Monoamine Transporters: Vulnerable and Vital Doorkeepers

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Abstract

Transporters of dopamine, serotonin and norepinephrine have been empirically used as medication targets for several mental illnesses for the last decades. These protein-targeted medications are effective only for subpopulations of patients with transporter-related brain disorders. Since the cDNA clonings in early 1990’s, molecular studies of these transporters have revealed a wealth of information about the transporters’ structure activity relationship (SAR), neuropharmacology, cell biology, biochemistry, pharmacogenetics and the diseases related to the human genes encoding these transporters among related regulators. Such new information creates a unique opportunity to develop transporter-specific medications based on SAR, mRNA, DNA and perhaps transporter trafficking regulation, for a list of highly relevant diseases including substance abuse, depression, schizophrenia and Parkinson’s disease.

I. Introduction

Monoamine transporters are transmembrane proteins located in plasma membranes of monoaminergic neurons, including the dopamine transporter (DAT), serotonin transporter (SERT, also expressed in platelets), and norepinephrine transporter (NET) (1, 2). These proteins use ion (Na+, Cl−) gradients as energy sources to move monoamines into or out of neurons. The major function of these transporters is to terminate monoamine transmission by inward transport of substrates away from the synaptic cleft. In the membrane of intracellular synaptic vesicles is the vesicular monoamine transporters 1 and 2 (VMAT1 and VMAT2), which use a proton gradient as the energy source to sequester cytosolic monoamines into the vesicles and then release the monoamines into synaptic cleft by exocytosis. Therefore, the overall function of these four transporters is to regulate tempor-spatial components of monoamine transmission. Loss of a transporter could cause severe disease or lethality. For instance, two loss-of-function DAT mutants, L368Q and P395L, cause infantile parkinsonism-dystonia in humans (3). Complete deletion of the VMAT2 gene causes developmental defect and embryonic lethality in mice (4-6).

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Because of the exclusive expression of each transporter in the corresponding neurons, these transporters are often used as markers of specific neurons. DAT is expressed in dopaminergic neurons that project mainly from VTA and substantia nigra to pre-frontal cortex, nucleus accumbens and striatum; SERT plays its role in the pons and upper brain stem; NET is localized in the locus coeruleus and the lateral tegmental group that project to many other brain regions. VMAT1 is expressed transiently during brain development and VMAT2 is the main vesicular transporter in these monoaminergic neurons (7). Importantly, these monoaminergic neurons intervene with each other and with many other types of neurons and innervate various brain regions including cortex, hippocampus, amygdala and hypothalamus.

The extensive distribution of these transporters determines their central roles in neurotransmission and ideal medication targets for a spectrum of monoamine-related neuropsychiatric disorders, including attention deficit hyperactivity disorder (ADHD), depression, anxiety, addiction, narcolepsy, fatigue, obesity, eating disorder, other mood disorders, schizophrenia (SCZ), bipolar disorder and Parkinson’s disease. On the other hand, the central roles have also presented these plasma membrane proteins as functional targets for drugs of abuse such as alcohol, cocaine, methamphetamine and MDMA (3,4-methylenedioxyamphetamine or Ecstasy). In this chapter, we summarize the recent progress in our understanding of the contribution of these monoamine transporters to brain function and diseases.

II. Clinical benefits: demonstration of the medical roles of monoamine transporters

Due to amino acid sequence and proposed structural similarity among the three plasma membrane transporters (DAT, SERT and NET), many monoamine transporter inhibitors have affinity for all three transporters. Unlike the other sections below that discuss individual transporters, this section categorizes the main diseases and their treatments with monoamine transporter inhibitors.

Depression

Depression is the most common disease that is treated by directly targeting the norepinephrine (8), serotonin (9), dopamine (e.g. 10-11) transporters, and/or some combination of the three (e.g., 12).

First developed in the 1950s in an attempt to improve the effectiveness of chlorpromazine and tricyclic antidepressants (TCA) function by inhibiting the reuptake of serotonin, norepinephrine, and dopamine through blocking each respective neurotransmitter transporter (SERT, NET, DAT) (13). Each class of drugs acts on all three of these monoamine systems, with most TCAs primarily inhibiting NET and SERT (14). These medications were then superseded by the selective serotonin reuptake inhibitors (SSRI) as antidepressants. As the most commonly prescribed antidepressant medication, SSRIs are posited to work more effectively within the complex central nervous-neural circuit-gene system in the epidemiology of depression (15), and thus have far less adverse side effects in comparison to TCAs and MAOIs (9). Hundreds of placebo controlled trials have demonstrated benefits in moderate to severe depression, particularly in those with symptoms of more acute major depressive episodes and dysthymia (9, 16, 17) and melancholic depression (18, 19). SSRIs also possess strong therapeutic activity for various DSM-IV-TR disorders (e.g. panic) as described below.

The most recently utilized class of antidepressants falls under selective norepinephrine/ dopamine reuptake inhibitors (SNDRIs), with bupropion (Welbutrin) as the most commonly
prescribed one. Bupropion is an effective and generally well-tolerated option in the
treatment of moderate to severe Major Depressive Disorder (e.g., 20). In addition, bupropion
has been shown to be as effective as many common psychopharmacological medications in
managing symptoms of depression (21). Trials have demonstrated that SSRIs appear to be
more effective in the treatment of moderate/acute depression while SNDRIs may be
advantageous in the treatment of chronic depression (14).

**Obsessive Compulsive Disorder (OCD) and other Anxiety Disorders**

Clomipramine, an inhibitor of SERT > NET > DAT and some receptors, was discovered by
Spanish psychiatrist Lopez-Ibor in 1960’s to be effective for treating OCD symptoms and
the efficacy was subsequently confirmed by many other trials (see 22-24). SSRIs have been
used for OCD but the clomipramine effect size appeared to be larger than the SSRIs (e.g.,
25). Regardless, clomipramine and SSRIs remain an integral part of “best practice”
management of OCD.

In fact, drugs acting on the monoamine transporters, specifically SERT, have efficacy in
other anxiety disorders too. Panic Disorder w/agoraphobia was originally named in DSM III
in 1980 following research in the USA described as “pharmacodissection” using the NET
inhibitor imipramine (26). Recent studies show benefits for norepinephrine and serotonin
uptake inhibitors in treatment of panic agoraphobia (27, 28), Social Anxiety Disorder (29),
Generalized Anxiety Disorder (30), and Post Traumatic Stress Disorder (31).

**Chronic pain syndrome**

In addition to new interventions (e.g. the use sodium oxybate in the treatment of
fibromyalgia pain and insomnia), TCAs, SNDRIs and SSRIs are promising medications for
fibromyalgia pain (32, 33). TCAs have been shown to improve the symptoms of pain, poor
sleep and fatigue associated with fibromyalgia but still possess greater side effects (e.g., 34).
The results of SNRIs are more promising (35), although current effect sizes are smaller than
trials using TCAs. The efficacy of pain treatments seemed to be better achieved by balanced
inhibition of multiple monoamine transporters (33).

**Attention Deficit Hyperactivity Disorder (ADHD)**

Atomoxetine, a NET inhibitor, has been shown in randomized, clinical trials to significantly
reduce ADHD symptoms in both comorbid and noncomorbid children (36), adolescents, and
adults with ADHD (37). While stimulants including the DAT inhibitors methylphenidate
and amphetamine remain the most frequently prescribed medication in treating ADHD,
SNDRIs are currently the leading second line alternatives (38, 39).

