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Regulation of Monoamine Transporters: Influence of Psychostimulants and Therapeutic Antidepressants

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Lankupalle D. Jayanthi[1] and Sammanda Ramamoorthy[1]

[1]Department of Neurosciences, Division of Neuroscience Research, Medical University of South Carolina, Charleston, SC 29425

ABSTRACT

Synaptic neurotransmission in the central nervous system (CNS) requires the precise control of the duration and the magnitude of neurotransmitter action at specific molecular targets. At the molecular level, neurotransmitter signaling is dynamically regulated by a diverse set of macromolecules including biosynthetic enzymes, secretory proteins, ion channels, pre- and postsynaptic receptors and transporters. Monoamines, 5-hydroxytryptamine or serotonin (5-HT), norepinephrine (NE), and dopamine (DA) play an important modulatory role in the CNS and are involved in numerous physiological functions and pathological conditions. Presynaptic plasma membrane transporters for 5-HT (SERT), NE (NET), and DA (DAT), respectively, control synaptic actions of these monoamines by rapidly clearing the released amine. Monoamine transporters are the sites of action for widely used antidepressants and are high affinity molecular targets for drugs of abuse including cocaine, amphetamine, and 3,4-methylenedioxymetamphetamine (MDMA) "Ecstasy." Monoamine transporters also serve as molecular gateways for neurotoxins. Emerging evidence indicates that regulation of transporter function and surface expression can be rapidly modulated by "intrinsic" transporter activity itself, and antidepressant and psychostimulant drugs that block monoamine transport have a profound effect on transporter regulation. Therefore, disregulations in the functioning of monoamine transporters may underlie many disorders of transmitter imbalance such as depression, attention deficit hyperactivity disorder, and schizophrenia. This review integrates recent progress in understanding the molecular mechanisms of monoamine transporter regulation, in particular, posttranscriptional regulation by phosphorylation and trafficking linked to cellular protein kinases, protein phosphatases, and transporter interacting proteins. The review also discusses the possible role of psychostimulants and antidepressants in influencing monoamine transport regulation.

Corresponding Author: Lankupalle D. Jayanthi, Department of Neurosciences, Medical University of South Carolina, Charleston, SC 29425. Tel: (843) 792-8542; Fax: (843) 792-4423; E-mail: jayanthi@musc.edu

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NEUROBIOLOGY OF MONOAMINE TRANSPORTERS

The catecholamines dopamine (DA) and norepinephrine (NE) derived from tyrosine and the indolamine 5-hydroxytryptamine or serotonin (5-HT) derived from tryptophan, packaged into synaptic vesicles and released into synapse in response to depolarizing stimuli, activate pre- and postsynaptic receptors and elicit synaptic responses. Presynaptic plasma membrane transporters for 5-HT (SERT), NE (NET), and DA (DAT), respectively, control synaptic actions of these monoamines by rapidly clearing the released amine, thereby dictating the kinetics of postsynaptic responses and receptor desensitization. Drugs that modulate the activity of biogenic monoamine transporters produce profound behavioral effects, leading to their therapeutic use in depression, obsessive-compulsive disorder, and other mental diseases and also to their abuse as stimulants.

SERT

Altered serotonergic neurotransmission and serotonin (5-HT) have long been associated with psychiatric disorders including depression, suicide, impulsive violence, and alcoholism.[1,2] Presynaptic plasma membrane SERT controls serotonergic neurotransmission by rapid clearance of released 5-HT. Reduced binding of imipramine and paroxetine to brain and platelet SERTs in patients with depression and suicide victims indicates that altered SERT function might contribute to aberrant behaviors. Drugs that block SERT, including tricyclic antidepressants such as imipramine, and selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Prozac), paroxetine (Paxil), sertraline (Zoloft), and citalopram (Celexa) are successfully used in the treatment of depression. SSRIs rapidly inhibit the uptake of 5-HT. However, the therapeutic efficacy of antidepressants develops slowly during several weeks of continuous treatment. These observations suggest that the elevation of synaptic 5-HT induces alterations in compensatory or intracellular regulatory pathways impacted by 5-HT signaling

