overlapping with the dopamine site needs to be considered.

Almost all of dopamine carrier substrates are phenethylamine derivatives and positively charged at physiological pH. These features have been used as a guide to find residues of the substrate-binding site at the carrier. Thus, charged, aromatic, and polar residues become most attractive targets for mutagenesis. For the dopamine transporter, $Asp^{79}$ of the rat variant remains the only residue reported to date, mutation of which results in an increase in both $K_m$ for dopamine uptake and the $K_i$ for dopamine to compete for [$^3$H]CFT binding site (Kitayama et al., 1992). In homologous human norepinephrine and rat serotonin carriers, replacement of the aligned aspartates with uncharged amino acids also causes a disruption of transport (Barker et al., 1999). It is reasoned that the positively charged amino group of catecholamine substrates might interact with the negatively charged carboxylic acid of $Asp^{79}$. This hypothesis is indirectly supported by a recent mutagenesis study on the rat serotonin transporter (Barker et al., 1999). Thus, in the rat mutant with glutamate substitution for the aligned aspartate (D98E), serotonin displays a sixfold loss of affinity as an inhibitor of the binding of [$^{125}$I] 2β-carbomethoxy-3β-(4-iodophenyl) tropane (RTI-55), a cocaine analog (Barker et al., 1999), again supporting the importance of this residue in substrate recognition. Moreover, $Glu^{98}$, which extends the acidic side chain by one carbon as compared with $Asp^{98}$, selectively increases the potency of two analogs with shorter alkylamine side chains, gramine and dihydroxybenzylamine. The observation with dihydroxybenzylamine suggests that catecholamines might be accomodated by the dopamine transporter in a similar way.

Other residues that could serve as substrate binding sites are aromatic residues of the dopamine transporter. As cationic molecules, transporter substrates could bind to the π faces of aromatic rings, producing noncovalent cation–π interactions (Dougherty, 1996). Alternatively, the aromatic ring of substrates could recognize phenyl ring side chains on the dopamine transporter through π–π interactions (Burley and Petsko, 1985). In search of such interactions, a large number of aromatic residues of the dopamine transporter, including most of the phenylalanine and tryptophan residues in transmembrane domains, have been mutated (Lin et al., 1999a,b). Mutation of $Phe^{155}$, a residue located on transmembrane domain 3 and unique to the dopamine transporter family, strikingly raises the $K_m$ for dopamine uptake and reduces the $V_{max}$. Intriguingly, it also enhances the potency of dopamine in inhibiting [$^3$H]CFT binding more than 30-fold (Lin et al., 1999a), indicating that this mutated dopamine transporter could still fully recognize dopamine. Thus, changes beyond dopamine recognition need to be considered, likely involving translocation steps impacting the $K_m$ value.

## 5. Substrate transport: inward and outward

Among 62 single or double/triple dopamine transporter mutants published so far, 14 of the 54 showing near normal membrane expression or CFT binding display reductions in turnover rates for dopamine to less than one-third of wild-type values (Table 2). Except for $Phe^{155}$ in

Table 2
Effect of DAT mutations on dopamine transport or/and CFT binding[a]

| Mutated residues[b] | Conservation in[c] | | Location | Other changes | Reference |
|---|---|---|---|---|---|
| | DATs | NET and SERT | | | |
| *Reduce DA turnover rate* | | | | | |
| F69 (A) | yes | yes | rDAT-TM1 | DA $K_m$ ⇓ | Lin et al., 1999a |
| F105 (A) | no (CeDAT) | no (SERT) | rDAT-TM2 | | Lin et al., 1999a |
| F114 (A) | yes | yes | rDAT-TM2 | | Lin et al., 1999a |
| F155 (A) | yes | no (NET, SERT) | rDAT-TM3 | DA $K_m$ ⇑, DA $K_i$ for CFT binding ⇓ | Lin et al., 1999a |
| F319 (A) | yes | yes | rDAT-TM6 | | Lin et al., 1999a |
| F331 (A) | no (CeDAT) | yes | rDAT-TM6 | DA $K_m$ ⇓ | Lin et al., 1999a |
| F410 (A) | yes | yes | rDAT-TM8 | | Lin et al., 1999a |
| F530 (A) | no (CeDAT) | yes | rDAT-TM11 | DA $K_m$ ⇓ | Lin et al., 1999a |
| S356–359 (AG)[d] | yes | yes | rDAT-TM7 | DA $K_i$ for CFT binding ⇑[e] | Kitayama et al., 1992 |
| *Reduce CFT binding affinity* | | | | | |
| D385 (N) | no (CeDAT) | no (NET) | hDAT-EL4 | | Loland et al., 1999 |
| F361 (A) | yes | yes | rDAT-TM7 | DA $K_i$ for CFT binding ⇓ | Lin et al., 1999a |
| *Reduce both* | | | | | |
| D79 (AGE) | yes | yes | rDAT-TM1 | DA $K_i$ for CFT binding ⇑ | Kitayama et al., 1992 |
| E218 (Q) | no (CeDAT) | no (SERT) | hDAT-EL2 | | Loland et al., 1999 |
| F76 (A) | yes | no (SERT) | rDAT-TM1 | DA $K_i$ for CFT binding ⇓ | Lin et al., 1999a |
| Y251 (A) | yes | yes | rDAT-TM4 | $MPP^+$ $K_m$ and $V_{max}$ ⇓ | Kitayama et al., 1998 |
| Y273 (A) | yes | yes | rDAT-TM5 | $MPP^+$ $V_{max}$ ⇓ | Kitayama et al., 1998 |

[a] only mutants are listed that are significantly different from wild-type proteins by at least threefold and that have near normal membrane expression and/or $B_{max}$ of CFT binding. TM, transmembrane domain; EL, extracellular loop; DAT, dopamine transporter; NET, norepinephrine transporter; SERT, serotonin transporter.
[b] amino acid substitutes used are indicated in paranetheses; sometimes multiple substitutes were used.
[c] if a transporter does not contain the aligned residue, it is indicated in parentheses.
[d] only the activity of double mutants was tested.
[e] only for glycine substitution.

