Brain Plasma Membrane Monoamine Transporter in Health and Disease

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Contents

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Abstract

Precise control of monoamine neurotransmitter levels in the central nervous system (CNS) is crucial for proper brain function. Dysfunctional monoamine signaling is associated with several neuropsychiatric and neurodegenerative disorders. The plasma membrane monoamine transporter (PMAT) is a new polyspecific organic cation transporter encoded by the *SLC29A4* gene. Capable of transporting monoamine neurotransmitters with low affinity and high capacity, PMAT represents a major uptake$_2$ transporter in the brain. Broadly expressed in multiple brain regions, PMAT can complement the high-affinity, low-capacity monoamine uptake mediated by uptake$_1$ transporters, the serotonin, dopamine, and norepinephrine transporters (SERT, DAT, and NET, respectively). This chapter provides an overview of the molecular and functional characteristics of PMAT together with its regional and cell-type specific expression in the mammalian brain. The physiological functions of PMAT in brain monoamine homeostasis are evaluated in light of its unique transport kinetics and brain location, and in comparison with uptake$_1$ and other uptake$_2$ transporters (e.g., OCT3) along with corroborating experimental evidences. Lastly, the possibility of PMAT's involvement in brain pathophysiological processes, such as autism, depression, and Parkinson's disease, is discussed in the context of disease pathology and potential link to aberrant monoamine pathways.

Keywords

5-HT · Autism · Brain · Catecholamine · Choroid plexus · Depression · Monoamine neurotransmitter · MPP^+ · OCT3 · Organic cation · PMAT · *Slc29a4* knockout · Uptake$_1$ · Uptake$_2$

1 Introduction

The plasma membrane monoamine transporter (PMAT) is the newest addition to the broadly selective organic cation and monoamine transporters. Unlike the organic cation transporters (OCTs) in the SLC22 family, PMAT belongs to the mammalian solute carrier 29 (SLC29) gene family, which primarily encodes the equilibrative nucleoside transporters (ENTs) (Baldwin et al. 2004; Kong et al. 2004; Ho and Wang 2014). Initially named ENT4, we first identified the existence of this transporter from the draft human genome database around 2000–2001 and had initially hypothesized it to function as a nucleoside or nucleobase transporter (Kong et al. 2004). We subsequently cloned the full-length human ENT4 cDNA and expressed the transporter in several expression systems including MDCK and HEK cell lines and *Xenopus laevis* oocytes. However, despite the transporter's sequence similarity to the ENTs, extensive screening work did not detect significant transport activities for nucleosides, nucleobases, or other structurally related analogs. The turning point came in 2003 when a postdoctoral fellow, Dr. Karen Engel, in our laboratory discovered by serendipity that 1-methyl-4-phenylpyridinium (MPP^+), a

dopaminergic neurotoxin and an OCT substrate, is robustly transported by ENT4. Using MPP^+ as a lead, we quickly discovered that the structurally related catecholamines (dopamine, norepinephrine, epinephrine) and indolamines (e.g., 5-HT) are all substrates for ENT4. We thus renamed ENT4 to "plasma membrane monoamine transporter (PMAT)" to reflect its true substrate profile.

In 2004, we reported the cloning and functional characterization of PMAT as a novel monoamine neurotransmitter transporter in the human brain (Engel et al. 2004). Shortly after, we found that besides monoamines, PMAT also transports a variety of structurally diverse organic cations (OC) and shares a remarkable functional resemblance to the OCTs (Engel and Wang 2005). The discovery of PMAT as a previously unknown monoamine and OC transporter has thus introduced a new member to organic cation and monoamine transporters. Since the initial cloning and characterization of PMAT, enormous advances have been made to our understanding of the molecular mechanisms, functional characteristics, cell and tissue-specific expression of PMAT. Critical reagents including antibodies, cell lines, and fluorescent probes have been developed. In 2013, we also generated the first mouse knockout model for Pmat (Duan and Wang 2013). With the availability of the molecular tools and animal models, the in vivo function of PMAT in the central nervous system and its significance in health and disease are just beginning to be understood. In this chapter, we summarize our current understanding of PMAT with an emphasis on its functional characteristics and potential roles in the central nervous system (CNS) in health and disease.