and hence neural plasticity. Therefore, changes in SERT kinetics and number will strongly influence the efficacy of antidepressants in the treatment of depression. Studies from DAT knockout (KO)[3] and SERT-DAT double knockout[4] mice demonstrate the involvement of the serotonergic system in the reward response and suggest that the interaction of cocaine with SERT might be necessary to initiate and maintain cocaine reward in DAT KO mice. Two polymorphic regions have been identified in the SERT promoter and implicated in anxiety, mood disorders, alcohol abuse, and in various neuropsychiatric disorders.[5,6] Recent studies indicate that an Ile to Val mutation at position 425 of hSERT found in obsessive compulsive disorder (OCD)-rich pedigrees leads to an increase in transport activity and insensitivity to stimulation by nitric oxide (NO) donors.[7,8] Thus, studies are just emerging to support the notion that impaired regulations might contribute to human disease conditions such as that seen in human variants of SERT coding region.

NET

Noradrenergic signaling is intimately linked with behavioral arousal[9] and is affected in stress-related paradigms linked to depression.[10] NE is an important chemical messenger in the nervous system and regulates affective states, learning and memory, endocrine, and autonomic functions. It has been implicated in depression, aggression, addiction, and cardiac and thermal regulation. NE acutely inhibits nociceptive transmission mediated by substance P (NK1), potentiates opioid analgesia, and underlies part of antinociceptive effects of tricyclic antidepressants.[11,12] NE transporter regulates noradrenergic signaling in central and peripheral nervous systems by mediating the clearance of NE and is an important target for antidepressants and psychostimulants.[13-16] Various biologic stimuli are known to regulate NE signaling, and alterations in NE signaling including NE clearance and NET density are observed in cardiovascular diseases and brain disorders.[17-20] Since reuptake into presynaptic nerve terminals is the predominant mechanism for terminating the action of released NE, changes in the activity of NET should have a significant impact on the concentration and duration of NE present in the synaptic cleft and consequently alter NE signaling. The psychostimulant supersensitivity[21] and potentiated opioid analgesia[22] in NET K/O animals indicate the importance of NET expression under physiological circumstances. A point mutation in the coding region of human NET is associated with peripheral orthostatic intolerance (OI), high plasma NE levels, and reduced systemic NE clearance.[23] This study demonstrates the impact of hNET coding variant on NE clearance contributing to a human disease condition.

DAT

DA signaling regulates many crucial functions, such as movement, emotion, and cognition.[24,25] DAT terminates dopaminergic neurotransmission by re-uptake of DA in presynaptic neurons and plays a key role in DA recycling. DAT can also provide reverse transport of DA under certain circumstances. Psychostimulants such as cocaine, and amphetamine and drugs used for ADHD such as methylphenidate exert their actions via DAT.[26,27] Recent molecular and pharmacological analyses using monoamine transport knockout mice have confirmed the physiological importance and requirement of presynaptic amine transporter expression for normal transmitter clearance, presynaptic transmitter homeostasis, postsynaptic response and response to drugs.[21,28,29] In particular, functional loss of DAT either through pharmacological inhibition[30,31] or gene knockout[32] results in profound physical, physiological, and behavioral changes. Altered DAT function or density has been implicated in various types of psychopathology, including depression, suicide, anxiety, aggression, and schizophrenia.[24,33-35] Associations between DAT gene polymorphisms and human disorders with possible links to DA system, including attention deficit hyperactivity disorder (ADHD) and consequences of cocaine and alcohol abuse have been reported.[2,36] Altered transport properties associated with some of the coding variants of DAT suggest that individuals with these DAT variants could display altered DA system.[37]