**Cigarette Smoking**

Reviews on the effect of cigarette smoking postulate that chronic exposure to nicotine elicits
depressogenic changes in serotonin formation and release in the hippocampus (40). These
changes may contribute to the symptoms of depression experienced by many smokers when
they first quit. The research examining this relationship has resulted in clinically significant
findings. For instance, medications such as bupropion and nortriptyline have been shown to
be efficacious in the treatment of cigarette smoking (41). Moreover, it has been shown that a
treatment modality which includes nicotine replacement/cessation therapy is recommended
for individuals who are highly nicotine dependent and who have a current or past history of
major depressive disorder (42).

In summary, DAT serves as a medication target for ADHD, depression, OCD, smoking
cessation, as well as narcolepsy and Parkinson’s disease that are not mentioned here; SERT
for depression, anxiety, OCD, pain; and NET for depression, OCD, anxiety and ADHD, diseases that affect approximately 20% of the populations.

III. Preclinical indications – behavioral pharmacology

During the last 40 years of preclinical and human psychopharmacology research, the monoamine transporters have been recognized to play a central role in modulating a wide variety of physiological and behavioral functions, including locomotion, autonomic function, and hormone regulation, and to make a fundamental contribution to emotional and cognitive function, neurotoxicity and mental disease. Such a central role is paralleled by the breadth of monoaminergic projections to the neocortex, basal ganglia and limbic forebrain. These pharmacologic and anatomical findings have also been instrumental for the identification and development of medications that are currently used as pharmacotherapies to treat a variety of behavioral disorders, such as major depression, OCD, anxiety, ADHD, and addiction.

DAT

The pioneering investigations of Arvid Carlsson and Kjell Fuxe established DA as a neurotransmitter in the late 1950s (43) and the topography of the dopaminergic innervation of the central nervous system was soon delineated. The mesolimbic DA system originating in the ventral tegmental area innervates the nucleus accumbens of the ventral striatum, where DA is postulated to participate in the control of exploratory activity, reward-related processes, and reinforcement, both natural and drug-induced (44, 45). In turn, the nigrostriatal contingent of DA fibers projecting to the dorsal striatum (i.e., caudate and putamen nuclei in humans) is linked to the control of movement, as revealed by the clinical phenomenology associated with basal ganglia disorders (46), and for “chunking” action repertoires and habits (47). Further, the innervation of the prefrontal cortex by the mesocortical DA pathway has been proposed to modulate various aspects of executive function, including working memory function, planning, and attention (48). Historically, the emerging view of DA as an important transmitter has been reinforced by two additional findings. First, the clinical efficacy of antipsychotic medications was observed to correlate with the binding affinity for DA D2 receptors. Second, observations showed that most abused drugs, especially psychomotor stimulants such as cocaine and amphetamine (AMPH) exert psychoactive effects through interactions with the DA system, some of which involved mainly the DAT.

Preclinical experiments and human data have demonstrated that the DAT is involved in the behavioral reinforcing and euphorogenic effects of stimulant drugs. The ability of cocaine-like drugs to maintain self-administration in rodents is correlated with their potency in inhibiting the DAT (49). The idea is that cocaine binding to DAT may increase extracellular DA concentration by blocking the reuptake activity and inducing the release of reserve pool of DA (50), activating DA receptors. Moreover, the self-reported “high” induced by stimulants in humans appears to be a function of both the rate of DAT occupancy by the stimulant and the speed of stimulant delivery into the brain (51). This evidence suggested that both the binding affinity for the DAT and the pharmacokinetic/pharmacodynamic properties are important characteristics that predict the psychopharmacological effects of drugs acting at the DAT. In addition, preclinical behavioral assays including tests of locomotor activity, conditioned place preference, drug discrimination and self-administration indicated that various DAT inhibitors differ from prototypical stimulants such as cocaine and AMPH. This evidence fueled speculation that it might be possible to design molecules that bind to the DAT, and prevent the actions of AMPH and cocaine at the DAT but lack psychomotor stimulant-like effects. However, AMPH- or cocaine-antagonism
without blocking DA reuptake activity has not been successful due to overlapping DAT sites for stimulant binding and DA recognition.

An alternative to antagonism approach is a substitute approach: a slow onset, long acting competing agonist could be used to treat stimulant addiction. As indicated, the specific pharmacokinetic/dynamic features and rate of DAT occupancy are important factors that influence the cocaine-like properties of DAT inhibitors (51). Different DA uptake inhibitors display specific modes of interaction with the DAT, leading to specific conformational changes of the protein (52, 53), and the different DAT conformations are related to the ability of the inhibitors to induce locomotor activity and substitute for cocaine in discrimination assays (54). It is currently believed that the rational design and development of high-affinity, long acting DAT ligands with specific pharmacokinetic/dynamic properties might lead to the discovery of optimal medications for stimulant addiction (55, 56). A variety of molecules based on the 3-aryltropanes (WIN compounds) (57), the 1,4-dialkylpiperazines (e.g., GBR 12909 and its analogues) (55), and analogues of benzztropine (BZT) (56) have all been synthesized, tested in vitro for binding at different transporters and receptors, and evaluated in preclinical models. Of these, BZT analogs exhibit ideal characteristics in preclinical assays, blocking the behavioral effects of cocaine and AMPH and exhibiting weak abuse liability in place preference and self-administration assays (58-60).

SERT

5-HT-containing neurons of the raphe regions located in the pons and upper brain stem extensively innervate the diencephalon and telencephalon, providing input to the hypothalamus, habenula, thalamus, amygdala, striatum, and cortical mantle (61). The 5-HT system is believed to regulate mood, emotion, learning, memory, sleep and appetite. Of the chemical neurotransmitters, 5-HT is perhaps the most widely implicated in the treatment of mental illnesses (62). The 5-HT transporter (SERT) is responsible for taking up 5-HT from the synapse and is indeed the target of an ample range of molecules which inhibit the uptake process. Two aspects have been decisive to confirm the key functions of 5-HT and the importance of inhibiting 5-HT transport as therapeutic principle. First, the discovery that 5-HT is structurally related to many psychotropic agents, including lysergic acid diethylamide (LSD) and psilocin (63) . Second, the finding that many drugs with psychoactive properties, including cocaine, AMPH, ecstasy, TCAs and SSRIs, effectively interact with SERT to block 5-HT uptake (64). These findings, particularly the latter, confirmed a critical role for 5-HT in the regulation of mood and affect, and marked a milestone in neuropsychopharmacology and psychiatry research.

