ACS Chemical Neuroscience

Review

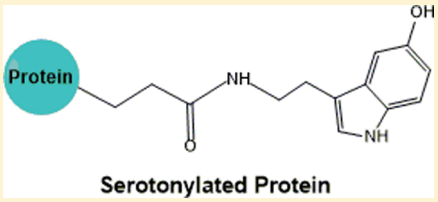
pubs.acs.org/chemneuro

Serotonylation and Transamidation of Other Monoamines

Nancy A. Muma* and Zhen Mi

Department of Pharmacology and Toxicology, University of Kansas School of Pharmacy, Lawrence, Kansas 66045, United States

ABSTRACT: Although serotonin was discovered over 65 years ago, it has been only within the past decade that serotonin was found to be involved in a covalent post-translational modification to proteins. The enzyme transglutaminase catalyzes the transamidation of serotonin to a protein-bound glutamine residue; the amino group of serotonin is covalently bound to the gamma carboxamide of glutamine. The term serotonylation is used to describe this transamidation reaction to serotonin. Not only can serotonin be a substrate for transamidation to proteins but also other monoamine neurotransmitters are substrates including histamine, dopamine, and noradrenaline. The term monoaminylation has been coined to describe the transamidation of monoamines to protein substrates. Small G proteins have emerged as the most common substrate for monoaminylation and are activated by this post-translational modification. Fibronectin and cytoskeletal proteins are also substrates for monoaminylation. Serotonylation and monoaminylation are involved in a number of physiological functions, including platelet activation, insulin release, smooth muscle contraction, and regulation of membrane localization of the serotonin transporter. Stimulation of 5-HT_{2A} receptors increases serotonylation and activates the small G protein Rac1, which plays a role in dendritic spine regulation. Monoaminylation is implicated in pathophysiological processes as well such as diabetes and hypertension. The availability of monoamines for monoaminylation is altered by antidepressants that target serotonin transporters, noradrenaline transporters, or the enzymatic degradation of monoamines as well as drugs of abuse such as cocaine and amphetamines. Further research on monoaminylation is needed to elucidate its physiological and pathophysiological roles and to explore monoaminylation as a novel target for drug therapy.



KEYWORDS: *Transamidation, serotonylation, monoaminylation, transglutaminase, serotonin transporter, organic cation transporter, plasma membrane monoamine transporter*

Serotonin (5-hydroxytryptamine, 5-HT) was first described in 1948 as a vasoconstrictor present in the serum;[1] it was then found in the gut mucosa in 1950[2] and isolated from the brain in 1953.[3] The anatomical distribution of neurons expressing 5-HT as a neurotransmitter in the brain was described about a decade later.[4,5] In the half-century since its discovery, there has been an enormous amount of research on 5-HT related to its important roles in diverse physiological functions including cognition, vascular tone, insulin release, and gastrointestinal mobility and in disease states including depression, psychosis, migraine, and anxiety as well as being a target for treatment for many of these disorders. 5-HT can interact through the cell surface either via one of the 16 5-HT receptors or through transporters including the 5-HT transporter (SERT), the organic cation transporters (OCTs), and the plasma membrane monoamine transporter (PMAT). Despite the plethora of research on 5-HT, it is been only in the past decade that serotonin has been discovered to regulate physiological functions through the covalent modification of substrates in a reaction termed serotonylation.

Serotonylation is the transamidation of 5-HT to a glutamine residue of a protein, a reaction catalyzed by the enzyme transglutaminase (TG, Figure 1). Serotonylation can take place intracellularly following 5-HT uptake into a cell via transporters or following intracellular synthesis of 5-HT.[6,7] Serotonylation of extracellular proteins, especially fibronectin, has also been demonstrated.[8] Furthermore, there is also evidence for a 5-HT receptor coordinated function for serotonylation in which stimulation of 5-HT_{2A} receptors results in increased serotonylation of downstream signaling proteins.[9]

While serotonylation is the covalent binding of 5-HT to a glutamine residue of a protein, other monoamine neurotransmitters, such as histamine (HA), dopamine (DA), and noradrenaline (NA), can also be bound to glutamine residues of a protein. The term monoaminylation has been coined to describe these transamidation reactions in which a monoamine is post-translationally added to proteins. In 2011, Walther and colleagues published a review on monoaminylation, emphasizing the evolutionary aspects of monoamines and the enzymes involved in their synthesis.[10] The current review places an emphasis on the role of monoamine transport in providing the monoamine substrates for monoaminylation and is more focused on the biological relevance of monoaminylation that has emerged over the last several years.

The transamidation reaction is catalyzed by TGs (EC 2.3.2.13), a family of enzymes that catalyze a covalent bond between free amines such as 5-HT or lysine residues in peptides or proteins and the γ-carboxamide group of peptide or

Special Issue: Serotonin Research

Received: December 11, 2014
Revised: January 23, 2015
Published: January 23, 2015

DOI: 10.1021/cn500329r
ACS Chem. Neurosci. 2015, 6, 961−969

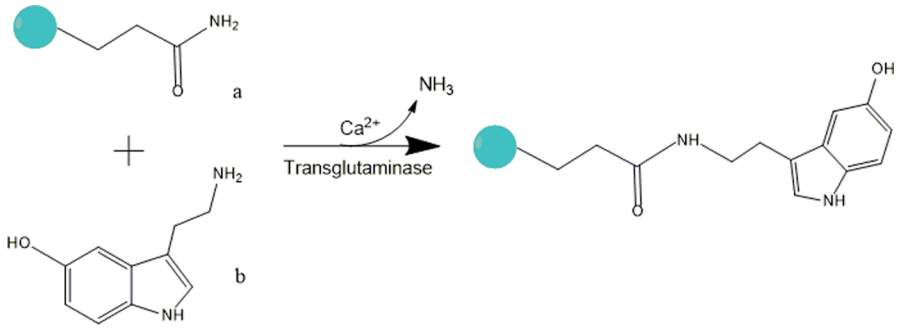


Figure 1. TG-catalyzed serotonylation reaction: (a) protein-bound glutamine residue and (b) serotonin.

protein-bound glutamines.[11,12] Alternatively, TG can catalyze the hydrolysis of the amide group on the protein-bound glutamine in a nucleophilic attack by water in place of a primary amine, resulting in deamidation.[13] In humans, there are nine TG genes that code for eight active enzymes (TG1−7 and factor XIIIa) and one catalytically inactive protein, erythrocyte membrane protein band 4.2 (Table 1). TG2 is ubiquitously expressed, in contrast to the other TGs which are more restricted in their distribution (Table 1), suggesting that TG2 may be more likely to be involved in monoaminylation.