MONOAMINE TRANSPORTERS AND ADDICTION

Monoamine transporters are also of interest in the field of addiction because they constitute high affinity molecular targets mediating the cellular and behavioral effects of psychostimulants such as cocaine and amphetamine.[38,39] Given the importance of monoamine transmission in various aspects of addiction, and the profound influence that monoamine transport has on the extracellular levels of monoamines, as well as intracellular monoamine homeostasis, long-term changes in transporter level, kinetics, or regulation would be expected to greatly influence spontaneous and drug-induced behaviors. This possibility is clearly evidenced by the large effects on drug-induced behaviors, as well as on neurotransmitter synthesis, storage, and release by removing monoamine transporter genes in mutant mice.[27,32,40,41] Receptors that are known to influence amine transporter functions, such as 5-HT1B (SERT), D2, opioid, and mGluR5 (DAT) have been implicated in cocaine sensitization.[42,43] Such changes in receptor-linked signaling cascades could influence the transporter phosphorylation and transport activity. Since the second messenger-linked pathways can be linked to presynaptic receptors, these pathways may provide positive/negative

feedback mechanisms in control of DA, 5-HT, and NE clearance in vivo.

Monoamine Transporters and Their Functional Regulation

Monoamine transporters belong to the Na^+, Cl^- -dependent, gamma-amino butyric acid (GABA)/norepinephrine transporter (GAT1/NET) gene family that also includes transporters for proline (PROT), taurine (TauT), glycine (GLYT), creatine (CreaT), betaine (BGT), and other "orphan" transporters. Monoamine transporters, in general, function by sequestering monoamines from specific nerve terminals. However, in several brain regions and under certain conditions, monoamine transporter functions can overlap.[44-46] Pharmacological and behavioral studies in genetic animal models with targeted disruption of monoamine transporters suggest functional overlap between these transporters. Consequently, pharmacological and physiological characterizations to distinguish their role in psychiatric illnesses and drug abuse require proper understanding of the mechanisms regulating monoamine transporter function.

Neural activity, hormones, growth factors, environmental factors, and pharmacological agents have been reported to regulate monoamine uptake, specific radioligand binding, and mRNA levels. These studies suggest the presence of endogenous regulatory mechanisms and such regulation could influence transporter expression and function at the level of gene transcription and translation (long-term or chronic regulation) or posttranslational protein modifications (short-term or acute regulation).

Regulation at the Gene Level

Structural analysis of transporters gene promoter region reveals several canonical transcription binding sites that may be important in controlling the responses of the transporter genes to regulatory factors. Notably, binding sites for transcription factors including TATA-like motif, an AP1 site, an element for CREB binding (CRE), AP-2, NF-IL6, NF-Kß, and SP1 sites have been identified for SERT. Relevant investigations performed on JAR cells have shown that activation of cAMP-dependent and independent pathways increase 5-HT uptake.[47-49]

The long-term modulation of SERT activity both in vivo and in vitro has been studied extensively and reviewed elsewhere.[50-52] Unlike SERT, very little is known about NET and DAT gene regulation. Earlier in vivo and in vitro studies demonstrate modulation of NE uptake by several factors such as insulin, atrial natriuretic peptide (ANP), angiotensine (ANG II), dexamethasone and nerve growth factor (NGF), and pharmacological substances such as desipramine and cocaine. Altered NET mRNA levels are shown for the NET modulation by insulin, dexamethasone, NGF, desipramine, and cocaine.[53-56] Recent reports showed an upregulation in the expression of DAT and NET genes following cocaine treatment. During pregnancy, cocaine exposure results in increased DAT mRNA levels in the fetal rhesus monkey brain.[57] Another study showed increased levels of NET binding sites in the placentas of rats treated with cocaine reflecting increased NET mRNA levels.[58] NET binding sites are also increased in stria terminalis of cocaine self-administered monkeys.[59] Although the actual cellular signaling pathways responsible for such regulation at the genetic level are yet to be identified, these studies suggest a significant functional role for the monoamine transporters not only in the monoaminergic neurotransmission, but also in the fetal development.