the rat dopamine transporter, all the other 13 residues are conserved throughout mammal dopamine and norepinephrine carriers. Except for $Glu^{218}$ in the human dopamine transporter, which is located on the second extracellular loop, all the other 13 residues are located in regions spanning transmembrane domain 1–8 and in transmembrane domain 11. Noticeably, 16 out of 17 substitutions of charged residues located on the extracellular surface of the human dopamine transporter displayed little or only modest reduction in turnover rates for dopamine (Norregaard et al., 1998; Loland et al., 1999). The influence of alanine substitution for tryptophan and proline residues in or near putative transmembrane domains has also been assessed. Many of those substitutions impaired dopamine translocation (Itokawa et al., 1998; Lin et al., 1999a,b). These data are generally in favor of the view that determinants important for substrate translocation appear closely associated with transmembrane segments. However, the role of extracellular and intracellular loops remains to be established for the dopamine transporter. In the serotonin carrier, external loops have been shown to participate in the conformational changes required for translocation, although they are not the primary determinants for ligand recognition (Smicun et al., 1999).

Simultaneous mutation of the two serines in position 356 and 359 in transmembrane domain 7 of the rat dopamine carrier reduces dopamine transport (Kitayama et al., 1992). Conservation of these two serines in catecholamine transporters and dopamine $D_2$ and β-adrenoceptors raises the hypothesis that the two serines might form hydrogen bonds with the two hydroxyl groups of dopamine (Strader et al., 1994). However, results obtained from single and simultaneous mutation of the aligned serines in the homologous norepinephrine transporter argue against a direct hydrogen bond interaction between substrates and the two serines (Danek Burgess and Justice, 1999).

Using site-directed chemical modification, the cytoplasmic loop residue $Cys^{342}$ has been identified to be in a conformationally sensitive part associated with the permeation way or cytoplasmic gating of the human dopamine transporter (Chen et al., 2000). $Cys^{342}$ is located in a region (transmembrane domain 5–8) hypothesized to be critical for substrate translocation. *m*-Tyramine specifically increased the rate of reaction of relatively permeant methane-thiosulfonate-ethylamminum (MTSEA), rather than impermeant methane-thiosulfonate-ethyltrimethylammonium (MTSET), with X-342C, a cysteine-less mutant construct with $Cys^{342}$ restored. This effect was observed only under conditions allowing inward translocation and it was blocked by cocaine. The observations indicate that substrate translocation involves structural rearrangement of the human dopamine transporter protein, which likely ex-

poses either the thiol side chain of $Cys^{342}$ or other endogenous cysteine residues the exposure of which require the presence of $Cys^{342}$ to intracellular MTSEA. Conformationally sensitive and translocation associated residues have also been reported for homologous GABA and serotonin transporters (Golovanevsky and Kanner, 1999; Chen and Rudnick, 2000).

Effort has also been made to probe the dopamine transporter structures participating in the transport of dopaminergic neurotoxic $MPP^+$. Amino acid substitutions in the rat transporter have suggested that some serine and tyrosine residues located in transmembrane domain 7 or 11 appear more influential on interaction with $MPP^+$ than with dopamine. Thus, alanine substitution for $Ser^{350}$ and $Ser^{353}$ preferentially elevate $V_{max}$ for $MPP^+$ transport, whereas alanine substitution for $Ser^{527}$, $Tyr^{533}$, and $Ser^{538}$ mainly reduce $K_m$ for $MPP^+$ transport (Kitayama et al., 1993). Whether this effect is specific for $MPP^+$ transport, or, as found for the aligned transmembrane serine in transmembrane domain 11 of the human dopamine transporter (Chen and Justice, 2000), whether this also applies to the transport of other substrates, remains to be clarified. Phenylalanine substitution of $Tyr^{533}$ in transmembrane domain 11 of the rat dopamine transporter, where the corresponding residue is phenylalanine in the human carrier, increases the velocity of $MPP^+$ uptake. The difference in $MPP^+$ uptake kinetics between the wild-type and the Y533F mutant was similar to the difference observed between rat and human dopamine transporters (Mitsuhata et al., 1998b). Therefore, $Tyr^{533}$ may be important for the species difference in the sensitivities to toxicity of $MPP^+$ between humans and rats. The transport of $MPP^+$ in 29 rat dopamine carrier mutants with alanine substitution for phenylalanine has also been examined (Mitsuhata et al., 1998a). Evidence for mutations that strongly inhibit $MPP^+$ transport without affecting dopamine transport has not yet been found.

A common feature of dopamine carrier substrates is that they can be transported in both directions. There are essentially two ways to unveil the outward transport of internal substrates: first, by changing transmembrane ion gradients (Pifl et al., 1997) and second, via adding substrates to the external medium (Eshleman et al., 1994; Wall et al., 1995; Johnson et al., 1998; Jones et al., 1998a,b; Sitte et al., 1998). In the latter case, external substrates, by undergoing inward transport, enhance transporter availability at the inner face of the membrane and, hence, bring about release of cytosolic substrates by inducing outward transport. This has been demonstrated by recent studies with the norepinephrine transporter (Chen and Justice, 1998). Additionally, inward $Na^+$ current accompanying substrate uptake may also be the trigger for dopamine transporter-mediated release (Sonders et al., 1997; Pifl and Singer, 1999). Substrate-induced dopamine efflux and uptake of the inducer appear to depend on common structural features of the inducer. As inferred from the $K_m$ or $K_i$ values for uptake and for inducing dopamine efflux, both effects are reduced by β-hydroxylation, stereoselective to α-methylation, but relatively insensitive to a switch of a single phenolic hydroxyl group from the m- to p-position. Thus, individual substrates have similar $K_m$ for uptake and for inducing dopamine efflux, resulting in identical rank order of $K_m$ in efflux and uptake assays (Chen and Justice, 2000). These findings provide strong support for the concept that outward transport of internal substrates is closely coupled with inward transport of external substrates.