Over the last decades, tremendous strides have been made in the treatment of major depressive illness, anxiety and eating disorders. The introduction of the first generation of monoamine transporter inhibitors, the TCAs, which target both NET and SERT with variable affinity, revolutionized the management of affective disorders. However, the widespread activity of TCAs at multiple biological sites, including not only monoamine transporters but also noradrenergic, histaminergic and muscarinic receptors, associates with unwanted side-effects (65). The development of SSRIs, such as fluoxetine, citalopram and fluvoxamine, led to fewer side-effects, while retaining full clinical efficacy. In animal models of depression, such as the learned helplessness and forced swimming tests, SSRIs have systematically displayed strong activity suggestive of clinical efficacy (66, 67). These models provide opportunities to assay new compounds and investigate the mechanisms underlying preclinical efficacy. Indeed, in spite of adequate treatment with current antidepressant medication, a large proportion of patients do not receive full symptom remission when treated with SSRIs, and new approaches are presently pursued. A promising lead for improved therapeutic effects is the development of triple uptake inhibitors targeting
SERT, NET and DAT (SNDRIs). Several of these compounds are in advance stages of development, though none has yet been approved for human use. In animal models, the SNDRI, DOV 21,947, inhibits reuptake of 5-HT, NE and DA (EC$_{50}$ of 12, 23, and 96 nM at human recombinant transporters, respectively) and exhibits antidepressant effects in the forced swim and tail suspension test (68). DOV 102,677, another SNDRI (EC$_{50}$ of 129, 103 and 133 nM at human recombinant transporters, respectively) was as effective as methylphenidate in reducing the amplitude of the startle response in juvenile mice, in addition to showing an antidepressant profile (69). Preclinical indications suggest that “triple inhibitors” may produce a more rapid onset of action and greater efficacy than traditional antidepressants (70). In addition, an emerging literature indicates that non-classical transporters such as the plasma membrane monoamine transporter (PMAT) and organic cation transporters (OCTs), which subserve promiscuous uptake of biogenic amines, may constitute new targets for improved antidepressant action, alone or in combination with SSRIs (71).

It is noticed that NET and SERT are also binding sites for psychomotor stimulants including cocaine, AMPH, methamphetamine or ecstasy (3,4-methylenedioxymethamphetamine, MDMA). Although DA seems to underlie the reinforcing effects more closely, several studies have suggested that these binding activities contribute to at least some of the reinforcing properties (72-76), warranting medication targets for drug addiction.

**NET**

There are two major noradrenergic neuronal clusterings in the brain: the locus coeruleus and the lateral tegmental group, which provide extensive innervation to the striatum, amygdala, hypothalamus, thalamus, cerebellum, and neocortex (77). Such widespread ascending projections have been implicated in the modulation of arousal, sleep, and cognitive processes (78, 79). An important additional function of the NE system is to control the endocrine and the autonomic nervous system, which play a fundamental role in anxiety and the stress response (80).

The NE system is an important target for a wide range of drugs used for the treatment of mood, anxiety and behavioral disorders, including major depression, generalized anxiety disorder and ADHD. The neurobiological links between stress, anxiety and depression have long been postulated, but the identification of such relationship has only begun to emerge. The locus coeruleus is uniquely placed to integrate both exteroceptive cues and internal visceral/endocrine information, and to influence stress- and fear-related anatomical structures, including amygdala, periaqueductal gray and neocortex (81). A great deal of evidence suggests that the NE system and the corticotrophin-releasing factor (CRF) pathways co-regulate their activation in response to fear and stress (82). Pharmacological inhibition of NE transport with NET blockers that display little or no activity at other monoamine transporters exerts activity in several animal models of stress and depression. Atomoxetine and reboxetine, which exhibit 50-to-100-fold preference at human NET versus other monoamine transporters (83), show strong activity in animal models of depression that is predictive of therapeutic effects in humans. For example, the therapeutic-like effects of reboxetine have been assayed in the olfactory bulbectomized (OB) rat model of depression, reducing immobility time in the forced swim test and hyperactivity in the open field (84). Reboxetine also attenuates the physiological responses associated to swim stress, including 5-HT elevations in amygdala and prefrontal cortex and activation of hypothalamic pituitary adrenal axis (85). The anti-depressant-like effects of reboxetine in the forced swim test are blocked by 6-hydroxydopamine lesions of the ventral noradrenergic bundle, suggesting that enhanced noradrenergic activity mediates the effects of reboxetine (86). Chronic social stress can produce depressive-like symptoms in mice and rats including decreased locomotor and exploratory behavior, reduced sucrose preference and increased immobility in the forced
swim test. Such disturbances elicited by chronic social stress are ameliorated by reboxetine (87).

Altered NET activity likely contributes to ADHD as well, consistent with the fact that selective NET inhibitors are primarily prescribed for treatment of ADHD (88). Reboxetine and atomoxetine enhance attentional performance, and reverse or attenuate some cognitive deficits in animal models of ADHD. Rats trained in a five-choice serial reaction time task increased the percentage of correct responses and decreased the number of premature responses under the influence of reboxetine (89) or atomoxetine (90). In the spontaneously hypertensive rat, a genetic model for ADHD, atomoxetine ameliorated the learning deficits that typically exhibit these rats (91). Further, atomoxetine is an effective treatment for ADHD, especially in patients with comorbid disorders and those who do not tolerate well stimulant drugs (92).

**VMAT2**

In virtually all tissues, a large proportion of monoamines present are located within specialized subcellular particles referred to as synaptic vesicles. The VMAT2 is responsible for the translocation of DA, 5-HT and NE among others from the cytoplasm into these synaptic vesicles. Such vital function points to these transporters as important players in monoaminergic transmission and potential target for the development of treatments for neuropsychiatric illnesses.

Some of the neuropharmacological and neurotoxic effects of various stimulant compounds are likely to result from interference with VMATs. In purified striatal tissue, cocaine treatment rapidly and reversibly increased both the V(max) of DA uptake and the B(max) of VMAT2 ligand (dihydrotetrabenazine, DTBZ) binding, with other DAT inhibitors, such as GBR 12935, similarly increasing vesicular DA uptake (93). As assessed in ex vivo fractions from striatal tissue, cocaine shifted VMAT2 protein from a synaptosomal membrane fraction to a vesicle-enriched fraction (94). The cocaine-induced VMAT2 redistribution, reduction in DA release, and decrease in total DA transport are mediated by DA D2 receptors, which are activated following accumulation of extracellular DA (95). However, the effects of AMPH and its analogs, including methamphetamine (METH) and MDMA, are radically different to those of cocaine. AMPH-like molecules enter into the cytoplasm through both uptake by monoamine transporters and diffusion across the cell membranes, impede vesicular DA sequestration and promote reverse DA transport and release (96). Contrary to cocaine, METH exposure rapidly decreased vesicular uptake and DTBZ binding (93) and caused a redistribution of VMAT2 from a vesicular-enriched fraction to a location outside the synaptosomal preparation (94). MDMA also induced an abrupt decrease in vesicular DA transport in striatal vesicles prepared from treated rats (97). The importance of VMAT2 in mediating drug-induced physiological and behavioral effects has been confirmed in mice deficient in VMAT2. VMAT2 heterozygous mice are supersensitive to cocaine and AMPH, but do not develop sensitization to cocaine (4). Collectively, these observations suggest that cocaine and AMPH-related compounds differentially affect vesicular DA transport and subcellular localization of VMAT2. These findings may have important implications for understanding the behavioral effects and neurotoxic profiles of psychomotor stimulants.

VMAT2 may also be a pivotal player regulating the balance between toxicity and neuroprotection. The maintenance of low levels of cytosolic monoamines is important to prevent oxidative damage associated with deamination (98). Such critical patrolling function requires effective reuptake of monoamines by VMAT2. Therefore, inhibitors of VMAT2 could produce toxic effects in monoaminergic neurons, a prediction that is consistent with the distinct neurotoxic profile of METH and MDMA. Moreover, VMAT2 is involved in N-
methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity. The active metabolite of MPTP, N-methyl-4-phenylpyridinium (MPP+), is a substrate for VMAT2. These data suggest that pharmacological agents that increase VMAT-2 activity may afford neuroprotection and constitute new treatments for neurodegenerative disorders. The quantitative assessment of VMAT2 density by PET scanning has been clinically useful for early diagnosis and monitoring of neurological and neuropsychiatric conditions, including Parkinson’s and Alzheimer’s diseases and drug addiction (99).