Regulation at the Protein Level

Reuptake of monoamine neurotransmitters into presynaptic terminals via the transporters is the principle mechanism for terminating the monoaminergic neurotransmission. Therefore, changes in the transporter activity or expression should have a significant impact on the duration and concentration of monoamines present in the synaptic cleft. These changes resulting in the regulation of transport function, in turn, would influence pre- and postsynaptic responses to released monoamines. The regulation of monoamine transporter function and expression reviewed here suggests that signaling proteins such as kinases, phosphatases, and transporter interacting proteins, as well as transporter ligands have a potential role in the regulation of transporters for appropriate transporter function and expression.

Amino acid sequence analysis of SERT, DAT, and NET proteins reveal numerous consensus sites for protein kinases as well as putative interactive motifs in cytoplasmic domains suggesting that second messengers are able to play a role in posttranslational regulation of monoamine transporters. Prior to the cloning of monoamine transporters, evidence suggests a pivotal role for second messenger-linked pathways in acute modulation of neurotransmitter uptake.[52] Potential pathways linked to second messenger-linked pathways might regulate transporter function acutely. Regulation of transport function can occur directly by phosphorylation of transporter protein. Phosphorylation of transporter protein might in turn change its intrinsic transport activity, turnover rate, plasma membrane insertion by modulating exocytic fusion, or sequestration from the plasma membrane by modulating endocytic machinery. Alternatively, transporters can be regulated through their association with other interacting proteins by phosphorylation dependent/or independent pathways. Substrate or antagonist binding may also modulate transport activity via its influence on transporter phosphorylation, trafficking, and interaction with transporter-interacting proteins.

Mechanisms of Acute Monoamine Transporter Regulation

Posttranslational modifications such as glycosylation and phosphorylation regulate monoamine transporter function and expression levels.[60-63] In addition, variations in monoamine transporter gene sequences known as polymorphisms may also alter transporter expression levels, activity, and/or regulation. Recent studies demonstrate that the activity and expression of monoamine transporters are regulated by phosphorylation and dephosphorylation of the transporter proteins. Thus, monoamine transporters are dynamically regulated by intracellular and extracellular regulation of phosphorylation state, which in turn should have a significant impact on the duration and concentration of monoamines present in the synaptic cleft.

Altered Transporter Surface Expression and Intrinsic Activity

Among various stimuli that acutely regulate monoamine transporters, the majority regulate transport function by altering transporter surface expression. In a variety of preparations, including synaptosomes and cell lines, application of protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (β-PMA) selectively reduces the transport capacity (Vmax) for transport of DA, NE, and 5-HT.[61-65] The most consistent finding is that activators of PKC or the agents that maintain phosphorylation state such as phosphatase inhibitors rapidly reduce amine transport capacity. The major kinetic alterations typically observed in acute modulation paradigms are changes in Vmax, with little or no significant change in substrate affinity (Km). The reduction in Vmax values suggests silencing of plasma membrane resident amine transport protein or changing cell surface expression levels by regulating endocytosis or recycling or plasma membrane insertion of transporter proteins. First evidence for altered cell surface expression for monoamine transporter as the result of PKC activation has come from studies of human SERT stably expressed in human embryonic kidney (HEK-293) cells. Protein phosphatase inhibitor, okadaic acid, also regulates SERT activity, which suggests that cellular phosphatases monitor phosphorylation-dependent SERT regulation.[66] PKC activation by β-PMA also leads to a decrease in 5-HT transport capacity and SERT currents under voltage clamp.[67] Analogous kinase-mediated transporter redistribution has been detected for DAT and NET.[63-65,68,69] Of interest, dominant negative mutant of dynamin 1, which is shown to block clathrin-mediated endocytosis, is able to block PKC-dependent DAT internalization.[70] These observations suggest that PKC regulates DAT internalization by clathrin-mediated and dynamin-dependent cellular mechanisms. They also provide evidence that internalized DAT is targeted to endosomal/lysomal pathways for degradation. However, other studies show that internalized DAT is directed to recycling pool,[71] and plasma membrane DAT sequestration can occur due to a combination of accelerated internalization or reduced recycling.[72] However, the molecular mechanisms responsible for different PKC-dependent DAT internalization pathways (recycling endosomes vs degradative lysosomes) between these studies are unknown. Recently, clathrin-dependent mechanisms were demonstrated for both constitutive and PKC-mediated DAT internalization.[73] Nonclassical, distinct endocytic signals drive constitutive and PKC-regulated DAT internalization.[74] The DAT internalization signal is conserved across SLC6 neurotransmitter carriers and is functional in the homologous norepinephrine transporter. Together, the above studies suggest the involvement of unconventional mechanisms in DAT and NET internalization.