Despite the similarities in substrate structural–activity relationship between efflux and uptake, the exchange between external substrates and internal dopamine is extremely unequal. Simultaneous monitoring of tyramine uptake and induced dopamine efflux reveals that the initial exit rate of internal dopamine is only 6% of the initial entry rate of external tyramine. Recently, the contribution of the dopamine carrier protein itself to the slow outward transport has been revealed by studying the human protein mutant S528A (Chen and Justice, 2000). Thus, a structural perturbation with alanine substitution for $Ser^{528}$ dramatically accelerates dopamine efflux induced by various substrates. Further analysis reveals that the mutation does not change the structure–activity relationships, $V_{max}$, or cation dependence for the uptake of external substrates, but selectively enhances the efflux kinetics of internal dopamine. Additionally, simultaneous monitoring of tyramine uptake and resultant dopamine efflux demonstrates that $Ala^{528}$ facilitates the dopamine efflux relative to tyramine uptake. These experiments suggest that the structural requirements of the human dopamine carrier protein differ for inward and outward transport, which may partially responsible for the extremely unequal exchange between external substrate and internal dopamine. It is possible that $Ser^{528}$ plays a role in stabilizing a conformation unfavorable for outward transport. Another evidence that the transporter conformation may differ between inward and outward transport comes from the study on effects of covalent sulfhydryl modification on dopamine uptake by X-342C. The m-tyramine-induced increase in the reactivity of X-342C is not observed under conditions allowing outward transport only (Chen et al., 2000), implying that Cys-342 is not accessible to sulfhydryl reagents during outward transport. Evidence is also emerging from mutagenesis studies on the human norepinephrine transporter in that a single mutation, $Ser^{357}$ to alanine, differentially affects dopamine uptake and tyramine-induced dopamine efflux (Danek Burgess and Justice, 1999).

In physiological context, a restricted outward transport would enhance the efficiency of the overall dopamine uptake process. It can be speculated to also have significance in pathological processes. For example, release of preloaded [$^3$H]$MPP^+$ is facilitated in COS cells expressing certain mutant rat dopamine transporters (S350A/S353A, Y273A, and Y533F), which show less sensitivity to $MPP^+$

toxicity. This suggests that re-distribution of $MPP^+$ due to influx/efflux turnover through the dopamine transporter might be a subtle factor in determining $MPP^+$ toxicity (Kitayama et al., 1998).

## 6. Inhibitor structure and recognition

Among inhibitors, most information is available for cocaine and the related analog CFT, as described in the next section. General indications as to which dopamine transporter domains interact with various inhibitors have been obtained in chimera studies. Thus, chimeras between the transporters for norepinephrine and dopamine constructed in two different laboratories (Giros et al., 1994; Buck and Amara, 1995) point to transmembrane domains 5–8 for conferring inhibitor sensitivity to a variety of compounds, including the antidepressants desipramine, nisoxetine, nortyptiline, nomifensine; the anorectic drug mazindol; the stimulants amphetamine, cocaine; and the diphenyl-piperazine derivatives that potently inhibit dopamine uptake such as GBR 12909, GBR 12935 and LR1111. Proteolysis of the dopamine transporter labeled with the photoaffinity label [$^{125}$I]1-(2-(diphenylmethoxy)-ethyl]-4-[2-(4-azido-3-iodophenyl)ethyl]-piperazine (DEEP), a derivative of the highly potent dopamine uptake inhibitor GBR-12935, shows labeling of the amino half of the dopamine transporter, in a region including transmembrane domains 1 and 2 and the 6-amino acid-linker (Vaughan, 1995; Vaughan and Kuhar, 1996). Interestingly, the same region interacts with [$^{125}$I]GA II 34, a benztropine photoaffinity analog (Vaughan et al., 1999), contrasting with the region that interacts with the cocaine analog [$^{125}$I]4′-azido-3′-iodophenylethyl ester (RTI-82), which encompasses transmembrane domains 4–7 (Vaughan, 1995; Vaughan and Kuhar, 1996) (see next section).

Protection by compounds against attack of the dopamine transporter by sulfhydryl reagents has been monitored in attempts to delineate differences in binding domains for inhibitors and substrates (for references and discussion see Povlock and Amara, 1997), but this approach has not uncovered specific regions of the transporter involved in binding interactions. A recent extension of the protection strategy has been the use of more selective sulfhydryl reagents targeting cysteine residues in the form of methanethiosulfonates (MTS) such as MTSEA and MTSET (see also previous section). Exposure to cocaine enhances the accessibility of $Cys^{90}$ to MTS in an human dopamine transporter mutant with many cysteines removed, and this paradoxically enhances rather than reduces subsequent [$^3$H]CFT binding (Ferrer and Javitch, 1998). It is likely that $Cys^{90}$ also contributes binding increases with MTS treatment of wild-type human dopamine transporter, in addition to binding decreases mediated by other cysteine residues reacting with MTS, as the C90A mutant displays a much weaker protection by cocaine than the wild-type (Reith et al., 2000).

## 7. Relationship between substrate permeation way and cocaine binding sites

This issue has been explored extensively by chimeric, photoaffinity labeling, and site-directed mutagenesis strategies. In identification of regions essential for function and transport inhibition, of most interest are structural components important for cocaine recognition but not for dopamine transport. This could provide significant insights into a transporter site that might recognize a dopamine-sparing cocaine antagonist and could even provide information about the structure of the cocaine antagonist accommodated by the site (Xu et al., 1995; Uhl et al., 1998). There is, however, abundance of evidence that domains on the carrier for dopamine transport and for cocaine analog binding are closely associated even though they are not identical.