2 Molecular and Functional Characteristics of PMAT

2.1 Molecular Features

The human PMAT protein is encoded by the *SLC29A4* gene located at chromosome 7p22.1. Human PMAT is a membrane protein of 530 amino acid residues and has a predicted molecular mass of 58 kDa. Based on hydropathy analysis, an 11-transmembrane domain (TM) model with a long intracellular N-terminus and a short extracellular C-terminus has been proposed (Fig. 1). There are six consensus sites for protein kinase C phosphorylation (S6, S262, S268, S306, S476, and T338) and one cAMP-dependent kinase phosphorylation site (T199), in addition to one potential N-linked glycosylation site (N523) (Engel et al. 2004). Homologs of PMAT are also found in mouse and rat (Dahlin et al. 2007; Okura et al. 2011). Those homologs share 86–87% amino acid sequence identity with human PMAT and are predicted to have a similar membrane topology as the human protein. At the protein level, human and rodent PMATs exhibit a low but significant sequence identity (~20%) to the ENTs. In the TM regions, sequence identity between PMAT and the ENTs increases up to 35–40%. In contrast, the sequence identity of PMAT to the OCTs is only 11–14% (Zhou et al. 2007b).

The main substrate-binding domain in PMAT was identified by constructing and analyzing domain-swapping chimeric transporters between human PMAT and

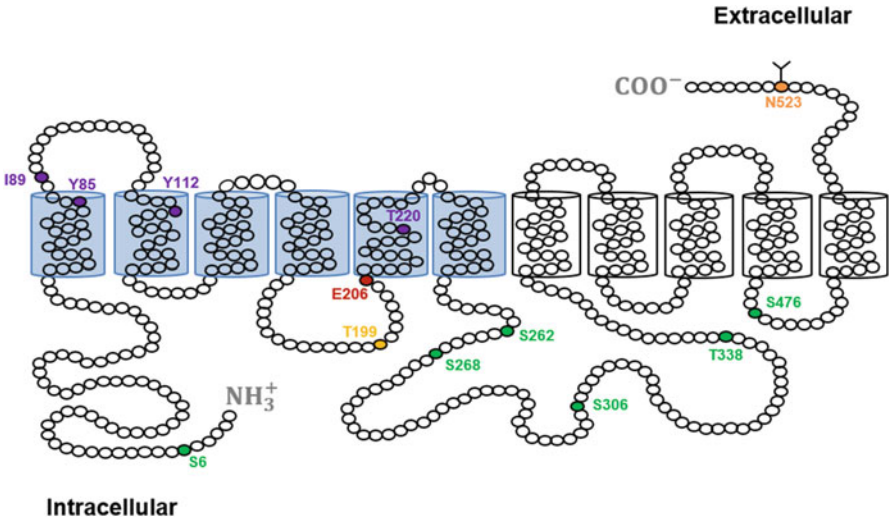


Fig. 1 Proposed topology of human PMAT with 11-TM domains. Circles represent individual amino acid residues. TM1-6 important for substrate recognition are shaded in blue. Purple: residues involved in substrate recognition (Y85, I89, Y112, T220). Red: residue involved in charge recognition (E206). Green: potential sites for protein kinase C phosphorylation (S6, S262, S268, S306, T338, S476). Yellow: potential site for cAMP-dependent protein kinase phosphorylation (T199). Orange: potential N-glycosylation site (N523)