In summary, monoamine transporters represent brain pathways underlying many behaviors associated with addiction, depression, anxiety, social management and Parkinson’s disease among others.

IV. Molecular study: cDNA cloning and structure activity relationship (SAR)

Molecular characterization of the transporters was not possible until their cDNAs were cloned in early 1990’s. By using various strategies, several laboratories cloned the cDNAs and revealed the amino acid sequences, enabling the immediate study of structural and activity relationship. With the help of site-directed mutagenesis analyses and elucidation of crystal structure of the bacterial homolog Leucine transporter (LeuT<sub>Aa</sub>) (100), functional residues in monoamine transporters are being uncovered for ion transport, substrate specificities and drug binding sites.

cDNA cloning and characterization

Structural study of monoamine transporters was not possible until early 1990’s when the transporter cDNAs were cloned, which was initiated by successful purification and cloning of GABA transporter GAT 1 (101, 102). By direct expression of human SK-N-SH cell cDNA pools in COS-1 cells and screening for radio-labeled norepinephrine analog, m-iodobenzylguanidine ([<sup>125</sup>I] mIBG) positive transfected cells, Amara’s laboratory cloned the hNET cDNA (103). Based on conservative amino acid sequences between GAT1 and NET, degenerative oligonucleotides were used to amplify cDNA fragments from brain mRNA pools to screen cDNA libraries and clone the DAT and SERT (104-106). Due to structural differences from the plasma membrane transporters, the vesicular VMAT1 and VMAT2 transporters were cloned based on their abilities to take up neurotoxin (expressing cells survived in the presence of the toxin) or radio-labeled substrate in the expressing cells vs non-expressing cells(107, 108).

With the cDNAs cloned, all transporters were subsequently characterized and their basic features are summarized in Table 1. The plasma membrane and vesicular monoamine transporters belong to different families and have different biophysical properties, such as ion dependence, action as symporter (NET, DAT, and SERT) or action as antiporters (VMAT1 and VMAT2), rank order of substrates, membrane localization, drug binding sites and affinities. They also share some common biophysical properties, such as uptake activities for monoamines, 12 transmembrane domains (TMs), co-expression in monoamine neurons. The active transport activities of the plasma membrane and the vesicular transporters are unidirectional to the cytoplasm and the vesicular lumen, respectively, under normal neurotransmission because of the extracellular sodium gradient created by Na<sup>+</sup>/K<sup>+</sup> ATPase and the intravesicular proton gradient created by H<sup>+</sup> ATPase.

SAR of the plasma membrane transporters

No crystal structures are solved for any of the monoamine transporters. Immediately after the cDNA cloning, a large number of site-directed mutagenesis studies were carried out to identify amino acid residues important for substrate and inhibitor recognition and interaction with ions. Subsequently, a crystal structure of the bacterial transporter, Aquifex aeolicus
leucine transporter (leuTα) (100) that shares amino acid sequence identity of 20-25% with the plasma membrane monoamine transporters (109, 110), was solved and has been used as a template to model the structures of SERT (110-114), DAT (110, 113, 115, 116) and NET (110, 113, 117). These SAR and molecular modeling studies have revealed TMs 1, 3, 6 and 8 as important binding domains. Specifically, residues Asp98, Tyr267 and Tyr289 of SERT are conserved among the three plasma membrane transporters and postulated to comprise the substrate binding cavity. Six SERT-specific residues including Asp98, Tyr95, Gly338, Ile172, Ser336 and Gly442 appear to interact with the substrate (118-121). The acidic side chain of Asp98 is predicted to interact with substrate formation a salt bridge with the positively charged amine of 5-HT (111). Tyr95 and Gly338 are predicted to be involved in interaction with the aliphatic side chain of 5-HT. The residue Tyr95 probably stabilizes the substrate by stacking interaction between the aromatic ring of Tyr95 and the 5-HT (111). Other functional residues interact with ions. Ala96, Asp98 (D79 in DAT and D75 in NET), Asn101, Ser336 and Asn368 of SERT are postulated to coordinate the first Na+ ion binding site (111). Other conserved residues including Gly94, Val97, Leu434, Asp437 and Ser438 of SERT are predicted to coordinate the second Na+ ion (111). The Cl-binding site in SERT consists of Tyr121, Ser336, Asn368 and Ser372 that are conserved among the three plasma membrane transporters (122, 123). Replacements of Asn368 and Ser372 with negatively charged residues Glu or Asp lead to the transportation of substrate 5-HT in the absence of Cl− ion (122, 123), indicating that the negatively charge amino acids can function as the Cl− ion. In particular, Cl− has been shown to be involved in facilitating a conformational change in SERT (124) and DAT (125) for alternating substrate binding.

In summary, the functional cloning and biochemical analysis of both plasma membrane and vesicular monoamine transporters are building up a solid foundation for the future study of those important drug targets and clinical and pathological relevant molecules.

V. Protein regulations

Monoamine transporters share other common features including oligomerization and cytoplasmically localized N- and C-terminals, in addition to 12 TMs (126-131). Oligomerization appears necessary for the transporter complex to efficiently exit the endoplasmic reticulum and cytoplasmic tails mediate subcellular trafficking of the transporter proteins (129, 132). It has also been suggested that the amphetamine-induced substrate efflux depends on the oligomeric nature of monoamine transporters (133).

Subcellular localization

Different transporters are localized at different subcellular sites. NET is primarily expressed within the cytoplasm in the majority of prefrontal cortex nerve terminals (134). Interestingly, the cytoplasmic localization of NET correlates with the absence of detectable Tyrosine Hydroxylase immunoreactivity, suggesting that most noradrenergic neurons in the prefrontal cortex operate in a low-activity state because they can neither synthesize, nor recapture noradrenaline. Manipulations that increase in vivo stress responses result in an increase of both the plasma membrane expression of NET and Tyrosine Hydroxylase immunoreactivity, illustrating the importance of trafficking mechanisms in the regulation of neuronal activity. By contrast, DAT and SERT are largely expressed at the plasma membrane of nerve terminals and specifically to perisynaptic areas (135, 136), supporting volume transmission as the primary transmission mode for dopamine and serotonin. Recent observations suggest that dopamine and serotonin can also be released from dendrites (137-139); however, the role of DAT and SERT in the regulation of postsynaptic monoamine release has not been well established.
Drug regulation

The subcellular localization can be regulated by psychostimulants including cocaine and amphetamine (AMPH) (140). It is reported that DAT cell surface levels are increased in response to cocaine without known mechanisms (141, 142). The transport of AMPH-like drugs results in a complex cascade of events leading to the redistribution of vesicular monoamine into the cytosol, reversal of transport, and consequent transporter-mediated monoamine release (143, 144). Phosphorylation of the amino terminus of DAT by Protein Kinase C (PKC) and Calmodulin-Dependent Kinase II (CAMK II) has recently been shown to be essential for the AMPH-induced DA efflux (145-147). AMPH regulated DAT trafficking bidirectionally between cell surface and intracellular vesicle by mechanisms to be dissected (148, 149).