Chimeric proteins constructed from domains of the dopamine and norepinephrine transporter (Buck and Amara, 1994, 1995; Giros et al., 1994) indicate the importance of sequences in transmembrane regions 1–3 and 9–11 for the apparent affinity of substrates, while sequences in transmembrane regions 5–8 contribute to substrate translocation and inhibitor interaction. In particular, regions encompassing transmembrane domain 1 as well as regions encompassing transmembrane segments 5–8 appear important for the recognition of cocaine and RTI-55, a phenyltropane analog of cocaine. In other studies using chimeras between human and bovine dopamine carrier, dopamine transport and CFT binding are dramatically reduced when the bovine region encompassing transmembrane domain 3 is substituted for that of the human carrier (Lee et al., 1998). Likewise, results from cysteine-scanning mutagenesis approaches also suggests that transmembrane domain 3 of the rat serotonin transporter contains residues associated with substrate and cocaine binding (Chen et al., 1997a,b). These studies indicate that cocaine's interactions are likely to involve several domains, including those shown to be important for substrate transport. In accordance, photoaffinity labeling studies have shown that [$^{125}$I]RTI 82, a cocaine-like compound, is incorporated in a region containing transmembrane domains 4–7 (Vaughan, 1995; Vaughan and Kuhar, 1996), the same region implicated in dopamine translocation (Giros et al., 1994). Consistent with chimeric and photoaffinity labeling studies, mutation of several residues affects both dopamine transport and cocaine analog binding, as inferred from the changes in $V_{max}$ for dopamine transport and $K_d$ for CFT binding (Table 2). Four of them are indeed located in regions important for dopamine transport (transmembrane domain 1 and domains 4–5). As replacement of $Asp^{79}$ of the rat dopamine transporter, or the aligned aspartate of the

rat serotonin transporter, strongly affects the binding affinity of both amine substrates and cocaine analogs containing an amine nitrogen, a direct ionic interaction of the wild-type aspartate with positive charged amine groups of these compounds remains a possibility (Kitayama et al., 1992; Barker et al., 1999; Miller et al., 1999a).

On the other hand, evidence for nonidentical domains is also growing. As shown in Table 2, quite a number of mutations have displayed selective impact on dopamine transport or cocaine analog binding. These observations suggest that it is possible to dissociate domains influencing dopamine translocation from those influencing cocaine analog recognition. However, strong evidence for structures selectively modulating binding of dopamine or cocaine analogs has not been obtained. For example, among those mutations selectively affecting dopamine transport, none have been reported to lower the ability of dopamine to compete for cocaine analog binding, although some of them cause a higher $K_m$ for dopamine transport. It is not unlikely that the initial step in transport, substrate recognition, is less perturbed than subsequent steps involving conformational changes. Interestingly, mutation of $Phe^{361}$ in the rat dopamine carrier results in reduced cocaine analog binding with preservation of membrane expression and dopamine transport. $Phe^{361}$ is highly conserved in monoamine transporters, situated on transmembrane segment 7, and close to the serines implicated in dopamine translocation. This encourages the possibility to develop small molecules selectively occluding cocaine recognition without affecting dopamine transport (Lin et al., 1999a). In this context it is important to note that Meltzer et al. (1998) point to the three-dimensional volume of cocaine-like compounds of the bicyclo[3,2,1]octane series rather than specific functionalities for dopamine transporter inhibition, based on a wide-ranging set of newly synthesized compounds that lack a protonatable nitrogen in the 8-position or lack an 8-group capable of hydrogen bonding. In this view (Meltzer et al., 1998), the possibility of developing a drug that interferes with cocaine binding while allowing the passage of dopamine depends on the position of the cocaine recognition site in the "pore" which is postulated to be formed by a circular arrangement of the 12 transmembrane domains (Edvardsen and Dahl, 1994).

It has been noted that changes in binding affinity of a cocaine analog ($K_d$) do not always parallel changes in its potency to inhibit dopamine uptake ($K_i$). For example, in mutant human dopamine transporter in which the C-terminus is truncated or substituted, both cocaine and CFT inhibit dopamine uptake with a $K_i$ similar to that in wild-type, while their binding affinities are at least four to fivefolds lower than those in wild-type (Lee et al., 1996a,b). Likewise, CFT potently inhibits dopamine uptake by COS-dopamine transporter in which the carrier appears prematurely truncated after transmembrane domain 6, even though no specific [$^3$H]CFT binding is observed in those cells (Sugamori et al., 1999). In our preliminary work with mutants of the human dopamine carrier, a single mutation almost completely abolished CFT binding but retained high potency of CFT in inhibiting dopamine transport. These observations suggest that the apparent lack of cocaine analog binding does not necessarily mean a poor recognition by the carrier. Changes in dopamine transporter structure may alter the association and dissociation rates for cocaine analogs binding such that their binding cannot be detected by classical equilibrium binding methods. Another possibility is that certain mutants display conformational states during the uptake cycle that have a high affinity for cocaine analogs, whereas such conformational states are not prevalent under the conditions of the binding assay. Be that as it may, in searching for residues specific for cocaine binding, it is necessary to examine the potency of cocaine analogs to inhibit dopamine transport in mutant human dopamine carriers in addition to assessing radiolabeled cocaine analog binding.