Recent studies from our laboratory provided the first evidence for lipid raft localization and raft-mediated internalization of NET and SERT.[63,75] SERT and NET are also expressed in nonneuronal tissues such as placenta and intestinal epithelial cells.[76-78] PKC activation stimulates lipid raft-mediated internalization of native NETs expressed in placental trophoblasts.[63] Of interest, PKC-, but not mitogen-activated protein kinase (p38 MAPK)-mediated SERT regulation in rat brain involves raft-mediated distribution.[75] The presence of NET and SERT in lipid rafts suggests that signaling machinery specific to lipid rafts may be linked to PKC-mediated transporter downregulation. Raft-associated sorting has been proposed to underlie several cellular processes including signal transduction, protein sorting, and membrane trafficking.[79] Receptors such as NK1R and TrkB, several channel proteins, many components of G protein coupled receptor (GPCR) signal transduction proteins such as, adenylyl cyclase, Akt1, PLC, activated PKC, PP2Ac, nonreceptor tyrosine kinases, and other signaling molecules such as syntaxin 1A (a SNARE protein), α-synuclein, and the PKC binding protein PICK1 (protein interacting with C kinase) have been shown to be associated with lipid rafts.[79,80] The localization of receptors such as NK1R that regulate monoamine transporter function and potential transporter-interacting proteins (syntaxin 1A, PP2Ac, PKC), as well as transporters in lipid raft microdomains, raises the possibility that lipid rafts may *act as morphological* "*conveners*" *of signal transduction* by placing various signal transduction molecules near the transporter molecule. For example, transporter-interacting proteins may "*guide*" targeting the transporter to lipid rafts and that phosphorylation of a "*motif/site*" within the transporter may act as a "*signal*" for fostering protein-protein interactions and redistribution. Thus, protein redistribution from plasma membrane microdomains may be one of the

several mechanisms by which synaptic plasticity and neurotransmitter homeostasis are maintained.[81,82]

NET,[83] DAT,[84,85] and SERT[75,86] are also regulated by MAPKs. While studies on DAT[85] and SERT[75] implicate MAPK effects on transporter trafficking, other studies on NET and SERT indicate modulation of transporter catalytic activity. In our study,[75] p38 MAPK-mediated SERT redistribution involves enhanced plasma membrane insertion of the transporter. Numerous studies have indicated alterations in the 5-HT levels, SERT binding sites, and serotonergic neuronal firing in response to numerous stressors.[87] Thus, the regulation of SERT by p38 MAPK, a stress-induced kinase, may provide a novel presynaptic mechanism in maintaining appropriate synaptic 5-HT levels during stressful conditions. Several studies indicate that SERT is upregulated by cGMP/PKG-mediated pathway through altered surface expression.[8,86,88] Of interest, MAPK effects on SERT intrinsic activity are also sensitive to PKG activity.[86] Together, these studies suggest that transporters are subject to multiple, interacting modes of kinase- dependent modulation via trafficking-dependent and independent mechanisms.