ENT1 (Zhou et al. 2007b). A chimera consisting of TM1-6 of PMAT and TM7-11 of hENT1 behaved like PMAT, transporting MPP^+ but not uridine (a nucleoside). These data suggest that although the C-terminal half of PMAT likely contributes to the formation of the substrate permeation pathway, the major substrate recognition site in PMAT is located within its N-terminal half (Fig. 1). Site-directed mutagenesis analyses further identified that a negatively charged glutamate residue (E206) on TM5 is critical for the cation selectivity of PMAT (Zhou et al. 2007b). T220, another residue in TM5, also directly impacts PMAT's interaction with its substrates. Helical wheel analysis further revealed an amphipathic pattern of residue distribution on TM5, with E206 and T220 clustered closely in the center of the hydrophilic face, suggesting that TM5 is a critical component of the substrate permeation pathway in PMAT (Zhou et al. 2007b). In addition, Y85, Y112, and I89 on TM1-2 were also identified to play important roles for PMAT to interact with its cationic substrates and inhibitors (Ho and Wang 2010; Ho et al. 2012b).

2.2 Substrate Specificity

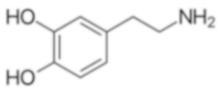
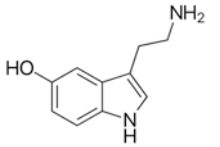
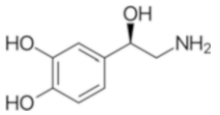
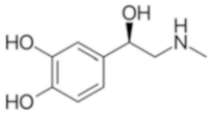
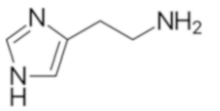
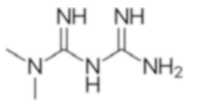
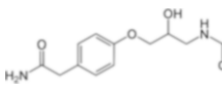
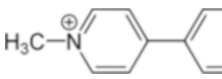
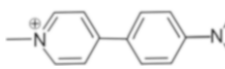
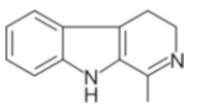
Nearly all PMAT substrates identified to date are small and hydrophilic organic cations (OCs). Biogenic amines, cationic drugs, and neurotoxins with diverse chemical structures are some of the OCs transported by PMAT (Engel and Wang

2005; Zhou et al. 2007c; Ho et al. 2011). Table 1 illustrates the structures of selected PMAT substrates along with their apparent binding affinities (K_m). As seen by the structures shown in Table 1, many of the PMAT substrates are OCs that contain one or more aromatic rings. However, the presence of the aromatic groups is not a required feature for interacting with this transporter, as some aliphatic OCs such as metformin and tetraethylammonium (TEA) are also transported by PMAT (Engel and Wang 2005; Zhou et al. 2007c). As a general observation, the presence of a positively charged nitrogen at physiological pH and a balance between hydrophilicity and hydrophobicity seem to be important determinants for interaction with PMAT. The diverse structures of compounds able to interact with PMAT indicate that this transporter functions as a polyspecific organic cation transporter. The OCTs from the SLC22 family are also considered polyspecific transporters, and a significant overlap is seen in the molecules transported by PMAT and OCT1-3. For instance, both metformin and the neurotoxin MPP^+, which are considered prototype substrates of OCTs, are efficiently transported by PMAT (Engel et al. 2004; Zhou et al. 2007c). In addition, the monoamine neurotransmitters, some of the most studied PMAT substrates, are known to be transported by OCT1-3 (Gründemann et al. 1999).

Despite the substantial overlap in substrates, there are also significant and important differences in the specificity and kinetics of PMAT and OCTs. For example, ASP^+, a fluorescent analog of MPP^+, is an excellent substrate for OCT1, 2, and 3, and is used as a fluorescence probe for these transporters (Ciarimboli et al. 2005; Kido et al. 2011; Duan et al. 2015). However, ASP^+ is not a PMAT substrate. On the other hand, another MPP^+ analog, APP^+ (IDT307), is robustly transported by PMAT (Duan et al. 2015). TEA is considered a good substrate for OCT1 and 2, but is a moderate to poor substrate for PMAT and OCT3. For the shared substrates, transport kinetics may also differ significantly among these transporters. For example, while the monoamine neurotransmitters are all transportable substrates for human PMAT and OCT3, the two transporters show marked differences in their apparent affinity and transport efficiency towards these substrates (Duan and Wang 2010). hPMAT shows a kinetic preference for serotonin and dopamine whereas hOCT3 appears to be more efficient towards histamine, norepinephrine, and epinephrine (see Sect. 5.3).