Conformational hypotheses, in addition to models with overlapping and separate domains in the interaction with dopamine and cocaine analogs, are also raised by information obtained from structure–function studies. Reaction of $Cys^{90}$ or $Cys^{306}$ with impermeant methanethiosulfonate derivatives enhances dopamine uptake and CFT binding to a similar extent. Because removal of $Cys^{90}$ or $Cys^{306}$ does not affect dopamine uptake and CFT binding, chemical modification of these cysteines seems to induce a conformational change in favor of both dopamine transport and cocaine binding. A difference between the effects of the covalent sulfhydryl modification of $Cys^{90}$ or $Cys^{306}$ on binding and transport is that the reaction of MTSET with $Cys^{90}$ or $Cys^{306}$ decreases the $K_d$ of [$^3$H]CFT binding (Ferrer and Javitch, 1998), but it increases the $V_{max}$ of dopamine uptake (Chen et al., 2000). Thus, although the mechanism is unclear, the conformational alteration induced by modification of $Cys^{90}$ or $Cys^{306}$ likely increases the propensity of the transporter to exist in a particular conformation that binds CFT with higher affinity and simultaneously also increases the turnover rate for transport. If cocaine bound with higher affinity to an inactive conformation of the transporter, dopamine transport could not be facilitated by a modification that increased the propensity of the transporter to exist in such a cocaine-preferring state. These data suggest that cocaine may bind with higher affinity to a conformational state along the transport cycle and that a modification that increases the propensity of the transporter to exist in such a state increases CFT binding and also enhances dopamine transport by facilitating transition along the transport cycle.

Taken together, using different approaches, the structural–function studies show that domains for dopamine and cocaine analogs are formed by interactions with multiple residues, some of which are separate in the primary structure but may lie close together in the still unknown tertiary structure. A recent survey of alanine substitution mutants altering polar residues in transmembrane domains

of the rat dopamine transporter has provided interesting data to support this possibility (Itokawa et al., 1999). Calculation of changes in Gibbs free energies associated with interactions between combined transmembrane domain mutants ($\Delta\Delta G_{int}^{\circ}$) reveals that combined mutations in transmembrane segments 4 + 5 yield some of the largest $\Delta\Delta G_{int}^{\circ}$ values for dopamine uptake, supporting models demonstrating their juxtaposition. Furthermore, transmembrane domain 4 + 11 mutants provide the largest $\Delta\Delta G_{int}^{\circ}$ for CFT binding, implying contributions of residues in transmembrane segment 4 and 11 to a polar pocket important for cocaine recognition (Itokawa et al., 1999).

## Acknowledgements

We would like to thank the National Institute on Drug Abuse (DA 08379 and DA 11978 to M.E.A.R.).

## References

Amara, S.G., Sonders, M.S., 1998. Neurotransmitter transporters as molecular targets for addictive drugs. Drug Alcohol Depend. 51, 87–96.

Barker, E.L., Moore, K.R., Rakhshan, F., Blakely, R.D., 1999. Transmembrane domain 1 contributes to the permeation pathway for serotonin and ions in the serotonin transporter. J. Neurosci. 19, 4705–4717.

Berfield, J.L., Wang, L.C., Reith, M.E.A., 1999. Which form of dopamine is the substrate for the human dopamine transporter: the cationic or the uncharged species? J. Biol. Chem. 274, 4876–4882.

Bönisch, H., 1998. Transport and drug binding kinetics in membrane vesicle preparation. In: Reith, M.E.A. (Ed.), Neurotransmitter Transporters: Structure, Function, and Regulation. Humana Press, Totowa, NJ, pp. 259–278.

Bönisch, H., Hammermann, R., Brüss, M., 1998. Role of protein kinase C and second messengers in regulation of the norepinephrine transporter. Adv. Pharmacol. 42, 183–186.

Brüss, M., Wieland, A., Bönisch, H., 1999. Molecular cloning and functional expression of the mouse dopamine transporter. J. Neural. Transm. 106, 657–662.

Buck, K.J., Amara, S.G., 1994. Chimeric dopamine-norepinephrine transporters delineate structural domains influencing selectivity for catecholamines and 1-methyl-4-phenylpyridinium. Proc. Natl. Acad. Sci. U. S. A. 91, 12584–12588.

Buck, K.J., Amara, S.G., 1995. Structural domains of catecholamine transporter chimeras involved in selective inhibition by antidepressants and psychomotor stimulants. Mol. Pharmacol. 48, 1030–1037.

Burley, S.K., Petsko, G.A., 1985. Aromatic–aromatic interactions in proteins. Science 229, 23–28.

Chen, J.-G., Liu-Chen, S., Rudnick, G., 1997a. External cysteines in the serotonin transporter. Biochemistry 36, 1479–1486.

Chen, J.G., Sachpatzidis, A., Rudnick, G., 1997b. The third transmembrane domain of the serotonin trasnporter contains residues associated with substrate and cocaine binding. J. Biol. Chem. 272, 28321–28327.

Chen, J.-G., Liu-Chen, S., Rudnick, G., 1998. Determination of External loop topology in the serotonin transporter by site-directed chemical labeling. J. Biol. Chem. 273, 12675–12681.

Chen, J.-G., Rudnick, G., 2000. Permeation and gating residues in serotonin transporter. Proc. Natl. Acad. Sci. U. S. A. 97, 1044–1049.

Chen, N., Justice, J.B. Jr., 1998. Cocaine acts as an apparent competitive inhibitor at the outward-facing conformation of the human norepinephrine transporter: kinetic analysis of inward and outward transporter. J. Neurosci. 18, 10257–10268.

Chen, N., Trowbridge, C.C., Justice, J.B. Jr., 1999. Cationic modulation of human dopamine transporter: dopamine uptake and inhibition of uptake. J. Pharmacol. Exp. Ther. 290, 940–949.

Chen, N., Ferrer, J.V., Javitch, J.A., Justice, J.B. Jr., 2000. Transport-dependent accessibility of a cytoplasmic loop cystine in the human dopamine transporter. J. Biol. Chem. 275, 1608–1614.

Chen, N., Justice, J.B. Jr., 2000. Differential effect of structural modification of human dopamine transporter on the inward and outward transport of dopamine. Mol. Brain Res., in press.

Danek Burgess, K.S., Justice, J.B. Jr., 1999. Effects of serine mutations in transmembrane domain 7 of the human norepinephrine transporter on substrate binding and transport. J. Neurochem. 73, 656–664.

Dougherty, D.A., 1996. Cation–pi interactions in chemistry and biology: a new view of benzene, phe, Tyr, and Trp. Science 271, 163–168.