Altered Transporter Phosphorylation

DAT, SERT, and NET proteins are phosphorylated in response to PKC activation.[61-63] DAT proteins become phosphorylated in transfected cells and synaptosomal preparations following treatment with PKC activators and PP2A inhibitors.[61,89] SERTs are also phosphorylated following PKC and PKA activation and PP2A inhibition. The PKC-dependent phosphorylation shows a close temporal correlation with reduction in transport capacity.[62,90] SERT substrates and antagonists influence PKC-dependent SERT phosphorylation and surface redistribution.[90] Our recent studies indicate that while PKC activation increases SERT basal phosphorylation, p38 MAPK inhibition decreases SERT basal phosphorylation indicating a role for p38 MAPK-induced phosphorylation in constitutive SERT expression.[75]

DAT is phosphorylated on N-terminal serine residues.[91] The PKC-mediated internalization of monoamine transporters occurs in parallel to an enhanced phosphorylation of the transporter. Although the transporters undergo phosphorylation in response to PKC activation or PP1/2A inhibition, it is not known whether phosphorylation of the transporter leads to its internalization. However, recent studies using phospho-site mutants of transporters provided positive as well as negative correlations between phosphorylation and transporter functional regulation.[92-95] Studies demonstrate that tyrosine kinase activity is also critical in maintaining NET,[83] DAT,[96] and GAT[97,98] cell surface levels. Phosphorylation of Y107 and Y317 on GAT1 is required for tyrosine kinase-mediated GABA transport regulation.[98] Of interest, the reciprocal relationship between PKC- and tyrosine kinase-mediated GAT1 phosphorylation suggests that a balance between these 2 states of phosphorylation may dictate relative abundance of GAT1 on the cell surface.[99]

While a part of N-terminal domain appears to interact with syntaxin 1A involved in PKC-mediated regulation,[100] determinants within the C-terminus of human NET (hNET) dictate NET trafficking, stability, and activity.[101] Of interest, nonclassical endocytic signals dictate constitutive and PKC-regulated internalization of DAT.[73,74] These signals are conserved in NET. However, it is not known whether there is a relationship between NET phosphorylation and PKC-induced transporter regulation. NET protein contains multiple consensus sites for several kinases including PKC that are distinct from those present in DAT or SERT, and therefore, NET may be regulated by mechanisms that are different for those of DAT and SERT. Recent work from our laboratory (Jayanthi et al, unpublished data, 2005) demonstrates that a PKC-site mutant of hNET is resistant to PKC-mediated downregulation and phosphorylation. This study provides the first evidence that phosphorylation of a PKC motif on a monoamine transporter is linked to transporter internalization. In addition, this PKC-site mutant of hNET exhibits altered transport properties such as increased affinity for NE and substrate-like ligand D-amphetamine, and decreased affinity for inhibitory ligands such as cocaine, desipramine, and nisoxetine. Phospho-site mutations could contribute to altered NE transport process due to physical conformational changes occurring as the result of mutation or altered phosphorylation state itself. Such changes can result in altered binding or recognition of the ligands. Recent studies on DAT harboring mutations in the second intracellular loop reported altered ligand binding properties attributed to structural changes in physical conformation of DAT protein.[102-105] Such investigations would prove useful in developing antidepressant drugs with higher efficacy and reduced potential side effects.

Altered Protein-Protein Interactions

As described earlier, agents that maintain phosphorylated state modulate amine transporter activity. For example, protein phosphatase 1/2A (PP1/PP2A) inhibitor, okadaic acid (OK), downregulates SERT, DAT, and NET activity. Phosphorylation state and regulation of amine transporter is a balance between the actions and localization of protein kinases and protein phosphatases. The pronounced effect of PP1/PP2A inhibition on amine uptake and amine transporter phosphorylation suggests the possible physical association of PP1/PP2A as a regulatory complex with amine transporter proteins. Indeed, physical complexes containing biogenic amine transporter and PP2Ac proteins were recently demonstrated.[66] SERT/PP2A complex is disrupted by PP1/

PP2A inhibitors as well as PKC activators and can be stabilized by SERT substrate 5-HT, suggesting that modulation of SERT/phosphatase association is involved in regulated transporter phosphorylation and trafficking. Similar transporter and PP2Ac associations were found for DAT and NET proteins. Analogous to SERT studies, PP2Ac association with NET in vas deferens is also decreased by PKC activation and PP1/PP2A inhibition.