DAT regulators

At the cell membrane, the function of monoamine transporters can be regulated also by multiple second messenger systems including Protein Kinase A, Protein Kinase C, Protein Kinase G, Tyrosine Kinases, Phosphatases, Calcium and Calmodulin-dependent Kinases, as well as Arachidonic Acid (for review see 150-154). Of these; the best characterized effect is the down-regulation of transporter activity by Protein Kinase C (PKC) activators through internalizing the proteins for degradation (155, 156). Interestingly, the down-regulation of DAT function by PKC activation is not a direct result of transporter phosphorylation as mutant transporter molecules that lack PKC phosphorylation sites still internalize after PKC activation (157). Endocytic signals have been identified in the intracellular carboxy terminus of DAT (158-160). In looking at the mechanisms associated with the internalization of DAT, Sorkin and colleagues (161) found that PKC activation significantly increases transporter ubiquitination through a mechanism involving the E3 ubiquitin ligase Nedd4-2 and the clathrin-coated pits adaptor proteins Epsin, Eps15, and Eps15R. Three lysine residues located in the amino terminus of DAT are required for both the PKC-induced ubiquitination and down-regulation of transporter function (162). Another study showed that the protein-ubiquity E3 ligase Parkin enhances DAT uptake and cell surface expression of the transporter via a mechanism involving an enhanced ubiquitination and degradation of misfolded DAT (163). Thus, a model is emerging in which ubiquitination is an important signal that links the plasma membrane transporter to the endocytic and degradation machinery (164). In addition to second messengers, other proteins also regulate DAT for transporter synthesis and assembly, subcellular localization, or modulating intrinsic activities such as uptake, efflux, and conductances associated with transporters.

The first DAT interacting protein identified was the synaptic Protein Interacting with C Kinase (PICK1) (165). PICK1 is a PDZ domain-containing protein which plays an important role in the targeting and clustering of several receptors and ion channels at synapses. Specifically, PICK1’s PDZ motif binds to a class I PDZ binding site located at the extreme carboxyl terminal of DAT, which may facilitate the targeting of DAT to nerve terminals. Although PICK1 also binds the carboxyl terminus of NET and SERT in vitro, the physiological significance of these interactions is unknown (166). Other regulators include α-Synuclein (167-169), Hic-5 (166), PP2A (170), NEDD4-2 (161, 171), GPR37 (172), Parkin (163, 173), PKC-β (146), and dopamine D2 receptors (174). In all these cases, manipulations that alter the expression levels of the interacting protein resulted in changes in the DAT cell surface levels. Interestingly, the ability of DAT to co-immunoprecipitate with the D2 receptor appears to be decreased in post-mortem brain tissue from schizophrenic patients (175). The increasing number of proteins that influence trafficking mechanisms leaves questions as to whether each of these proteins interacts with DAT at different steps in the endocytic, recycling, degradation pathways and/or individual pathway steps require multiple interactions.
More recently identified DAT regulators include PKC-β (146), CAMKII-α (147), Syntaxin 1A (176, 177), and Synaptogyrin-3 (178). PKC-β, CAMKII-α, and Syntaxin 1A all promote DA efflux in response to AMPH. Inhibition of PKC-β blocks AMPH induced DA release in cultured cells (146). Consistently, PKC-β knock-out mice have less AMPH stimulated DA efflux and reduced locomotor activity in response to AMPH (179). On the other hand, studies have shown that CAMKII-α binds directly to the carboxyl terminus of DAT (147). This association between CaMKII-α and the carboxyl terminus of DAT and promotes phosphorylation of the amino terminus of DAT. Interestingly, CAMKII-α activation is necessary to enable Syntaxin 1A to bind directly to the amino-terminus of DAT and promotes DA efflux (177). Synaptogyrin-3 is also a synaptic vesicle protein and it modulates the transporter function via N-terminus-N-terminus binding (178). Functional studies showed that Synaptogyrin-3 overexpression in catecholaminergic PC12 and MN9D cells resulted in increases in DAT activity without changes in cell surface density. These results were not recapitulated in cells devoid of a vesicular dopamine system, suggesting that the DAT/Synaptogyrin-3 interaction play a role in docking synaptic vesicles at the plasma membrane near DAT to facilitate a more efficient loading of the vesicles with extracellular DA after release. Indeed, studies also showed an interaction between DAT and VMAT2 (178), perhaps for a rapid and efficient coupling between the two transporters.

**SERT and NET regulators**

SERT and NET are also regulated by several proteins in trafficking as well as intrinsic activity. For example, PP2A and PKC interact with SERT, in addition to DAT and NET (170). The PKC-induced internalization of SERT was shown to be prevented by serotonin (180) suggesting a feedback mechanism where the transmitter can control the levels of plasma membrane transporter “on demand.” Syntaxin 1A has also been identified as a SERT/NET interacting protein and might have a dual role regulating both transporter trafficking and intrinsic channel activity (181, 182). Additional regulators include Hic-5 (166, 183), α-Synuclein (168, 169), the Secretory Carrier Membrane Protein 2 (184), Neuronal Nitric Oxide Synthase (185), and the cGMP-dependent Protein Kinase alpha (186). The latter is an important link between the regulation of transporter function and PKG activation. Activation of adenosine A3 receptors results in two PKG-dependent signaling pathways regulating SERT function. One of these mechanisms leads to an increase in catalytic activity; whereas, the second one is p38MAPK-dependent and involves an increase in the cell surface levels of the transporter. In addition to PP2A, other known regulators of NET include PICK1 (165), Hic5 (166), α-Synuclein (169), Syntaxin 1A (187) and 14-3-3 proteins (188).

**VMAT2 regulators**

Much less is know about regulation of VMAT2, except its physical interaction with dopamine synthesizing enzymes such as tyrosine hydroxylase (TH) and aromatic amino acid decarboxylase (AADC) (189).

In summary, the activities of plasma membrane transporters are regulated by both small molecules and proteins at two main levels: cell surface expression and intrinsic activity. These regulators compromise an expanding community associated with various mechanisms yet to be fully understood. Thus, our current understanding of how monoamine transporters and homeostasis are regulated will surely be dynamic in the years to come as we continue to identify and investigate additional interactions with monoamine transporters.
VI. Animal genetics

The cDNA cloning has enabled animal genetic studies since middle 1990’s. Findings from these genetic and modeling studies are consistent with the results from the pre-clinical research and confirm the roles of monoamine transporters in brain function and behaviors.

DAT

The essential role of DAT in the brain is demonstrated by gene knockout studies. Complete deletion (z−/−) causes dramatic affects including hyperdopaminergia with delayed clearance of extracellular dopamine, abnormal brain development, spontaneous hyperactivity and high response to and/or lack of habituation to novelty, motor and learning deficits, sleep disturbances and loss of cocaine’s abuse-related effects (190-200).