Earles, C., Schenk, J.O., 1999. A multisubstrate mechanism for the inward transport of dopamine by the human dopamine transporter expressed in HEK cells and its inhibition by cocaine. Synapse 33, 230–238.

Edvardsen, Ø., Dahl, S.G., 1994. A putative model of the dopamine transporter. Mol. Brain Res. 27, 265–274.

Eshleman, A.J., Henningsen, R.A., Neve, K.A., Janowsky, A., 1994. Release of dopamine via the human transporter. Mol. Pharmacol. 45, 312–316.

Eshleman, A.J., Neve, R.L., Janowsky, A., Neve, K.A., 1995. Characterization of a recombinant human dopamine transporter in multiple cell lines. J. Pharmacol. Exp. Ther. 274, 276–283.

Ferrer, J.V., Javitch, J.A., 1998. Cocaine alters the accessibility of endogenous cysteines in putative extracellular and intracellular loops of the human dopamine transporter. Proc. Natl. Acad. Sci. U. S. A. 95, 9238–9243.

Giros, B., Mestikawy, S.E., Bertrand, L., Caron, M.G., 1991. Cloning and functional characterization of a cocaine-sensitive dopamine transporter. FEBS 295, 149–154.

Giros, B., Mestikawy, S.E., Godinot, N., Zheng, K., Han, H., Yang-Feng, T., Caron, M.G., 1992. Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. Mol. Pharmacol. 42, 383–390.

Giros, B., Wang, Y.-M., Suter, S., McLeskey, S.B., Pifl, C., Caron, M.G., 1994. Delineation of discrete domains for substrate, cocaine, and tricyclic antidepressant interactions using chimeric dopamine-norepinephrine transporters. J. Biol. Chem. 269, 15985–15988.

Giros, B., Jaber, M., Jones, S., Wightman, R.M., Caron, M.G., 1996. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379, 606–612.

Golovanevsky, V., Kanner, B.I., 1999. The reactivity of the γ-aminobutyric acid transporter GAT-1 toward sulfhydryl reagents is conformationally sensitive. J. Biol. Chem. 274, 23020–23026.

Hersch, S.M., Yi, H., Heilman, C.J., Edwards, R.H., Levey, A.I., 1997. Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra. J. Comp. Neurol. 388, 211–227.

Huff, R.A., Vaughan, R.A., Kuhar, M.J., Uhl, G.R., 1997. Phorbol esters increase dopamine transporter phosphorylation and decrease transport $V_{max}$. J. Neurochem. 68, 225–232.

Itokawa, M., Lin, Z., Uhl, G.R., 1998. Transmembrane proline resides and dopamine transport function. Neurosci. Soc. Abstr. 24, 605.

Itokawa, M., Lin, Z., Cai, N., Wu, C., Kitayama, S., Wang, J.B., Uhl, G.R., 1999. Dopamine transporter transmembrane polar mutants: evidence for interactions important for transporter functions. Neurosci. Soc. Abstr. 25, 159.

Jayanthi, L.D., Apparsundaram, S., Malone, M.D., Ward, E., Miller, D.M., Eppler, M., Blakely, R.D., 1998. The *Caenorhabditis elegans* gene T23G5.5 encodes an antidepressant- and cocaine-sensitive dopamine transporter. Mol. Pharmacol. 54, 601–609.

Johnson, R.A., Eshleman, A.J., Meyers, T., Neve, K.A., Janowsky, A.,

1998. [$^3$H]Substrate- and cell-specific effects of uptake inhibitors on human dopamine and serotonin transporter-mediated efflux. Synapse 30, 97–106.

Jones, S.R., Gainetdinov, R.R., Jaber, M., Giros, B., Wightman, R.M., Caron, M.G., 1998a. Profound neuronal plasticity in response to inactivation of the dopamine transporter. Proc. Natl. Acad. Sci. U. S. A. 95, 4029–4034.

Jones, S.R., Gainetdinov, R.R., Wightman, R.M., Caron, M.G., 1998b. Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. J. Neurosci. 18, 1979–1986.

Kilty, J.E., Lorang, D., Amara, S.G., 1991. Cloning and expression of a cocaine-sensitive rate dopamine transporter. Science 254, 578–579.

Kitayama, S., Shimada, S., Xu, H., Markham, L., Donovan, D.M., Uhl, G.R., 1992. Dopamine transporter site-directed mutations differentially alter substrate transport and cocaine binding. Proc. Natl. Acad. Sci. U. S. A. 89, 7782–7785.

Kitayama, S., Wang, J.-B., Uhl, G.R., 1993. Dopamine transporter mutants selectively enhance $MPP^+$ transport. Synapse 15, 58–62.

Kitayama, S., Dohi, T., Uhl, G.R., 1994. Phorbol esters alter functions of the expressed dopamine transporter. Eur. J. Pharmacol. 268, 115–119.

Kitayama, S., Mitsuhata, C., Davis, S., Wang, J.-B., Sato, T., Morita, K., Uhl, G.R., Dohi, T., 1998. $MPP^+$ toxicity and plasma membrane dopamine transporter: study using cell lines expressing the wild-type and mutant rat dopamine transporters. Biochim. Biophys. Acta 1404, 305–313.

Lee, F.J.S., Pristupa, Z.B., Ciliax, B.J., Levey, A.I., 1996a. The dopamine transporter carboxyl-terminal tail. J. Biol. Chem. 271, 20885–20894.

Lee, S.-H., Rhee, J., Koh, J.-K., Lee, Y.-S., 1996b. Species differences in functions of dopamine transporter: paucity of $MPP^+$ uptake and cocaine binding in bovine dopamine transporter. Neurosci. Lett. 214, 199–201.

Lee, S.-H., Son, H., Chang, M.Y., Kang, S.H., Chin, H., Lee, Y.S., 1998. Protein kinase C-mediated regulation of dopamine transporter is not achieved by direct phosphorylation on the transporter. Neurosci. Soc. Abstr. 24, 607.