While direct phosphorylation and/or dephosphorylation of transporters by cellular protein kinases and phosphatases is involved in the dynamic regulation and expression of transporters, other integral membrane proteins also appear to play an important role in trafficking and catalytic function of transporters. First evidence for the physical association of GAT1 GABA transporter with atachment protein receptor (t)-SNARE protein syntaxin-1A as a complex comes from studies by Quick and his coworkers.[106,107] Of interest, this association is modulated by PKC-dependent manner and the interaction of GAT1 with syntaxin-1 is required for GAT1 regulation by PKC activation. Syntaxin-1A association not only regulates PKC-dependent trafficking of GAT1 but also appears to influence catalytic function of the transporter.[108] Recently, it has been shown that syntaxin-1A interacts with N-terminal cytoplasmic domain of GAT1 GABA transporter and decreases transport rates.[108] Like GAT1, SERT and NET are also physically associated with syntaxin-1A.[100,109] Using noradrenergic tissues such as vas deferens and transfected cell lines, Sung et al[100] reported that syntaxin-1A is found in NET immunoprecipitates. Activation of PKC or phosphatase inhibition decreases syntaxin-1A level with NET. Altering intracellular Ca^{2+} also regulates syntaxin-1A/NET interaction. Together these findings suggest an important role for syntaxin-1A in neurotransmission by regulating both transmitter release and reuptake.

Signals following presynaptic receptor stimulation may influence transporter function and expression by regulating the stability of transporter heteromeric complexes. Studies by Torres et al show the interaction of PDZ domain containing protein PICK1 with C-terminal cytoplasmic domains of DAT and NET.[110] Co-expression of DAT and PICK1 in HEK-293 cells enhances DAT activity and PICK1 co-immunoprecipitates with DAT. Confocal microscopy analysis reveals colocalization of both DAT and PICK1 in heterologous system and in dissociated dopaminergic neurons. Since PICK1 is a PKC binding protein, it is possible that PICK1 acts like an adaptor to bring PKC near DAT proteins, which in turn could regulate PKC-evoked DAT phosphorylation, trafficking, and function. Lee and coworkers recently identified the interaction of C-terminal cytoplasmic domain of DAT with α-synuclein.[111] α-Synuclein is enriched in dopaminergic nerve terminals and has been implicated in Parkinson's and other neurodegenerative disorders.[112] α-Synuclein binds directly to C-terminal region of DAT and facilitates the plasma membrane clustering and increase in DAT activity.[113] This study suggests that α-synuclein/DAT complex regulates normal dopaminergic neurotransmission, and altered interactions may result in abnormal DAT function causing dopaminergic neurodegeneration seen in Parkinson's disease. DAT also interacts with LIM domain-containing adapter protein, Hic-5.[114] Hic-5 is known to interact with signaling molecules such as nonreceptor protein tyrosine kinases FAK and FYN. This raises the possibility that Hic-5 may be involved in tyrosine kinase-mediated DAT regulation. Together, these studies indicate that protein-protein interactions play an important role in influencing transporter phosphorylation, trafficking, and intrinsic activity.

Influence of Substrates and Antagonists on Monoamine Transport Regulation

Another important observation is that the amine transporter substrates and antagonists influence transporter trafficking and plasma membrane residency. Since transporters are subjected to phosphorylation by multiple kinase-dependent modulations, it is possible that substrates may also influence transporter surface expression by regulating kinase-mediated transporter phosphorylation. For example, Ramamoorthy and Blakely provided the evidence that SERT substrates such as serotonin, amphetamines, fenfluramine, and antagonists such as antidepressants and cocaine control PKC-dependent SERT phosphorylation and surface redistribution.[90] SERT transporting substrates, 5-HT, amphetamine, and fenfluramine, protect SERT from PKC-linked phosphorylation and sequestration. The effect of 5-HT is SERT-dependent but not 5-HT receptor-dependent, and the nontransported ligands such as cocaine and antidepressants block the effect of 5-HT. Amphetamines substitute for substrates in suppressing PKC-mediated SERT phosphorylation. Such action could override homeostatic transporter sequestration processes and provide for psychostimulant sensitization by increasing the number of psychostimulant targets available to a subsequent stimulus. On the other hand, nonpermeant SERT ligands including SSRIs and cocaine that prevent 5-HT permeation block the effect of 5-HT, thus SSRIs may have therapeutic utility in disease states, not only by preventing 5-HT uptake but also by shifting the cellular distribution of SERT. Amphetamines trigger DAT internalization,[115] and while acute cocaine exposure increases DAT surface levels,[116,117] amphetamine-induced DAT internalization is blocked by cocaine.[115] This inverse relationship between antagonist binding and substrate translocation suggests that various transporter conformational states may promote or preclude their engagement with