DATkd (knock-down) mice expressing 10% of wild-type DAT levels displayed similar effects including hyperactivity with delayed habituation and increased stereotypy, and deficits in response inhibition, but not prepulse inhibition of startle (201-204). DATkd mice also consumed more food and showed behaviors suggesting increased “motivation” towards food reward (205-207). The DAT-reduced mice thus may model aspects of manic or obsessive-compulsive behavior, Tourette’s syndrome, ADHD, and perhaps of over-eating. Consistently, mice overexpressing DAT by 20-30% showed phenotypes opposite to hyperdopaminergic DAT-deficient strains, including lower spontaneous activity with prompter habituation in a novel environment, increased neurotoxicity of MPTP (208), and increased amphetamine-induced dopamine release, hyperactivity and conditioned place preference (CPP) (208, 209). These findings support the results from the DAT-deficient mice.

Recent DAT transgenic studies have demonstrated that DAT is the functional target of cocaine in the brain. Mice carrying a cocaine-insensitive mutant DAT (DAT-CI) failed to self-administer cocaine under various conditions whereas food, D-amphetamine, and a direct dopamine agonist maintained operant behavior at levels comparable to wild-type mice (210). Cocaine failed to increase extracellular accumbens dopamine or induce locomotor activity stimulation, stereotypies, or conditioned place preferences in the DAT-CI mice (211-213).

SERT

The contribution of SERT to various rodent behaviors is extensively documented by both gene knockout in mice and inactivation (N-ethyl-N-nitrosourea or ENU mutagenesis) in rats. Complete loss of functional SERT caused spontaneous serotonin syndrome-like behaviors (tremor, Straub tail and backward movement) and hypersensitivity to its induction by serotonergic or opioid drugs, reduced motor coordination, strength, and locomotor activity, an anxiety-like and depressive-like phenotype and decreased aggression and other social interactions (214-227). In agreement with the large literature implicating 5-HT in the pathophysiology and treatment of mood and anxiety disorders, SERT-deficient (−/− and +/-) mice showed an anxiogenic phenotype, which was ameliorated by a 5-HT1A antagonist (215, 220, 228, 229). Cognitive assays showed SERT−/− rodents may perform better in emotionally guided/motivated learning, but worse in “neutral” tasks - perhaps related to the anxiety-like phenotype (230, 231). Consistently, a SERT-overexpressing mouse line showed a phenotype opposite to the SERT−/− in many respects: reduced extracellular and total 5-HT levels, and decreased anxiety-like behaviors (232, 233). It is noteworthy that genetic deletion thus had effects opposite to SSRI treatment in many respects, suggesting compensatory mechanisms in the mutant animals, or perhaps complex adaptations to chronic SSRI treatment.

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NET

NET−/− mice generally behaved like anti-depressant-treated wild-types in several assays, and showed no or reduced further effect of most antidepressants, corroborating the role of norepinephrine systems in the action of some antidepressant drugs (234-236). Furthermore, NET−/− mice were resistant to depressive-like effects of stressors, behaviorally and neurochemically (236). Also, consistent with the mild antinociceptive effects of α2 agonists and tricyclic antidepressants, NET−/− mice showed α2-mediated enhancement of antinociceptive effects of morphine and endogenous opioids (237). Like DAT−/− mice, NET−/− mice were less vulnerable to MPTP-induced neurotoxicity (238). Studies in NET−/− mice have also implicated noradrenergic systems in epilepsy and/or actions of anticonvulsants (239, 240), as well as confirming roles in cardiovascular and respiratory functions (241). Thus NET−/− mice offer a depression-resistant phenotype, and may also be valuable to the fields of analgesia, epilepsy, and aspects of resistance to neurotoxicity/Parkinson’s.

VMAT2

Complete deletion of VMAT2 is lethal within a few days of birth, and in vivo studies have concentrated on the viable VMAT2+/− mice with reduced VMAT2 expression (4-6). VMAT2+/− mice were behaviorally hypersensitive to the acute stimulant effects of cocaine, amphetamine, methamphetamine and ethanol (4, 6, 242). Paradoxically, dopamine-releasing, rewarding and sensitizing effects of psychostimulant drugs and ethanol were reduced in the VMAT2+/− mice (4, 6, 243, 244). As expected, VMAT2+/− mice were found more vulnerable than wild-types to neurotoxicity/neurodegeneration induced by MPTP, L-DOPA, or methamphetamine (6, 243, 245-247). VMAT2+/− mice also showed depressive-like behaviors (244, 248, 249).

In addition to the knockout approach, a transgenic mouse strain with very low levels of VMAT2 expression (5% of wild-type) was generated (250). Unlike the VMAT2−/− mice, these “VMAT2 LO” (low) mice are viable, but represent a more pronounced VMAT2 deficit than the previously available VMAT2+/− mice. Findings from VMAT2 LO mice corroborated and extended those from VMAT2+/− mice regarding their Parkinson’s-like phenotype, including increased susceptibility to MPTP- or methamphetamine-induced neurotoxicity/neurodegeneration (250, 251). Further, VMAT2 LO mice showed an age-dependent phenotype reminiscent of pre-parkinsonian symptoms and Parkinson’s disease, including olfactory deficits, anxiety- and depressive-like behaviors, progressive loss of striatal dopamine, nigral dopaminergic cell loss, α-synuclein accumulation and L-DOPA-responsive motor deficits (250, 252-254). Thus VMAT2 mutant mice may be useful to model Parkinson’s and related depressive states, oxidative stress, and amphetamines addiction.

In summary, despite concerns regarding both compensatory mechanisms and conflicting findings, an impressive database now suggests a striking amount of agreement between genetic manipulations in animals and traditional pharmacological approaches. For example, knockout mice lacking monoamine transporters frequently exhibit a phenotype akin to normal animals treated with monoamine reuptake inhibitors. Mice lacking the DAT, but not the SERT or NET, are insensitive to cocaine in assays of hyperactivity and self-administration, and this is even clearer for mice with a “knockin” of a cocaine-insensitive but functional DAT. Such findings bolster the utility of mutant mice generally for behavioral and in vivo pharmacology studies, and suggest that DAT mutants may be used to study aspects of hyperdopaminergic states that may have relevance for ADHD, SCZ, bipolar disease, addiction and Parkinson’s. Correspondingly, SERT mutants may be especially valuable for studying hyper-serotonergic states relevant to anxiolysis and antidepressant
treatments, as well as abuse and toxicity of hallucinogens and stimulants such as LSD and
MDMA and methamphetamine. Likewise NET mutants offer tools relevant to
cardiovascular and respiratory functions, antidepressants, analgesics, anticonvulsants, and
neurotoxicity. VMAT mutants have been used mostly to clarify the functions of that protein,
and the potential role in Parkinson’s-related neurodegeneration and mood disorders.

VII. The transporter genes as risk factors

Human genetic studies of the transporters did not start until the cDNAs were cloned and the
Human Genome Project began in early 1990’s. During the last one and a half decades, a lot
of progress has been made to elucidate DNA sequence polymorphisms in the transporter
genes and their correlations with gene activities and ultimately with genetic etiologies of
diseases. We attempt to summarize the most recent progresses in genetic associations with
disease (see 255 for a review on non-synonymous mutations).

Gene structures

The human monoamine transporters are each encoded by single copy genes located on
different chromosomes. The human DAT gene or hDAT/SLC6A3 (53.2 kb on Chr 5) is the
largest; hSERT/SLC6A4 has 41.4 kb (Chr 17); hNET/SLC6A2 is 48.6 kb (Chr 16), hVMAT1/
SLC18A1 (38.4 kb on Chr8) and hVMAT2/SLC18A2 (38.3 kb on Chr 10) the smallest in size,
without considering their external regulatory regions such as promoters. Each of first four
transporter genes has 15 exons and 14 introns (hVMAT1 has 16 exons and 15 introns).