Li, L.B., Reith, M.E.A., 1999. Modeling of the interaction of $Na^+$ and $K^+$ with the binding of dopamine and [$^3$H]WIN 35,428 to the human dopamine transporter. J. Neurochem. 72, 1095–1109.

Li, L.B., Reith, M.E.A., 2000. Interaction of $Na^+$, $K^+$, and $Cl^-$ with the binding of amphetamine, octopamine, and tyramine to the human dopamine transporter. J. Neurochem., in press.

Lin, Z., Wang, W., Kopajtic, T., Revay, R.S., Uhl, G.R., 1999a. Dopamine transporter: transmembrane phenylalanine mutations can selectively influence dopamine uptake and cocaine analog recognition. Mol. Pharmacol. 56, 434–447.

Lin, Z., Itokawa, M., Wang, W., Uhl, G.R., 1999b. Selective influences on dopamine uptake and cocaine analog binding by alanine substitutions for dopamine transporter transmembrane domain residues. Neurosci. Soc. Abstr. 25, 283.

Loland, C.J., Norregaard, L., Gether, U., 1999. Defining proximity relationship in the tertiary structure of the dopamine transporter. J. Biol. Chem. 274, 36928–36934.

Meiergerd, S.M., Schenk, J.O., 1994. Striatal Transporter for dopamine: Catechol structure-activity studies and susceptibility to chemical modification. J. Neurochem. 62, 998–1008.

Melikian, H.E., Ramamoorthy, S., Tate, C.G., Blakely, R.D., 1996. Inability to N-glycosylate the human norepinephrine transporter reduces protein stability, surface trafficking, and transport activity but not ligand recognition. Mol. Pharmacol. 50, 266–276.

Melikian, H.E., Buckley, K.M., 1999. Membrane trafficking regulates the activity of the human dopamine transporter. J. Neurosci. 19, 7699–7710.

Meltzer, P.C., Blundell, P., Madras, B.K., 1998. Structure activity relationships of inhibition of the dopamine transporter by 3-arylbicyclo[3,2,1]octanes. Med. Chem. Res. 8, 12–34.

Miller, G.M., Gracz, L.M., Goulet, M., Yang, H., Panas, H.N., Meltzer, P.C., Hoffman, B.J., Madras, B.K., 1999a. Point mutations of the human dopamine transporter (hDAT) reveal differential binding of an amine and a non-amine ligand. Neurosci. Soc. Abstr. 25, 283.

Miller, G.W., Gainetdinov, R.R., Levey, A.I., Caron, M.G., 1999b. Dopamine trasnporters and neuronal injury. TIPS 20, 424–429.

Mitsuhata, C., Lin, Z., Kitryama, S., Dohi, T., Uhl, G.R., 1998a. Molecular mapping of the sites on rat dopamine transporter for specific recognition of 1-methyl-4-phenylpyridium. Neurosci. Soc. Abstr. 24, 606.

Mitsuhata, C., Kitayama, S., Morita, K., Vandenbergh, D., Uhl, G.R., Dohi, T., 1998b. Tyrosine-533 of rat dopamine transporter: involvement in interactions with 1-methyl-4-phenylpyridinium and cocaine. Mol. Brain Res. 56, 84–88.

Nguyen, T., Amara, S.G., 1996. N-Linked oligosaccharides are required for cell surface expression of the norepinephrine transporter but do not influence substrate or inhibitor recognition. J. Neurochem. 67, 645–655.

Nirenberg, M.J., Vaughen, R.A., Uhl, G.R., Kuhar, M.J., Pickel, V.M., 1996. The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. J. Neurosci. 16, 436–447.

Norregaard, L., Frederiksen, D., Nielsen, E.Ø., Gether, U., 1998. Delineation of an endogenous zinc-binding site in the human dopamine transporter. Eur. Mol. Biol. J. 17, 4266–4273.

Patel, A.P., 1997. Neurotransmitter transporter protines. In: Reith, M.E.A. (Ed.), Neurotransmitter Transporters: Structure, Function, and Regulation. Humana Press, Totowa, NJ, pp. 241–262.

Pifl, C., Agneter, E., Drobny, H., Reither, H., Singer, E.A., 1997. Induction by low $Na+$ or $Cl-$ of cocaine sensitive carrier-mediated efflux of amines from cell transfected with the cloned human catecholamine transporters. Br. J. Pharmacol. 121, 205–212.

Pifl, C., Singer, E.A., 1999. Ion dependence of carrier-mediated release in dopamine or norepinephrine transporter-transfected cells questions the hypothesis of facilitated exchange diffusion. Mol. Pharmacol. 56, 1047–1054.

Povlock, S.L., Amara, S.G., 1997. The structure and function of norepinephrine, dopamine, and serotonin transporters. In: Reith, M.E.A. (Ed.), Neurotransmitter Transporters: Structure, Function, and Regulation. Humana Press, Totowa, NJ, USA, pp. 1–28.

Pristupa, Z.B., Wilson, J.M., Hoffman, B.J., Kish, S.J., Niznik, H.B., 1994. Pharmacological heterogeneity of the cloned and native human dopamine transporter: disassociation of [$^3$H]WIN 35, 428 and [$^3$H]GBR 12, 935 binding. Mol. Pharmacol. 45, 125–135.

Pristupa, Z.B., McConkey, F., Liu, F., Man, H.Y., Lee, F.J.S., Wang, Y.T., Niznik, H.B., 1998. Protein kinase-mediated bidirectional trafficking and functional regulation of the human dopamine transporter. Synapse 30, 79–87.

Qian, Y., Galli, A., Ramamoorthy, S., Risso, S., DeFelice, L.J., Blakely, R.D., 1997. Protein kinase C activation regulates human serotonin transporter in HEK-293 cells via altered cell surface expression. J. Neurosci. 17, 45–57.