cellular endocytic machinery dictating transporter surface levels.

Transporter substrates are known to regulate transporter function and expression.[75,118-120] Substrate-induced upregulation of GAT1 requires tyrosine phosphorylation and acute substrate exposure slows down the transporter internalization rate, resulting in increased surface levels from continued delivery to the plasma membrane.[98,118] D-amphetamine, which is also a substrate for SERT, increases SERT basal phosphorylation that is sensitive to p38 MAPK inhibition suggesting that p38 MAPK may govern substrate-mediated effects on SERT basal phosphorylation.[75] Substrate translocation or ligand occupancy may influence the equilibrium of protein conformation required for transporter phosphorylation or transporter association with other regulators. Thus, a feedback loop may exist providing a mechanism by which changes in extracellular neurotransmitter concentrations could rapidly modulate neurotransmitter transport capacity. This way, the control of transporter cell surface expression by its substrates or ligands would provide a novel homeostatic mechanism in the neuron to fine-tune transport capacity to match demands imposed by fluctuating levels of the neurotransmitter. Signaling pathways linked to presynaptic auto- and hetero-receptors could provide a positive/negative feedback control and provide a mechanism by which changes in synaptic neurotransmitters could rapidly modulate transport capacity of the transporter.

CONCLUSIONS

In summary, evidence suggests that amine transporters may be modulated by various biologic stimuli as well as pharmacological agents. The intracellular second-messenger systems mimic cellular protein kinases and phosphatases and subsequently regulate amine transporter gene and protein expression. The past few years of active research on transporter function, structure, expression, and regulation strongly support the idea that amine transporters are principle players in regulating normal and abnormal amine signaling in the central nervous system (CNS) and periphery and hence complex behavioral and physiological functions. An even more exciting discovery is that the regulation of transporter phosphorylation by kinases or phosphatases indeed plays a major role in dictating transporter function. Recent studies that demonstrate altered response to second-messenger and/or kinase-mediated regulations in human variants of monoamine transporters[8,121,122] signify the search for underlying mechanisms of transporter phosphorylation regulating amine transport in normal physiology and pathophysiology. Another important lead in the monoamine transporter research is that intrinsic transport capacity of a transporter molecule governs its own plasma membrane expression and function. There appear to be multiple mechanisms by which transporter substrates and antagonists can influence transporter function and expression. The variations in the transporter response to different signals and the various mechanisms they adopt to regulate transporter function suggest a need for future research to focus on physiologically relevant events. The capacity of transporter to fine-tune its function in response to extracellular neurotransmitter would maintain a constant level of neurotransmitter at the synaptic cleft. Altered pattern or a disturbance in the pre- and post-synaptic regulatory mechanisms could lead to abnormal neurotransmission and hence abnormal behavior or brain disorders. Drugs of abuse such as cocaine and amphetamines bind and inhibit biogenic amine transporters and might interfere with activity-dependent regulatory mechanisms and cause or initiate drug addiction or sensitization. Along with information delineating cis/trans acting elements and signals for acute and chronic amine transporter regulation, over the next few years, we will expect rapid progress in understanding the role of cellular regulation of amine transporters in amine signaling and behavior. These developments add further impetus to the drive for psychotherapeutic innovation and a better understanding of mechanisms of monoamine transporter regulation in drug addiction.

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