Which of these TG proteins is capable of catalyzing the monoaminylation reaction? Limited evidence suggests that TG2 is preferentially involved in monoaminylation. In a neuronal cell line, A1A1v cells, the reduction of TG2 using siRNA diminished serotonylation of Rac1 to below baseline levels.[9] Second, although TG2 was able to catalyze the monoaminylation of histamine to small G proteins *in vitro*, neither TG1 nor TG3 was capable of catalyzing the reaction.[14] However, several pieces of evidence taken together suggest that a TG other than TG2 may be able to catalyze serotonylation in vascular smooth muscle cells. Rat aorta and vena cava express TG1, TG2, and TG4; the selective TG2 inhibitor Z-DON inhibited 5-HT-induced and TG-dependent contraction to an extent that was less than that with the nonselective TG inhibitor cystamine. Furthermore, Z-DON reduced protein transamidation to an extent that was less than that of the nonselective TG inhibitor cystamine in rat aorta cells.[15] Additional experiments are needed to directly determine if TG1 or TG4 is capable of catalyzing monoaminylation in vascular smooth muscle as well as the ability of other TGs to do so in other cell and tissue types. However, FXIIIA has been shown to be capable of catalyzing serotonylation of extracellular fibronectin in osteoblast cell cultures.[16]

METHODS TO DETECT MONOAMINYLATION

Monoaminylated proteins are difficult to identify, visualize, and enrich from biological samples. Since effective antibodies against specific serotonylated proteins are difficult to make due to the high abundance of 5-HT in hosts,[17] in most reported studies, detection of TG-mediated protein serotonylation was carried out using immunoprecipitation of the target protein followed by immunoblotting with an anti-5-HT−BSA conjugate antibody.[18−20] Radioactive isotope-labeled monoamines have also been used in assays for detecting protein monoaminylation.[6,21] However, only low-energy β-emitting [^{3}H] and [^{14}C] can be used to isotopically label small amines, limiting the use of radioactive methods for precise characterization of monoaminylation.[10] Furthermore, to demonstrate serotonylation using these two approaches, it is necessary to demonstrate that the association of the substrate protein and monoamine is TG-dependent. TG dependence can be demonstrated using a pharmacological inhibitor such as cystamine or a molecular approach such as siRNA to reduce TG expression. A well-characterized antibody, 81D4, which is specific for peptide- or protein-bound *N*-ε-(γ-L-glutamyl)-L-lysine isopeptide, immunoprecipitates monoaminylated proteins as well.[7,9] In another study, 5-HT was labeled by EZ-Link Sulfo-NHS-LC-LC-biotin as a substrate to identify serotonylated proteins through tandem mass spectrometry.[22] However, because this commercial biotin analogue does not cross the plasma membrane, only extracellular proteins can be studied using this method. In two recent studies, an *in vivo* tagging method was reported to characterize protein serotonylation.[17,23] 5-HT was modified at the 5-hydroxyl moiety to generate propargylserotonin, which is able to cross the plasma membrane and is recognized by TG2. Using click chemistry, an alkyne-functionalized 5-HT derivative was bound to N3-PEG4-biotin and was used as a substrate for serotonylation; the labeled proteins were subsequently characterized using immunoblotting and mass spectrometry. Site-specific identification of protein serotonylation is achievable using this technique.

TARGETS FOR MONOAMINYLATION

Several groups of proteins are emerging as major targets for monoaminylation (Figure 2). The family of small G proteins is the largest group of target proteins for serotonylation described to date, including Rac1, Rab3a, Rab4, Rab27a, and RhoA.[6,7,9,21] Additionally, there is also one report describing monoaminylation of the heterotrimeric Gα proteins Go1 and Gq *in vitro*.[14] Monoaminylation of fibronectin has been reported in brain tissue,[8,24] which is a regulator of pulmonary artery smooth muscle cell proliferation and migration[25] and inhibits mineralization of osteoblasts.[16] Furthermore, cytoskeleton proteins, including α-actin, β-actin, γ-actin, myosin heavy chain, and the actin-binding protein filamin A, are another group of targets for monoaminylation.[22,26]

Platelet Activation. Serotonylation was first described to take place on platelets wherein, following platelet activation, 5-HT was found to be transamidated to fibrinogen and von Willebrand factor on the cell surface.[27] The term serotonylation was coined by Walther and colleagues in a subsequent study demonstrating that platelet activation also resulted in intracellular transamidation of 5-HT to the small G protein RhoA.[6] Furthermore, this study demonstrated that RhoA is activated by transamidation to serotonin. Not only was RhoA serotonylation demonstrated *in vivo* using [^{14}C]5-HT but also Rab4 could be labeled with [^{14}C]5-HT using skeletal muscle cytosol *ex vivo*.

DOI: 10.1021/cn500329r
ACS Chem. Neurosci. 2015, 6, 961−969

Table 1. Properties of TG Proteins[12,74,75]

TG	alternative names	tissue and subcellular distribution	biological functions	pathology
TG1	Keratinocyte TG, particulate TG, TGK	Stratified squamous epithelia of skin, upper digestive tract, lower female genital tract, brain; Plasma membrane	Cell envelope formation in keratinocyte differentiation	Autosomal recessive lamellar ichthyosis
TG2	Tissue TG, TGc or Gh, liver TG, endothelial TG, erythrocyte TG	Widely distributed in tissues and cell types; Cytosolic, nucleus, and plasma membrane	Many functions: endocytosis, apoptosis, cell survival signaling, cell adhesion, matrix stabilization	Celiac disease, neurodegenerative disorders, cataract, malignancies
TG3	Epidermal TG, hair follicle TG, bovine snout TG, callus TG	Hair follicles, epidermis, and brain; Cytosolic	Harden hair follicle, cell envelope formation during differentiation of keratinocytes	Impaired hair development and epidermal diseases
TG4	Prostate TG, androgen-regulated major secretory protein, dorsal prostate protein 1, vesiculase	Prostate gland, prostatic fluids; Extracellular	Reproduction and fertility	Possible link to aggressive prostate cancer phenotype[76]
TG5	TGX	Foreskin keratinocytes, epithelial barrier lining, skeletal muscle; Cytosolic	Cell envelope formation in keratinocyte differentiation	Ichthyosis and psoriasis
TG6	TGY	Human testes and lungs, and in the brain of mice	Not clearly defined	Not known
TG7	TGZ	Ubiquitous but predominately in testes and lungs	Not clearly defined	Not known
FXIIIA	Plasma TG, fibrin-stabilizing factor, Laki–Lorand factor, fibrinoligase	Platelets, astrocytes, macrophages, dermal dendritic cells, placenta, chondrocytes, synovial fluid, the heart, the eyes, osteoclast	Blood coagulation, wound healing, inflammation and bone synthesis	Clotting defect, impaired wound healing, miscarriage and tissue remodeling defects
Band 4.2	Erythrocyte membrane protein band 4.2	Erythrocytes, bone marrow, fetal liver, and spleen; Plasma membrane	Membrane integrity, cell attachment, signal transduction	Spherocytosis and altered ion transport in red blood cells

SERT Regulation. SERT is abundant in the plasma membrane of circulating platelets in addition to being located in the plasma membrane of serotonergic neurons in the brain, where it is a target of the selective serotonin reuptake inhibitors (SSRI) class of antidepressant drugs. The small G protein Rab4 plays a role in regulating the amount of SERT located on the cell surface of platelets.[28] Large increases in plasma 5-HT concentrations increase the activation of Rab4 and cause the sequestration of SERT in the cytoplasm of platelets.[29] The data from two studies are consistent with the transamidation of Rab4 by 5-HT being involved in the activation of Rab4.[28,29] However, further investigation is needed to demonstrate that the association of 5-HT and Rab4 is dependent on TG-catalyzed transamidation, thereby demonstrating serotonylation. The mechanisms of regulation of SERT by 5-HT plasma levels are relevant to human disease, as elevated concentrations of 5-HT are found in a subset of patients with cardiovascular disorders and can be associated with coronary artery disease and cardiac events.[29]

Insulin Release. Insulin release from pancreatic β-cells is dependent on serotonylation of two small G proteins: Rab3a and Rab27a.[21] Serotonylation of Rab3a and Rab27a was demonstrated by incorporation of [^{3}H]5-HT into these small G proteins, which could be prevented by the TG inhibitor and competitive substrate cystamine. 5-HT is stored and released along with insulin from granules in pancreatic β-cells. Peripheral tryptophan hydroxylase 1 (Tph1) knockout mice lack peripheral 5-HT since Tph1 is a necessary enzyme for the synthesis of 5-HT. Tph1 knockout mice are diabetic and are impaired in the release of insulin.[21] Similarly, TG2 knockout mice are glucose-intolerant and have impaired glucose-stimulated insulin release.[30,31] Furthermore, missense mutations in human TG2 that reduce the transamidation function are associated with early onset diabetes.[31] These studies examining human polymorphisms and using knockout mice support an important role for serotonylation in the release and control of insulin.