A remarkable feature of these gene structures is that at a DNA sequence level, these
transporter genes carry a lot of polymorphisms in our populations, approximately 7-14
polymorphisms per kb on average based on the current NCBI database information. The
average density of polymorphisms is 14.4 in hDAT, 9.0 in hSERT, 7.8 in hNET, 10.5 in
hVMAT1 and 8.4 in hVMAT2, expecting that these numbers will be increased by the ongoing
1000 Genomes Project. These high densities of DNA sequence polymorphisms suggest that
 genetic variation in the transporters contributes to monoamine-related inter-person variation
in brain function and diseases in extreme cases.

hDAT—DNA sequence variation in hDAT is correlated with its expressional activity and
other brain activity. The well-studied variable number tandem repeats located in the 3’
untranslated region (3’UTR) in Exon 15 (3’ VNTR of 40 bp) has been examined as to which
allele of this marker is associated with low expression levels (a risk factor). In vitro and
human imaging analyses have consistently shown that the 10-repeat allele is associated with
lower expression levels than the 9-repeat allele (256-258). In addition, an Intron 8 VNTR
and 5’ haplotypes are also correlated with expressional variations (259, 260). These
functional analyses of hDAT genotypes are consistent with genotype-dependent brain
activities including those in ADHD patients (261-264), suggesting that genetic variation in
the transporter gene may lead to altered brain function and even disorders.

Consistently, association studies have shown that various genetic markers throughout the
gene are associated with at least eleven different disorders such as ADHD, substance abuse,
depression, SCZ and bipolar disorder in different populations (Table 2). The genetic
variation also modulates treatment efficacy for ADHD, depression, SCZ and smoking
(312-317). A recent study has suggested that the 3’VNTR and Intron 8 VNTR are
differentially involved in ADHD in childhood versus persistent manner (318). Genotype
10/10 and haplotype 6-10 are associated more with childhood ADHD but 9/9 and 6-9 are
with a persistent form of this diseases in Europeans. A multiple SNP-formed haplotype is
associated with depression in Mexican-Americans (282). Intron 4 rs464049 is associated
with SCZ in Croatians (319).
Gene-gene interaction is implicated in related disorders. For example, \textit{hDAT} x \textit{DRD2} is found to contribute to smoking in Poles (320) and \textit{hDAT} x \textit{DRD3} to SCZ in Spanish (321). It is also shown to interact with its transcription factor NR4A2 in conferring risk for smoking (322). In addition, epigenetic and environmental involvement further complicates statistical evaluation of genetic associations with ADHD and Parkinson’s disease (298, 323, 324).

\textbf{hSERT}—The differences in \textit{hSERT} expression levels among different individuals could be as large as 6-fold in selected brain regions (325, 326). A number of lines of evidence have linked \textit{hSERT} polymorphisms to such differences: the promoter marker 5-HTTLPR has two alleles, \textit{s} and \textit{l} and \textit{in vitro}, postmortem and \textit{in vivo} studies have suggested that the \textit{s} allele is associated with lower expression levels of \textit{hSERT} (326-329).

Consistently with the genotype-dependent expression, genetic markers in \textit{hSERT} are shown to be associated with 20 different diseases such as ADHD, aggression, alcoholism, Alzheimer’s, autism, substance abuse, anxiety, OCD, depression, suicide, PTSD, SCZ, panic and unipolar disorders (Table 2). In addition, the genetic variations also modulate the treatment efficacy for ADHD and alcoholism and environmental effect (unemployment etc) on PTSD (282, 330-335). Furthermore, the genetic variations may confer risk for depression in patients with different diseases (336, 337).

\textbf{hNET}—While little information is available about genotypic correlation with \textit{hNET} expression levels in relevant brain regions, approximately 50 association studies have been done and revealed several positive signals. Findings from these studies suggest that this gene is associated with six diseases including ADHD, depression, drug abuse, hypertension, orthostatic intolerance and anorexia nervosa (Table 2). Overall association with ADHD and modulation of the treatment efficacy for this disease has been confirmed by genome-wide association study (GWAS) (338, 339). Only markers located in the 3’ side of the gene did not support \textit{hNET} association with ADHD (267), suggesting the promoter plays a major role in conferring the risk for ADHD. Future study using the promoter markers are expected to reveal more positive association signals.

\textbf{hVMATs}—Among the transporter genes of main interest here, \textit{hVMAT1} and \textit{hVMAT2} are the least studied so far. There are fewer than 20 published studies on these genes. Postmortem and imaging analyses suggested that \textit{hVMAT2} expression levels vary by more than 10-fold among individuals and were reduced in substance abusers (340, 341).

Limited number of association studies have shown that \textit{hVMAT2} is positively associated with four diseases including alcoholism (only disease whose association was replicated), depression, Parkinson’s disease and SCZ (Table 2). Consistently with the fact that \textit{hDAT} and \textit{hVMAT2} are coupled to each other in regulating DA transmission, \textit{hDAT} and \textit{hVMAT2} interact with each other in conferring risk for SCZ (302).

More recently, \textit{hVMAT1} is also implicated in brain activity and positively associated with SCZ and bipolar disorder but has not been analyzed for depression yet (309-311, 342).

In summary, the monoamine transporter genes are highly polymorphic in their chromosomal DNA sequences. Such polymorphisms contribute to the variable expressions and risk for at least 27 brain disorders. Remarkably consistent with the preclinical findings, all four studied transporter genes are associated with depression (Table 2). Three of these genes are associated with ADHD, alcoholism, Parkinson’s disease and SCZ.
VIII. Perspectives for medication development

Fifty years of medications demonstrate that the monoamine transporters are effective medication targets for a spectrum of brain disorders including ADHD, depression, OCD, anxiety, smoking and Parkinson’s disease. As illustrated in Figure 1, recent pharmacologic and molecular studies suggest that these transporters can be more widely and more effectively utilized for medication developments.

Medication issues

There are several caveats associated with current transporter protein-based medications such as those for depression, OCD and ADHD. First, although proven to effectively treat depression, the use of TCAs and MAOIs has been shown to elicit adverse side effects (343-346) and require high doses to achieve therapeutic effects (14). While comparably effective, more contemporary treatments seem to mollify these issues (e.g., 16). Second, approximately 30% of patients with depression fail to respond to antidepressant drug therapy (e.g., 344). Prospective, longitudinal research has shown that more than 75% of those who experience a first episode will relapse in their lifetimes (e.g., 347). Third, the conflicting body of literature on antidepressant tolerance must also be taken into account. Tolerance can emerge with long-term antidepressant treatment or on retreatment after discontinuation (348). Patients who are treated with antidepressants may be less likely to have a positive response to new antidepressant treatments (18). However, conflicting research has hinted that prior antidepressant treatment may have no effect on new interventions (349, 350) and that discontinuation of maintenance SSRI treatments can result in a far poorer prognosis than continued maintenance (351). Fourth, treatments of OCD with these medications do not display long-term benefits after the medications are terminated (352). Finally, the abuse potential of both AMPH and methylphenidate warrants investigation of novel DAT blockers with low abuse profile to treat ADHD. Similarly, the DAT is likely to become the target for future pharmacotherapies used for the management of several other disorders which feature dopaminergic dysregulation, including Parkinson’s disease, Lesch-Nyhan syndrome, Tourette’s syndrome and obesity (353).