Quan, H., Wang, Y.-M., Pifl, C., Caron, M.G., 1998. $Ca2+$-dependent phosphorylation of dopamine transporter upregulates its transporter activity. Neurosci. Soc. Abstr. 24, 608.

Ramamoorthy, S., Blakely, R.D., 1999. Phosphorylation and sequestration of serotonin transporter differentially modulated by psychostimulants. Science 285, 763–766.

Reith, M.E.A., Berfield, J.L., Ferrer, J.V., Javitch, J.A., 2000. Cocaine and other inhibitors of dopamine uptake promote different conformational changes in the dopamine transporter. Winter Conf. Brain Res. Abstr. 33, 99.

Shimada, S., Kitayama, S., Lin, C.-L., Patel, A., Nanthakumar, E., Gergor, P., Kuhar, M., Uhl, G., 1991. Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. Science 25, 576–578.

Sitte, H.H., Huck, S., Reither, H., Boehm, S., Signer, E.A., Pifl, C., 1998. Carrier-mediated release, transport rates, and charge transfer induced by amphetamine, tyramine, and dopamine in mammalian cells trans-

fected with the human dopamine transporter. J. Neurochem. 71, 1289–1297.

Smicun, Y., Campbell, S.D., Chen, M.A., Gu, H., Rudnick, G., 1999. The role of external loop regions in serotonin transport. J. Biol. Chem. 274, 36058–36064.

Sonders, M.S., Zhu, S.-J., Zahniser, N.R., Kavanaugh, M.P., Amara, S.G., 1997. Multiple ionic conductances of human dopamine transporter: the actions of dopamine and psychostimulants. J. Neurosci. 17, 960–967.

Strader, C.D., Fong, T.M., Tota, M.R., Underwood, D., 1994. Structure and function of G protein-coupled receptors. Annu. Rev. Biochem. 63, 101–132.

Sugamori, K.S., Lee, F.J.S., Pristupa, Z.B., Niznik, H.B., 1999. A cognate dopamine transporter-like activity endogenously expressed in a COS-7 kidney-derived cell line. FEBS Lett. 451, 169–174.

Sur, C., Schloss, P., Betz, H., 1997. The rat serotonin transporter: identification of cysteine residues important for substrate transport.

Tatsumi, M., Groshan, K., Blakely, R.D., Richelson, E., 1997. Pharmacological profile of antidepressants and related compounds at human monoamine transporters. Eur. J. Pharmacol. 340, 249–258.

Uhl, G., Lin, Z., Metzger, T., Dar, D.E., 1998. Dopamine transporter mutants, small molecules, and approaches to cocaine antagonist/dopamine transporter disinhibitor development. Meth. Enzymol. 296, 456–465.

Usdin, T.B., Mezey, E., Chen, C., Brownstein, M.J., Hoffman, B.J., 1991. Cloning of the cocaine-sensitive bovine dopamine transporter. Proc. Natl. Acad. Sci. U. S. A. 88, 11168–11171.

Vandenberg, D.J., Persico, A.M., Uhl, G.R., 1992. A human dopamine transporter cDNA predicts reduced glycosylation, display a novel repetitive element and provides racially-dimorphic Taq I RFLPs. Mol. Brain Res. 15, 161–166.

Vaughan, R.A., 1995. Photoaffinity-labeled ligand binding domains on dopamine transporters identified by peptide mapping. Mol. Pharmacol. 47, 956–964.

Vaughan, R.A., Kuhar, M.J., 1996. Dopamine transporter ligand binding domains. J. Biol. Chem. 271, 21672–21680.

Vaughan, R.A., Brown, V.L., McCoy, M.T., Kuhar, M.J., 1996. Species- and brain region-specific dopamine transporters: immunological and glycosylation characteristics. J. Neurochem. 66, 2146–2152.

Vaughan, R.A., Huff, R.A., Uhl, G.R., Kuhar, M.J., 1997. Protein kinase C-mediated phosphorylation and functional regulation of dopamine transporters in striatal synaptosomes. J. Biol. Chem. 272, 15541–15546.

Vaughan, R.A., Agoston, G.E., Lever, J.R., Newman, A.H., 1999. Differential binding of tropane-based photoaffinity ligands on the dopamine transporter. J. Neurosci. 19, 630–636.

Vrindavanam, N.S., Arnaud, P., Ma, J.X., Altman-Hamamdzic, S., Parratto, N.P., Sallee, F.R., 1996. The effect of phosphorylation on the functional regulation of an expressed recombinant human dopamine transporter. Neuroscience 216, 133–136.

Wang, J.B., Moriwaki, A., Uhl, G.R., 1995. Dopamine transporter cysteine mutants: second extracellular loop cysteines are required for transporter expression. J. Neurochem. 64, 1416–1419.

Wall, S.C., Gu, H., Rudnick, G., 1995. Biogenic amine flux mediated by cloned transporters stably expressed in cultured cell lines: amphetamine specificity for inhibition and efflux. Mol. Pharmacol. 47, 544–550.

Wu, X., Gu, H.H., 1999. Molecular cloning of the mouse dopamine transporter and pharmacological comparison with the human homologue. Gene 233, 163–170.

Xu, C., Coffey, L.L., Reith, M.E.A., 1995. Translocation of dopamine and binding of 2β-cabomethoxy-β-(4-fluorophenyl) tropane (WIN 35,428) measured under identical conditions in rat striatal synaptosomal preparations. Inhibition by various blockers. Biochem. Pharmacol. 49, 339–350.

Zhang, L., Coffey, L.L., Reith, M.E.A., 1997. Regulation of the functional activity of the human dopamine transporter by protein kinase C. Biochem. Pharmacol. 53, 677–688.

Zhu, S.J., Kavanaugh, M.P., Sonders, M.S., Amara, S.G., Zahniser, N.R., 1997. Activation of protein kinase C inhibits uuptake, currents and binding associated with the human dopamine transporter expressed in Xenopus Oocytes. J. Pharmacol. Exp. Ther. 282, 1358–1365.