Smooth Muscle Contraction. The involvement of receptor-independent serotonylation of proteins in contraction was demonstrated in rat aortic cells.[22] The major targets for serotonylation in aortic smooth muscle cells are proteins involved in contraction, including α-actin, β-actin, γ-actin, myosin heavy chain, and the actin-binding protein filamin A. Inhibition of TG-catalyzed serotonylation with cystamine reduced 5-HT-stimulated contraction of thoracic aorta. Serotonylation of actin was also demonstrated in other smooth muscle cells, specifically the stomach fundus and intestine. NE monoaminylation of proteins was suggested by the colocalization of NE and TG2 with α-actin fibers and the presence of NE−BSA antibody labeling of several protein bands in western blots prepared with homogenates of aorta and vena cava.[26] Inhibition of TG activity by cystamine reduced NE-induced contraction in the aorta and vena cava and produced a modest inhibition of KCl-induced contractions.[26] Further experiments are needed to directly demonstrate monoaminylation with NE in vascular smooth muscle cells.

Pulmonary Hypertension. Serotonylation of fibronectin and the small G protein Rho are elevated in pulmonary hypertension patients and experimental animal models of hypertension.[25] 5-HT has been known to play an important role in the pathophysiology of pulmonary hypertension. Indeed, SERT knockout mice do not develop pulmonary hypertension when given treatments, such as hypoxia, that would otherwise

DOI: 10.1021/cn500329r
ACS Chem. Neurosci. 2015, 6, 961−969

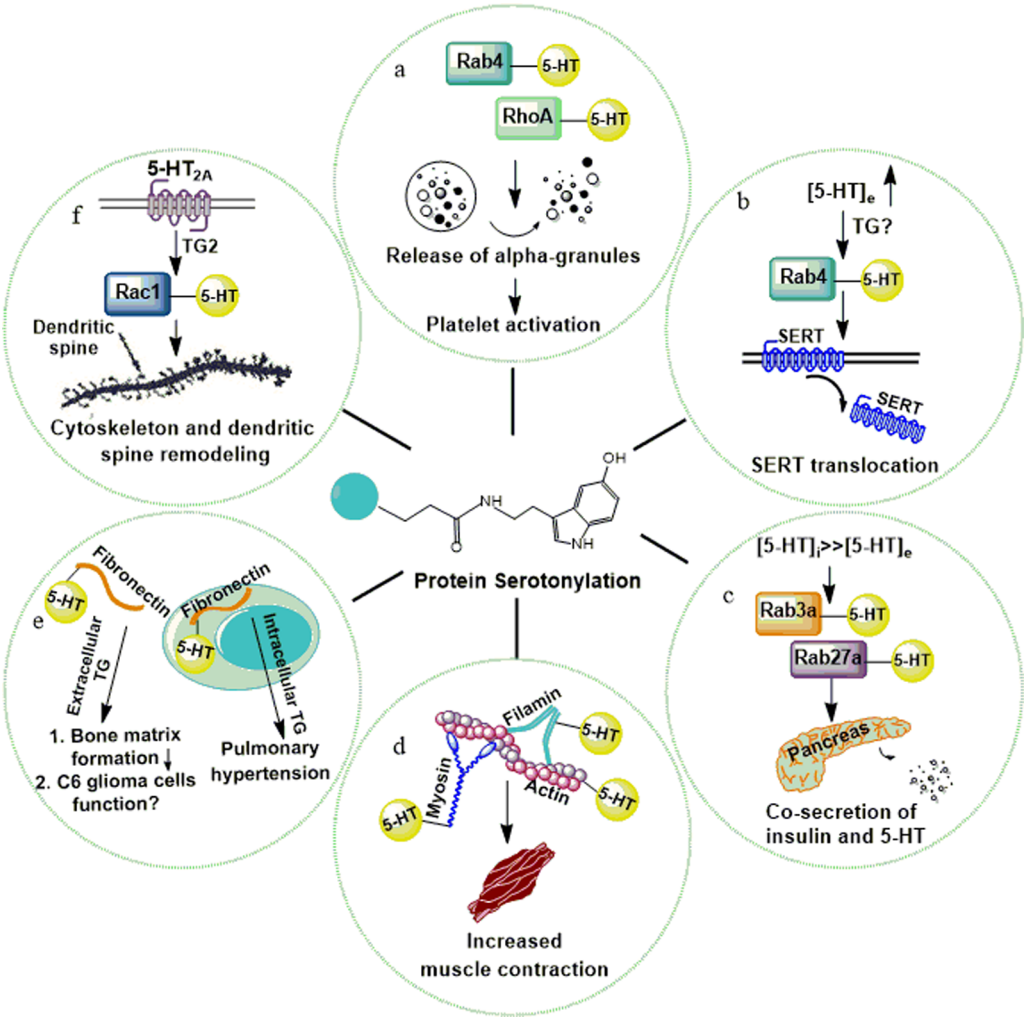


Figure 2. Serotonylation and monoaminylation are involved in the regulation of diverse physiological functions. (a) Serotonylation of the small G proteins RhoA and Rab4 in platelet cells activates the small G proteins and promotes the exocytosis of α-granules. (b) High extracellular 5-HT leads to covalent attachment of 5-HT to Rab4, rendering Rab4 constitutively active and causing the sequestration of SERT into the cytoplasm. (c) 5-HT regulates insulin secretion by serotonylation of the small G proteins Rab3a and Rab27a within pancreatic β-cells. (d) α-Actin, β-actin, γ-actin, myosin, and filamin A are serotonylated in aortic smooth muscle cells, enhancing arterial muscle contraction. (e) Extracellular matrix proteins such as fibronectin are monoaminylated by 5-HT, DA, and NA in C6 glioma cells. Serotonylation of intracellular fibronectin also plays a role in the etiology of primary pulmonary hypertension. (f) Serotonylation and activation of the small G protein Rac1 increases following 5-HT_{2A} receptor stimulation in neurons.

result pulmonary hypertension. Conversely, mice that overexpress SERT in the smooth muscle of the pulmonary and systemic vessels develop pulmonary hypertension by 8 weeks of age, and exposure to chronic hypoxia or treatment with monocrotaline-pyrrole, which induces hypertension, results in more severe pulmonary hypertension than that in wild-type mice.[33] 5-HT receptor signaling through 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{2A} is thought to contribute to pulmonary hypertension.[25] Moreover, inhibition of TG inhibited smooth muscle proliferation and migration responses to 5-HT, supporting serotonylation as being an important mediator of the role of 5-HT in pulmonary hypertension.[18,25]

Bone Extracellular Matrix. 5-HT has recently been found to be an important regulator of bone mass. Serotonylation of fibronectin in the extracellular matrix of osteoblast cultures inhibits stabilization of the extracellular matrix.[16] FXIIIA cross-links fibronectin in the extracellular matrix and thereby plays an important role in stabilizing collagen matrix. Serotonylation inhibits the protein cross-linking, acting as a competitive substrate, which could lead to weakening of the bone. Patients treated long term with SSRIs have an increased risk of bone fractures, perhaps due to the increased availability of 5-HT as a substrate for transamidation to fibronectin outcompeting the cross-linking function of FXIIIA.