In addition to the limited efficacy, the current medications cover only approximately a third of related diseases. The large body of preclinical neuropharmacologic, animal genetics and human association data suggests that monoamine transporters regulate brain pathways that underlie not only these diseases under transporter protein-based treatments but also twenty others such as addiction, mood disorder, PTSD, stress, hypertension, SCZ, bipolar disorder and anorexia nervosa, etc. (Table 2).

The caveats and limited coverage mirror a fact that the current medications have not benefited much from the molecular studies conducted during the last 15 years, warranting new searches for complementary medications. The new and expanding information from molecular studies suggests that it is very likely for monoamine transporters to serve as more effective medication targets and for more related diseases.

New targets for medications

Substance abuse and depression represent the main focus for future transporter-based medication development. Substance abuse is a worldwide epidemic but the treatment has benefited little from the elucidation that these monoamine transporters are the functional targets for substances of abuse. Depression is common and predicted by the World Health Organization (WHO) to be the second most debilitating disease next to heart disease by the year 2020. It is also one of the best studied diseases by different independent disciplines including neuropharmacology, animal genetics and human genetics. Animal genetic data
suggested that reduced or eliminated transporter expression causes depressive behaviors, suggesting that medications that up-regulate these transporters’ activity may prove effective in treating this disease. However, the current medications are all based on inhibition of the transporters and such an approach works to certain degree perhaps by inhibiting multiple transporters to balance the neurotransmission of different types at the same time. It remains difficult to boost the transporter activity in the plasma membranes. Understanding the molecular mechanisms could assist in evidence-based medication development.

First, with the help of structural models and SAR information, medicinal chemists are in an exciting position to explore the transport-sparing antagonism and develop more transporter-selective inhibitors. Such new inhibitors could develop into medications for drug abuse among others and would allow testing transporter-specific hypotheses in terms of roles the transporters play in brain function and diseases. Second, we ought to take advantage of the intracellular networks that regulate transporter expression in the plasma membrane. There are more than ten proteins that interact and regulate transporter trafficking and therefore could serve as new targets for medication development. Third, DNA sequence polymorphisms that underlie transporters’ genetic contribution to the diseases provide novel medication targets. These novel medication targets may require the dissection of related signaling cascades in the monoaminergic neurons or can be directly used in cell-based high throughput screening (HTS) for small molecule regulators. Finally, there is pauciity of information on translation and translocation efficiency and regulation of stability of the transporter mRNA molecules. mRNA represents an unexplored subject and another medication target. A major advantage of using nucleic acids as medication targets is the increased transporter specificity and target diversity. Therefore, it will not be surprising to see monoamine transporter-based new medication targets to be discovered, tested and utilized in the next decade.

Acknowledgments

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Figure 1. Schematic of clinical relevance of monoamine transporters (MATs in blue) or vesicular monoamine transporters (VMATs in brown): regulation (black arrow) of activity and expression by small molecules, proteins and DNA sequence polymorphisms (see “x”). Clinical roles of MAT inhibitors are indicated on the left hand side and diseases contributed by genetic variations listed on the right side. Green dot, monoamine. Horizontal arrow, MAT gene.
### Table 1

Biochemical and molecular properties of monoamine transporters

<table>
<thead>
<tr>
<th>Protein alias</th>
<th>DAT</th>
<th>SERT</th>
<th>NET</th>
<th>VMAT1</th>
<th>VMAT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrates</td>
<td>dopamine, norepinephrine, amphetamine, MPP+</td>
<td>Serotonin, MDMA</td>
<td>norepinephrine, dopamine, amphetamine</td>
<td>serotonin, dopamine, norepinephrine</td>
<td>serotonin, dopamine, norepinephrine</td>
</tr>
<tr>
<td>Rank</td>
<td>DA&gt;NE&gt;5HT</td>
<td>5HT&gt;MDMA</td>
<td>DA&gt;NE&gt;5HT</td>
<td>5HT&gt;DA&gt;NE</td>
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<tr>
<td>$K_M$</td>
<td>885 nM</td>
<td>320 nM</td>
<td>457 nM</td>
<td>1 μM</td>
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</tr>
<tr>
<td>Selected inhibitors</td>
<td>mazindol, amphetamine, cocaine</td>
<td>paroxetine, citalopram, chlorimipramine</td>
<td>mazindol, dopamine, cocaine</td>
<td>reserpine, tetrabemazine</td>
<td>reserpine, tetrabemazine</td>
</tr>
<tr>
<td>Ion</td>
<td>Na$^+$, Cl$^-$</td>
<td>Na$^+$, Cl$^-$, K$^+$</td>
<td>Na$^+$, Cl$^-$</td>
<td>H$^+$</td>
<td>H$^+$</td>
</tr>
<tr>
<td>Amino acids</td>
<td>619</td>
<td>653</td>
<td>617</td>
<td>521</td>
<td>515</td>
</tr>
<tr>
<td>Tissue distribution</td>
<td>mid brain</td>
<td>brain stem, mid brain, peripheral</td>
<td>brain stem</td>
<td>Adrenal gland, PC-12 cells</td>
<td>brain stem, mid brain, stomach</td>
</tr>
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<td>References</td>
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<td>(106)</td>
<td>(103)</td>
<td>(108)</td>
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*All transporters have 12 TMs.*
### Table 2

<table>
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<tr>
<th>Disease</th>
<th>hDAT</th>
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<th>hNET</th>
<th>hVMAT2</th>
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<td>√</td>
<td></td>
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<td>2. Aggression</td>
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<td>3. Alcoholism</td>
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<td>269-273</td>
</tr>
<tr>
<td>4. Alzheimer’s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>274</td>
</tr>
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<td>5. Angry</td>
<td>√</td>
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<td>275</td>
</tr>
<tr>
<td>6. Anorexia nervosa</td>
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<td></td>
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<td></td>
<td>276, 277</td>
</tr>
<tr>
<td>7. Anxiety</td>
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<td>278</td>
</tr>
<tr>
<td>8. Autism</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td>279</td>
</tr>
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<td>9. Bipolar disorder</td>
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<td></td>
<td></td>
<td></td>
<td>280</td>
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<td>10. Delirium</td>
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<td></td>
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<td>11. Depression</td>
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<td>16. Neuroticism</td>
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<td>17. OCD</td>
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<td>18. Orthostatic intolerance</td>
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<td>19. Pain</td>
<td>√</td>
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<tr>
<td>20. Parkinson’s disease</td>
<td>√</td>
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</tr>
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<td>21. PTSD</td>
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<td>22. Schizophrenia</td>
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<td>302, 303</td>
</tr>
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<td>24. Suicide behavior</td>
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<tr>
<td>25. TCI/TPQ HA</td>
<td>√</td>
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<td>26. Panic disorder</td>
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<td>27. Unipolar disorder</td>
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<td>hSERT</td>
<td>hNET</td>
<td>hVMAT2</td>
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ADHD, attention deficit hyperactivity disorder; IBS, irritable bowel syndrome; OCD, obsessive compulsive disorder; PTSD, post-traumatic stress disorder; TCI/TPQ HA, TCI/TPQ harm avoidance.

* hVMAT1 is also reported to be associated (309-311).