Extracellular and Glial Membrane Proteins. Similar to the serotonylation of cell surface proteins on platelets, serotonylation of cell surface proteins and extracellular proteins was demonstrated in C6 glioma cells.[8] The TG2-dependent incorporation of [^{3}H]5-HT and the autofluorescent synthetic monoamine monodansylcadaverine (MDC) were used to demonstrate serotonylation and possible monoaminylation in cell membrane proteins. A direct demonstration of the incorporation of other monoamines into fibronectin used [^{3}H]DA and [^{3}H]NA.[24] Not only were [^{3}H]DA and [^{3}H]NA incorporated into fibronectin in a TG-dependent manner but also there was competition between these monoamines for incorporation into fibronectin. The maximal amount of incorporation of [^{3}H]DA, [3H]NA, and [^{3}H]5-HT into

DOI: 10.1021/cn500329r
ACS Chem. Neurosci. 2015, 6, 961−969

fibronectin was similar, suggesting that the same glutamine sites are modified by each monoamine. However, the affinities for incorporation varied, with NA having the highest affinity (154 nM), followed by DA (448 nM) and then 5-HT (749 nM). The functional significance of the monoaminylation of fibronectin is unclear. However, the authors noted an increase in extracellular protein aggregation and accumulation with monoaminylation. This increase was not due to TG-catalyzed protein cross-linking alone since the addition of both TG and 5-HT resulted in a significantly greater effect than that from the addition of TG alone (ref 32 and personal communication, P. Schloss, Heidelberg University, Germany).

Neuronal Serotonylation. The small G protein Rac1 can be serotonylated in neuronal cells following stimulation of 5-HT_{2A} receptors.[9] Serotonylation of Rac1 results in its activation. 5-HT_{2A} receptors couple to the Gq/11 family of Gα proteins, resulting in activation of phospholipase Cβ, the production of inositol 1,4,5-triphosphate (IP3), and the release of calcium from the endoplasmic reticulum via the activation of IP3 receptors. Serotonylation of Rac1 following stimulation of 5-HT_{2A} receptors is dependent on phospholipase C and the subsequent increase in intracellular calcium.[7] An increase in intracellular calcium is not only necessary but also sufficient to induce the transamidation of Rac1,[7] suggesting that other mechanisms resulting in an increase in intracellular calcium may activate TGs to induce monoaminylation. The transamidating action of TG2 requires not only high calcium concentrations but also low GTP concentrations beyond the normal intracellular concentrations. Stimulation of G protein coupled receptors coupled to the Gq/11 family of Gα proteins increases intracellular calcium concentrations by producing IP3, which stimulates IP3 receptors to release calcium from the endoplasmic reticulum to increase intracellular concentration in a microdomain in the cell. Furthermore, activated Gα proteins bind GTP, perhaps lowering the GTP concentration to further contribute to the activation of TG's transamidating function in the microdomain. Further experiments are necessary to determine whether activation of other Gq/11 coupled receptors increases monoaminylation of small G proteins and results in their activation.

We recently found that the Rac1 Q61N mutant cannot be transamidated by activation via 5-HT_{2A} receptor stimulation and is constitutively active (unpublished results). Deamidation or transamidation of Rac1 and Cdc42 at Q61 and RhoA at Q63 prevents intrinsic and GAP-catalyzed hydrolysis of the small G proteins, rendering them constitutively active.[34−36] Taken together, these results suggest that serotonylation of small G proteins such as Rac1, Cdc42, and others increases their activation by inhibiting GTP hydrolysis. 5-HT_{2A} receptor stimulation produces a transient increase in dendritic spine size that is dependent on Pak1 activation[37] and TG activity, probably via transglutaminase-catalyzed serotonylation of Rac1 (unpublished results).

AVAILABILITY OF MONOAMINES FOR MONOAMINYLATION

In order for serotonylation or other monoaminylation reactions to occur intracellularly, a primary amine, such as 5-HT, must be present in the cell in addition to the presence of a transglutaminase enzyme. Watts and colleagues[22] demonstrated that 5-HT is present in aortic cells; 5-HT is synthesized in and can be transported into arterial smooth muscle cells by SERT.[38] SERT is present on the plasma membrane of platelets and provides 5-HT for serotonylation.[28,29] Similarly, SERT is present in the smooth muscle cells of the pulmonary and systemic vessels.[33] In the brain, SERT is present in 5-HT cells originating in the raphe nucleus and projecting throughout the adult brain. However, other transporters and other monoamines would likely be involved in postsynaptic 5-HT_{2A} receptor-dependent monoaminylation in adults. There are numerous transporters for monoamines that could contribute to the pool of substrate for monoaminylation reactions.

Monoamine Transporters. SERT, NET, and DAT (coded for by SLC6A4, SLC6A2, and SLC6A3 genes) are high-affinity, low-capacity monoamine transporters. In the brain, these transporters are expressed predominantly in neurons using these monoamines as neurotransmitters. SERT is located on the axonal plasma membrane of serotonergic neurons but not the plasma membrane of dendrites or cell bodies in adults.[39] Interestingly, SERT is not found at the synapse but is in the peri-synaptic region and along the axonal plasma membrane.[39] During development, SERT is distributed throughout the entire plasma membrane and is not limited to the axonal membrane of serotonergic neurons.[40] Moreover, SERT expression is not limited to serotonergic neurons during development but is broadly expressed in nonserotonergic neurons in the CA3 regions of the hippocampus, medial prefrontal cortex, the entire medial limbic cortex from the anterior cingulate cortex to the retrosplenial cortex, and the entire auditory pathway.[41] The high levels of expression of SERT in nonserotonergic neurons are transient, ceasing in nonraphe neurons by postnatal day 14 in mice. Relevant to 5-HT_{2A} receptor-stimulated serotonylation, 5-$HT_{2A/C}$ receptors appear to be expressed in neurons in limbic regions that express SERT including the medial prefrontal cortex, anterior cingulate cortex, and CA3 region of the hippocampus during development.[41] This transient SERT expression could supply the substrate necessary for serotonylation in neurons following stimulation of 5-HT_{2A} receptors during development. Our preliminary data suggest that 5-HT_{2A} receptor-stimulated serotonylation regulates dendritic spine size in cortical cultures (personal communication). We propose that 5-HT_{2A} receptor-mediated serotonylation is positioned to play a role in the regulation of dendritic spines during development. Depletion of serotonin using either neonatal *para*-chloroamphetamine or early postnatal treatment with 5,7-dihydroxytryptamine results in a reduction of dendritic spines in hippocampal dentate granule cells,[42] consistent with the hypothesis that serotonylation regulates dendritic spines during development. Since SERT is not expressed in 5-HT_{2A} receptor-expressing neurons in adults, intracellular 5-HT for serotonylation would have to be provided by another transporter. SSRI treatment increases the concentration of 5-HT in the synaptic region, setting the stage for low-affinity transporters to take up 5-HT.[43] Alternatively, other monoamines could be used as substrates for monoaminylation of small G proteins resulting from 5-HT_{2A} receptor activation. For example, 5-HT_{2A} receptors are expressed on DA neurons in the substantia nigra and ventral tegmentum[44,45] as well as in projection areas such as the prefrontal cortex.[46] DA would be available as a substrate in these cells for Rac1 monoaminylation and activation after 5-HT_{2A} receptor stimulation.

SERT, NET, and DAT share a high level of structural and sequence homology and functional overlap. NET and DAT are capable of uptake of NA and DA.[47,48] Similarly, SERT is capable of transporting DA and NE.[49,50] NET protein immunoreactivity is found in NA cell bodies, axons, and

DOI: 10.1021/cn500329r
ACS Chem. Neurosci. 2015, 6, 961−969

dendrites, primarily in the cytoplasm, and occasionally in the plasma membrane of axons and in synapses.[51,52] Drugs that alter NET activity such as antidepressants including desipramine and reboxetine or drugs of abuse including cocaine and amphetamines increase NA and DA in the synaptic cleft regions, providing sufficient concentrations for low-affinity transporters to act on the monoamines. DAT is located in DA cell bodies primarily in the cytoplasm and in the plasma membrane of dendrites and dendritic spines in the substantia nigra.[53,54] In the striatum, DAT is expressed in axonal plasma membranes. On dendrites, DAT immunolabeling was often found apposed to or near synapses from nonlabeled axons. In contrast, axonal labeling for DAT was primarily distant from synapses. The presence of DAT in dendrites and dendritic spines places this transporter in an optimal position to provide monoamines for monoaminylation reactions.

Organic Cation Transporters. Organic cation transporters (OCT1−3; gene symbol SLC22A1−3) are capable of high-capacity, low-affinity transport of monoamines as well as numerous physiological and xenobiotic compounds. OCTs are expressed in the kidney, liver, placenta, and brain. For a recent review of OCTs in brain, see Couroussé and Gautron.[55] In contrast, this review is limited to discussing OCTs in relation to monoaminylation. Both OCT2 and OCT3 could play a role in providing 5-HT and other monoamines for the monoaminylation reaction in 5-HT_{2A} receptor-containing neurons. OCT1 is not detected in rat brain.[56] OCT2 is expressed in neurons in the hippocampus, frontal and cingulate cortex, amygdala, dorsal raphe, and locus coeruleus.[57,58] Relevant to 5-HT_{2A}-stimulated serotonylation, OCT2 is expressed postsynaptically in dendrites and neuronal cell bodies.[58] OCT3 is the most abundant of the three OCTs in the brain.[56,59,60] With regard to supplying monoamines for monoaminylation in 5-HT_{2A} receptor-containing postsynaptic neurons, OCT3 is expressed in most monoamine projection areas, predominantly in neuronal cell bodies and proximal processes but not dendrites.[59,60]

Pharmacological OCT inhibitors increase extracellular concentrations of monoamines used alone and in conjunction with high-affinity transporter inhibition in various brain regions tested: frontal cortex, hypothalamus, and nucleus accumbens.[61−63] OCT3 expression and function is increased in $SERT^{+/-}$ knockout mice.[63] Neuroactive steroids have differential inhibitory effects on the OCTs; corticosterone, deoxycorticosterone, and progesterone inhibit OCT2 and 3 with an IC_{50} in the low micromolar range (10.5−1.1), whereas estradiol inhibits OCT3 with an IC_{50} of 1.1 μM but OCT2 with an IC_{50} of 85 μM.[64] Since corticosterone is a selective antagonist for OCT3 (not SERT or PMAT), Daws and Gould[65] hypothesized that early life stress could result in corticosterone inhibition of OCT3 during development, especially in the face of low expression of SERT, such as in SERT knockout mice or humans carrying the low-expressing S allele of SERT. Although Daws and Gould interpreted the transporter problem to be related to high extracellular levels of 5-HT, it is equally plausible that low intracellular levels of 5-HT or other monoamines could be problematic. In light of the role of intracellular 5-HT in serotonylation and dendritic spine regulation, low levels of intracellular 5-HT could play an important role in long lasting perturbations in serotonergic function.

OCT3 knockout mice have reduced levels of dopamine in all brain regions examined, minimal differences in histamine and 5-HT levels, and no differences in NA.[59] Limited behavioral changes were found in the OCT3 knockout mice, suggesting a possible increase in anxiety consistent with alterations in dopamine levels. Whether the knockout of OCT3 alters intracellular availability of monoamines for monoaminylation has not been explored.

OCT2 knockout mice have reduced levels of 5-HT in the hippocampus, hypothalamus, and striatum and reduced NE in the cortex, striatum, hippocampus, brainstem, and cerebellum.[66] The knockout mice had increased sensitivity to low-dose acute treatment with NET and SERT-selective inhibitors reboxetine and citalopram. Chronic administration of corticosterone was used to model depression and compare the OCT2 knockout mice to wild-type mice in the development of depressive-like behavior and response to treatment. The OCT2 knockout mice developed depressive-like behaviors similar to those of wild-type mice but did not demonstrate improved behavior with long-term treatment with the NET inhibitor venlafaxine.[66] The study's authors suggested that this demonstrates that OCT2 is necessary for the rescue of corticosterone-induced depression-like behavior by the NET inhibitor. Inhibition of SERT and or NET could increase the concentration of monoamines to a level sufficient to engage the low-affinity OCT2 transporters to increase the uptake of monoamines into the postsynaptic region by OCT2. In the OCT2 knockout mice, high extracellular levels of monoamines would be achieved by the NET inhibitor, but OCT2 would not be present to increase the postsynaptic intracellular levels of monoamines available for monoaminylation of small G proteins such as Rac1. Higher levels of intracellular monoamines available for monoaminylation and activation of the small G proteins may be needed for an antidepressive response to the NET inhibitor.

Plasma Membrane Monoamine Transporter. Plasma membrane monoamine transporter (PMAT, SLC29A4) is abundantly and widely expressed in the brain.[67] On the basis of its substrate specificity, it is primarily a monoamine transporter, especially for 5-HT and DA and to a lesser extent histamine and NA. PMAT is another high-capacity, low-affinity transporter for these monoamines. It is not inhibited by corticosterone as OCTs are.[68] Colocalization of PMAT and MAP2 labeling in neuronal processes indicates that dendrites express PMAT, including cells that do not synthesize monoamines.[69] PMAT is expressed in regions that receive dense monoaminergic input,[70] placing it in an ideal position to provide monoamines, especially 5-HT and dopamine, for monoaminylation following 5-HT_{2A} receptor activation.[9]

To summarize the transporter issues when considering the ability of a cell to provide monoamines for monoaminylation, it is not sufficient to match the expression of the high-affinity transporter for a particular monoamine with the monoamine of interest. It is important to consider the possibility that SERT, NET, and DAT are able to transport monoamines other than those for which they are named. The low-affinity monoamine transporters, particularly OCT2 and PMAT, appear to be strategically located and able to provide monoamines for monoaminylation in postsynaptic regions of neurons. These low-affinity, high-capacity transporters may be especially relevant in the presence of high-affinity uptake inhibitors for SERT, DAT, and NET. Indeed it is possible that one of the beneficial mechanisms by which SERT and NET inhibitors provide therapeutic effects is by increasing the concentration of synaptic monoamines available for uptake by postsynaptic low-affinity transporters, which then supply monoamines for monoaminylation.

DOI: 10.1021/cn500329r
ACS Chem. Neurosci. 2015, 6, 961−969

CONCLUSIONS

Over the past decade, the role of monoaminylation in physiological processes and disease states has begun to emerge. Serotonylation plays important roles in platelet activation[6] and regulation of membrane expression of the SERT in platelets.[28] Serotonylation is involved in insulin release[21] and as such can play a role in diabetes. Monoaminylation of cytoskeletal proteins is involved in smooth muscle contraction,[22] whereas serotonylation of fibronectin and small G proteins in smooth muscles in pulmonary vessels has been shown to be involved in pulmonary hypertension.[25] Similarly, fibronectin and small G proteins are monoaminylated in glia cells[8] and neurons,[9] respectively. The consequences of monoaminylation in these cells in the nervous system are less apparent, although monoaminylation of small G proteins in neurons appears to regulate dendritic spine morphology. Modulation of dendritic spines is an important factor in synaptic plasticity and is abnormal in psychiatric disorders including depression and schizophrenia.[71−73] Drugs currently used to treat these psychiatric disorders target the high-affinity transporters that can supply monoamines for monoaminylation. Taken together, monoaminylation is a novel drug target for a range of disorders from depression and schizophrenia to diabetes and hypertension. Clearly, further studies are needed to elucidate the roles of monoaminylation in normal physiology and pathophysiology.

AUTHOR INFORMATION

Corresponding Author

*E-mail: nmuma@ku.edu.

Notes

The authors declare no competing financial interest.

DEFINITIONS

Transamidation, the formation of a covalent bond between an amino group of a primary amine or the ε-amine group of a peptide-bound lysine residue and the γ-carboxamide group of a peptide-bound glutamine residue; Serotonylation, the transamidation of serotonin to a glutamine residue of a protein catalyzed by the enzyme transglutaminase; Monoaminylation, the transamidation of a monoamine to a glutamine residue of a protein catalyzed by the enzyme transglutaminase; Transglutaminase, a family of enzymes that catalyze the formation of an isopeptide bond between free amines and the acyl side chain of a peptide or protein bound glutamine; Serotonin transporter, a membrane-bound transporter protein that shuttles serotonin into cells with high affinity but low capacity; Organic cation transporter, a membrane-bound transporter protein capable of high-capacity low-affinity transport of monoamines; Plasma membrane monoamine transporter, a membrane-bound transporter protein capable of low-affinity high-capacity transport of monoamines, especially serotonin and dopamine

REFERENCES

(1) Rapport, M. M., Green, A. A., and Page, I. H. (1948) Serum vasoconstrictor, serotonin; isolation and characterization. *J. Biol. Chem. 176*, 1243−1251.

(2) Erspamer, V., and Ottolenghi, A. (1950) Antidiuretic action of enteramine. *Experientia 6*, 428.

(3) Twarog, B. M., and Page, I. H. (1953) Serotonin content of some mammalian tissues and urine and a method for its determination. *Am. J. Physiol. 175*, 157−161.

(4) Anden, N. E., Dahlstrom, A., Fuxe, K., Olson, L., and Ungerstedt, U. (1966) Ascending noradrenaline neurons from the pons and the medulla oblongata. *Experientia 22*, 44−45.

(5) Dahlstrom, A., and Fuxe, K. (1964) Localization of monoamines in the lower brain stem. *Experientia 20*, 398−399.

(6) Walther, D. J., Peter, J. U., Winter, S., Holtje, M., Paulmann, N., Grohmann, M., Vowinckel, J., Alamo-Bethencourt, V., Wilhelm, C. S., Ahnert-Hilger, G., and Bader, M. (2003) Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. *Cell 115*, 851−862.

(7) Dai, Y., Dudek, N. L., Li, Q., and Muma, N. A. (2011) Phospholipase C, Ca^{2+}, and calmodulin signaling are required for 5-HT2A receptor-mediated transamidation of Rac1 by transglutaminase. *Psychopharmacology 213*, 403−412.

(8) Hummerich, R., and Schloss, P. (2010) Serotonin—more than a neurotransmitter: transglutaminase-mediated serotonylation of C6 glioma cells and fibronectin. *Neurochem. Int. 57*, 67−75.

(9) Dai, Y., Dudek, N. L., Patel, T. B., and Muma, N. A. (2008) Transglutaminase-catalyzed transamidation: a novel mechanism for Rac1 activation by 5-hydroxytryptamine2A receptor stimulation. *J. Pharmacol. Exp. Ther. 326*, 153−162.

(10) Walther, D. J., Stahlberg, S., and Vowinckel, J. (2011) Novel roles for biogenic monoamines: from monoamines in transglutaminase-mediated post-translational protein modification to monoaminylation deregulation diseases. *FEBS J. 278*, 4740−4755.

(11) Mycek, M. J., Clarke, D. D., Neidle, A., and Waelsch, H. (1959) Amine incorporation into insulin as catalyzed by transglutaminase. *Arch. Biochem. Biophys. 84*, 528−540.

(12) Eckert, R. L., Kaartinen, M. T., Nurminskaya, M., Belkin, A. M., Colak, G., Johnson, G. V., and Mehta, K. (2014) Transglutaminase regulation of cell function. *Physiol. Rev. 94*, 383−417.

(13) Mycek, M. J., and Waelsch, H. (1960) The enzymatic deamidation of proteins. *J. Biol. Chem. 235*, 3513−3517.

(14) Vowinckel, J., Stahlberg, S., Paulmann, N., Bluemlein, K., Grohmann, M., Ralser, M., and Walther, D. J. (2012) Histaminylation of glutamine residues is a novel posttranslational modification implicated in G-protein signaling. *FEBS Lett. 586*, 3819−3824.

(15) Johnson, K. B., Petersen-Jones, H., Thompson, J. M., Hitomi, K., Itoh, M., Bakker, E. N., Johnson, G. V., Colak, G., and Watts, S. W. (2012) Vena cava and aortic smooth muscle cells express transglutaminases 1 and 4 in addition to transglutaminase 2. *Am. J. Physiol.: Heart Circ. Physiol. 302*, H1355−1366.

(16) Cui, C., and Kaartinen, M. T. (2015) Serotonin (5-HT) inhibits Factor XIII-A-mediated plasma fibronectin matrix assembly and crosslinking in osteoblast cultures via direct competition with transamidation. *Bone 72*, 43−52.

(17) Lin, J. C., Chou, C. C., Gao, S., Wu, S. C., Khoo, K. H., and Lin, C. H. (2013) An *in vivo* tagging method reveals that Ras undergoes sustained activation upon transglutaminase-mediated protein serotonylation. *ChemBioChem 14*, 813−817.

(18) Liu, Y., Wei, L., Laskin, D. L., and Fanburg, B. L. (2011) Role of protein transamidation in serotonin-induced proliferation and migration of pulmonary artery smooth muscle cells. *Am. J. Respir. Cell Mol. Biol. 44*, 548−555.

(19) Wei, L., Warburton, R. R., Preston, I. R., Roberts, K. E., Comhair, S. A., Erzurum, S. C., Hill, N. S., and Fanburg, B. L. (2012) Serotonylated fibronectin is elevated in pulmonary hypertension. *Am. J. Physiol.: Lung Cell. Mol. Physiol. 302*, L1273−L1279.

(20) Guilluy, C., Eddahibi, S., Agard, C., Guignabert, C., Izikki, M., Tu, L., Savale, L., Humbert, M., Fadel, E., Adnot, S., Loirand, G., and Pacaud, P. (2009) RhoA and Rho kinase activation in human pulmonary hypertension: role of 5-HT signaling. *Am. J. Respir. Crit. Care Med. 179*, 1151−1158.

(21) Paulmann, N., Grohmann, M., Voigt, J. P., Bert, B., Vowinckel, J., Bader, M., Skelin, M., Jevsek, M., Fink, H., Rupnik, M., and Walther, D. J. (2009) Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation. *PLoS Biol. 7*, e1000229.

DOI: 10.1021/cn500329r
ACS Chem. Neurosci. 2015, 6, 961−969

(22) Watts, S. W., Priestley, J. R., and Thompson, J. M. (2009) Serotonylation of vascular proteins important to contraction. *PLoS One 4*, e5682.
(23) Lin, J. C., Chou, C. C., Tu, Z., Yeh, L. F., Wu, S. C., Khoo, K. H., and Lin, C. H. (2014) Characterization of protein serotonylation via bioorthogonal labeling and enrichment. *J. Proteome Res. 13*, 3523−3529.
(24) Hummerich, R., Thumfart, J. O., Findeisen, P., Bartsch, D., and Schloss, P. (2012) Transglutaminase-mediated transamidation of serotonin, dopamine and noradrenaline to fibronectin: evidence for a general mechanism of monoaminylation. *FEBS Lett. 586*, 3421−3428.
(25) Penumatsa, K., Abualkhair, S., Wei, L., Warburton, R., Preston, I., Hill, N. S., Watts, S. W., Fanburg, B. L., and Toksoz, D. (2014) Tissue transglutaminase promotes serotonin-induced AKT signaling and mitogenesis in pulmonary vascular smooth muscle cells. *Cell. Signalling 26*, 2818−2825.
(26) Johnson, K. B., Thompson, J. M., and Watts, S. W. (2010) Modification of proteins by norepinephrine is important for vascular contraction. *Front. Physiol. 1*, 131.
(27) Dale, G. L., Friese, P., Batar, P., Hamilton, S. F., Reed, G. L., Jackson, K. W., Clemetson, K. J., and Alberio, L. (2002) Stimulated platelets use serotonin to enhance their retention of procoagulant proteins on the cell surface. *Nature 415*, 175−179.
(28) Ahmed, B. A., Bukhari, I. A., Jeffus, B. C., Harney, J. T., Thyparambil, S., Ziu, E., Fraer, M., Rusch, N. J., Zimniak, P., Lupashin, V., Tang, D., and Kilic, F. (2009) The cellular distribution of serotonin transporter is impeded on serotonin-altered vimentin network. *PLoS One 4*, e4730.
(29) Ziu, E., Mercado, C. P., Li, Y., Singh, P., Ahmed, B. A., Freyaldenhoven, S., Lensing, S., Ware, J., and Kilic, F. (2012) Down-regulation of the serotonin transporter in hyperreactive platelets counteracts the pro-thrombotic effect of serotonin. *J. Mol. Cell. Cardiol. 52*, 1112−1121.
(30) Bernassola, F., Federici, M., Corazzari, M., Terrinoni, A., Hribal, M. L., De Laurenzi, V., Ranalli, M., Massa, O., Sesti, G., McLean, W. H., Citro, G., Barbetti, F., and Melino, G. (2002) Role of transglutaminase 2 in glucose tolerance: knockout mice studies and a putative mutation in a MODY patient. *FASEB J. 16*, 1371−1378.
(31) Salter, N. W., Ande, S. R., Nguyen, H. K., Nyomba, B. L., and Mishra, S. (2012) Functional characterization of naturally occurring transglutaminase 2 mutants implicated in early-onset type 2 diabetes. *J. Mol. Endocrinol. 48*, 203−216.
(32) Hummerich, R. Ph.D. Thesis, Technical University Darmstadt, Germany, 2011.
(33) Guignabert, C., Izikki, M., Tu, L. I., Li, Z., Zadigue, P., Barlier-Mur, A. M., Hanoun, N., Rodman, D., Hamon, M., Adnot, S., and Eddahibi, S. (2006) Transgenic mice overexpressing the 5-hydroxy-tryptamine transporter gene in smooth muscle develop pulmonary hypertension. *Circ. Res. 98*, 1323−1330.
(34) Lerm, M., Selzer, J., Hoffmeyer, A., Rapp, U. R., Aktories, K., and Schmidt, G. (1999) Deamidation of Cdc42 and Rac by *Escherichia coli* cytotoxic necrotizing factor 1: activation of c-Jun N-terminal kinase in HeLa cells. *Infect. Immun. 67*, 496−503.
(35) Flatau, G., Landraud, L., Boquet, P., Bruzzone, M., and Munro, P. (2000) Deamidation of RhoA glutamine 63 by the *Escherichia coli* CNF1 toxin requires a short sequence of the GTPase switch 2 domain. *Biochem. Biophys. Res. Commun. 267*, 588−592.
(36) Schmidt, G., Goehring, U. M., Schirmer, J., Lerm, M., and Aktories, K. (1999) Identification of the C-terminal part of *Bordetella dermonecrotic* toxin as a transglutaminase for rho GTPases. *J. Biol. Chem. 274*, 31875−31881.
(37) Jones, K. A., Srivastava, D. P., Allen, J. A., Strachan, R. T., Roth, B. L., and Penzes, P. (2009) Rapid modulation of spine morphology by the 5-HT2A serotonin receptor through kalirin-7 signaling. *Proc. Natl. Acad. Sci. U.S.A. 106*, 19575−19580.
(38) Ni, W., Geddes, T. J., Priestley, J. R., Szasz, T., Kuhn, D. M., and Watts, S. W. (2008) The existence of a local 5-hydroxytryptaminergic system in peripheral arteries. *Br. J. Pharmacol. 154*, 663−674.
(39) Tao-Cheng, J. H., and Zhou, F. C. (1999) Differential polarization of serotonin transporters in axons versus soma-dendrites: an immunogold electron microscopy study. *Neuroscience 94*, 821−830.
(40) Zhou, F. C., Sari, Y., and Zhang, J. K. (2000) Expression of serotonin transporter protein in developing rat brain. *Dev. Brain Res. 119*, 33−45.
(41) Narboux-Neme, N., Pavone, L. M., Avallone, L., Zhuang, X., and Gaspar, P. (2008) Serotonin transporter transgenic (SERTcre) mouse line reveals developmental targets of serotonin specific reuptake inhibitors (SSRIs). *Neuropharmacology 55*, 994−1005.
(42) Yan, W., Wilson, C. C., and Haring, J. H. (1997) Effects of neonatal serotonin depletion on the development of rat dentate granule cells. *Dev. Brain Res. 98*, 177−184.
(43) Daws, L. C., Koek, W., and Mitchell, N. C. (2013) Revisiting serotonin reuptake inhibitors and the therapeutic potential of "uptake-2" in psychiatric disorders. *ACS Chem. Neurosci. 4*, 16−21.
(44) Ikemoto, K., Nishimura, A., Okado, N., Mikuni, M., Nishi, K., and Nagatsu, I. (2000) Human midbrain dopamine neurons express serotonin 2A receptor: an immunohistochemical demonstration. *Brain Res. 853*, 377−380.
(45) Nocjar, C., Roth, B. L., and Pehek, E. A. (2002) Localization of 5-HT(2A) receptors on dopamine cells in subnuclei of the midbrain A10 cell group. *Neuroscience 111*, 163−176.
(46) Li, Y., Huang, X. F., Deng, C., Meyer, B., Wu, A., Yu, Y., Ying, W., Yang, G. Y., Yenari, M. A., and Wang, Q. (2010) Alterations in 5-HT2A receptor binding in various brain regions among 6-hydroxydopamine-induced Parkinsonian rats. *Synapse 64*, 224−230.
(47) Moron, J. A., Brockington, A., Wise, R. A., Rocha, B. A., and Hope, B. T. (2002) Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. *J. Neurosci. 22*, 389−395.
(48) Carboni, E., Tanda, G. L., Frau, R., and Di Chiara, G. (1990) Blockade of the noradrenaline carrier increases extracellular dopamine concentrations in the prefrontal cortex: evidence that dopamine is taken up *in vivo* by noradrenergic terminals. *J. Neurochem. 55*, 1067−1070.
(49) Larsen, M. B., Sonders, M. S., Mortensen, O. V., Larson, G. A., Zahniser, N. R., and Amara, S. G. (2011) Dopamine transport by the serotonin transporter: a mechanistically distinct mode of substrate translocation. *J. Neurosci. 31*, 6605−6615.
(50) Vizi, E. S., Zsilla, G., Caron, M. G., and Kiss, J. P. (2004) Uptake and release of norepinephrine by serotonergic terminals in norepinephrine transporter knock-out mice: implications for the action of selective serotonin reuptake inhibitors. *J. Neurosci. 24*, 7888−7894.
(51) Schroeter, S., Apparsundaram, S., Wiley, R. G., Miner, L. H., Sesack, S. R., and Blakely, R. D. (2000) Immunolocalization of the cocaine- and antidepressant-sensitive l-norepinephrine transporter. *J. Comp. Neurol. 420*, 211−232.
(52) Miner, L. H., Schroeter, S., Blakely, R. D., and Sesack, S. R. (2003) Ultrastructural localization of the norepinephrine transporter in superficial and deep layers of the rat prelimbic prefrontal cortex and its spatial relationship to probable dopamine terminals. *J. Comp. Neurol. 466*, 478−494.
(53) Nirenberg, M. J., Vaughan, R. A., Uhl, G. R., Kuhar, M. J., and Pickel, V. M. (1996) The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. *J. Neurosci. 16*, 436−447.
(54) Hersch, S. M., Yi, H., Heilman, C. J., Edwards, R. H., and Levey, A. I. (1997) Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra. *J. Comp. Neurol. 388*, 211−227.
(55) Couroussé, T., and Gautron, S. (2015) Role of organic cation transporters (OCTs) in the brain. *Pharmacol. Ther. 146*, 94−103.
(56) Amphoux, A., Vialou, V., Drescher, E., Bruss, M., Mannoury La Cour, C., Rochat, C., Millan, M. J., Giros, B., Bonisch, H., and Gautron, S. (2006) Differential pharmacological *in vitro* properties of

DOI: 10.1021/cn500329r
ACS Chem. Neurosci. 2015, 6, 961−969

organic cation transporters and regional distribution in rat brain. *Neuropharmacology 50*, 941−952.
(57) Busch, A. E., Karbach, U., Miska, D., Gorboulev, V., Akhoundova, A., Volk, C., Arndt, P., Ulzheimer, J. C., Sonders, M. S., Baumann, C., Waldegger, S., Lang, F., and Koepsell, H. (1998) Human neurons express the polyspecific cation transporter hOCT2, which translocates monoamine neurotransmitters, amantadine, and memantine. *Mol. Pharmacol. 54*, 342−352.
(58) Bacq, A., Balasse, L., Biala, G., Guiard, B., Gardier, A. M., Schinkel, A., Louis, F., Vialou, V., Martres, M. P., Chevarin, C., Hamon, M., Giros, B., and Gautron, S. (2012) Organic cation transporter 2 controls brain norepinephrine and serotonin clearance and antidepressant response. *Mol. Psychiatry 17*, 926−939.
(59) Vialou, V., Balasse, L., Callebert, J., Launay, J. M., Giros, B., and Gautron, S. (2008) Altered aminergic neurotransmission in the brain of organic cation transporter 3-deficient mice. *J. Neurochem. 106*, 1471−1482.
(60) Gasser, P. J., Orchinik, M., Raju, I., and Lowry, C. A. (2009) Distribution of organic cation transporter 3, a corticosterone-sensitive monoamine transporter, in the rat brain. *J. Comp. Neurol. 512*, 529−555.
(61) Rahman, Z., Ring, R. H., Young, K., Platt, B., Lin, Q., Schechter, L. E., Rosenzweig-Lipson, S., and Beyer, C. E. (2008) Inhibition of uptake 2 (or extraneuronal monoamine transporter) by normetanephrine potentiates the neurochemical effects of venlafaxine. *Brain Res. 1203*, 68−78.
(62) Feng, N., Lowry, C. A., Lukkes, J. L., Orchinik, M., Forster, G. L., and Renner, K. J. (2010) Organic cation transporter inhibition increases medial hypothalamic serotonin under basal conditions and during mild restraint. *Brain Res. 1326*, 105−113.
(63) Baganz, N. L., Horton, R. E., Calderon, A. S., Owens, W. A., Munn, J. L., Watts, L. T., Koldzic-Zivanovic, N., Jeske, N. A., Koek, W., Toney, G. M., and Daws, L. C. (2008) Organic cation transporter 3: keeping the brake on extracellular serotonin in serotonin-transporter-deficient mice. *Proc. Natl. Acad. Sci. U.S.A. 105*, 18976−18981.
(64) Wu, X., Kekuda, R., Huang, W., Fei, Y. J., Leibach, F. H., Chen, J., Conway, S. J., and Ganapathy, V. (1998) Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake2) and evidence for the expression of the transporter in the brain. *J. Biol. Chem. 273*, 32776−32786.
(65) Daws, L. C., and Gould, G. G. (2011) Ontogeny and regulation of the serotonin transporter: providing insights into human disorders. *Pharmacol. Ther. 131*, 61−79.
(66) Bacq, A., Balasse, L., Biala, G., Guiard, B., Gardier, A. M., Schinkel, A., Louis, F., Vialou, V., Martres, M. P., Chevarin, C., Hamon, M., Giros, B., and Gautron, S. (2012) Organic cation transporter 2 controls brain norepinephrine and serotonin clearance and antidepressant response. *Mol. Psychiatry 17*, 926−939.
(67) Engel, K., Zhou, M., and Wang, J. (2004) Identification and characterization of a novel monoamine transporter in the human brain. *J. Biol. Chem. 279*, 50042−50049.
(68) Engel, K., and Wang, J. (2005) Interaction of organic cations with a newly identified plasma membrane monoamine transporter. *Mol. Pharmacol. 68*, 1397−1407.
(69) Dahlin, A., Xia, L., Kong, W., Hevner, R., and Wang, J. (2007) Expression and immunolocalization of the plasma membrane monoamine transporter in the brain. *Neuroscience 146*, 1193−1211.
(70) Vialou, V., Balasse, L., Dumas, S., Giros, B., and Gautron, S. (2007) Neurochemical characterization of pathways expressing plasma membrane monoamine transporter in the rat brain. *Neuroscience 144*, 616−622.
(71) Ota, K. T., and Duman, R. S. (2012) Environmental and pharmacological modulations of cellular plasticity: role in the pathophysiology and treatment of depression. *Neurobiol. Dis. 57*, 28−37.
(72) Roberts, R. C., Roche, J. K., and Conley, R. R. (2005) Synaptic differences in the patch matrix compartments of subjects with schizophrenia: a postmortem ultrastructural study of the striatum. *Neurobiol. Dis. 20*, 324−335.
(73) Glantz, L. A., and Lewis, D. A. (2000) Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch. Gen. Psychiatry 57*, 65−73.
(74) Lorand, L., and Graham, R. M. (2003) Transglutaminases: crosslinking enzymes with pleiotropic functions. *Nat. Rev. Mol. Cell Biol. 4*, 140−156.
(75) Odii, B. O., and Coussons, P. (2014) Biological functionalities of transglutaminase 2 and the possibility of its compensation by other members of the transglutaminase family. *Sci. World J. 2014*, 714561.
(76) Cao, Z., Wang, Y., Liu, Z. Y., Zhang, Z. S., Ren, S. C., Yu, Y. W., Qiao, M., Zhai, B. B., and Sun, Y. H. (2013) Overexpression of transglutaminase 4 and prostate cancer progression: a potential predictor of less favourable outcomes. *Asian J. Androl. 15*, 742−746.

DOI: 10.1021/cn500329r
ACS Chem. Neurosci. 2015, 6, 961−969