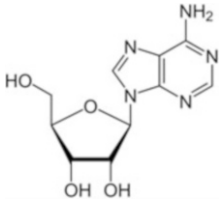
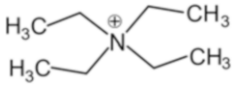
As stated earlier, PMAT (SLC29A4) was identified as the fourth member in the mammalian ENT family, which also contains ENT1, ENT2, and ENT3. ENT1 and 2 are classic nucleoside transporters and contribute to the cellular uptake of purine and pyrimidine nucleosides and their structural analogs (Baldwin et al. 2004; Kong et al. 2004; Ho and Wang 2014). ENT3 is an intracellular transporter essential in the transport of nucleosides into mitochondria and lysosomes (Govindarajan et al. 2009; Hsu et al. 2012). Although initially named ENT4, our functional studies have clearly shown that PMAT does not transport classical ENT substrates such as nucleosides, nucleobases, and nucleotides (Engel et al. 2004; Engel and Wang 2005). Among the naturally occurring nucleosides and nucleobases only adenosine is recognized as a substrate of PMAT; however, it is transported at an efficiency much lower than the OC substrates such as MPP^+ and serotonin (Zhou et al. 2010).

Table 1 Structure and apparent affinity (K_m) of selected PMAT substrates

Class	Substrate	K_m (μM)	Structure
Biogenic amines	Dopamine	406 ± 48[a] 329 ± 8[b]	
	Serotonin (5-HT)	283 ± 40[a] 114 ± 12[b]	
	Norepinephrine	1,078 ± 107[a] 2,606 ± 258[b]	
	Epinephrine	951 ± 59[a] 15,323 ± 3,947[b]	
	Histamine	4,379 ± 679[a] 10,471 ± 2,550[c]	
Organic cation drugs	Metformin	1,320[d]	
	Atenolol	907 ± 93[e]	
Neurotoxins and molecular analogs	MPP^+	111 ± 3[a] 33 ± 7[b]	
	APP^+ (IDT307)	*n.a.*	
	Harmalan	*n.a.*	

(continued)

Table 1 (continued)

Class	Substrate	K_m (μM)	Structure
Other substrates	Adenosine	413 ± 107[f]	
	TEA	8,759 ± 3,175[a] 6,593 ± 1,702[c]	

n.a. indicates value was not available in literature
Values from [a]Duan and Wang (2010)
[b]Engel et al. (2004)
[c]Engel and Wang (2005)
[d]Zhou et al. (2007b)
[e]Mimura et al. (2017)
[f]Zhou et al. (2010)

Table 2 K_i or IC_{50} values (μM) of selected inhibitors of human PMAT

Inhibitor	K_i or IC_{50} (μM)
Decynium-22 (D22)	0.10 ± 0.03[a]
GBR12935	7.9 ± 1.0[a]
Fluoxetine	22.7 ± 6.1[a]
Desipramine	32.6 ± 2.7[a]
Corticosterone	450.5 ± 76.5[a]
Rhodamine123	1.02 ± 0.12[b]
Quinine	26.9 ± 4.6[b]
Quinidine	25.3 ± 4.8[b]
Verapamil	18.6 ± 3.1[b]
Lopinavir	1.4 ± 0.2[c]
Ritonavir	6.0 ± 0.5[c]
Saquinavir	7.0 ± 1.0[c]

Values from [a]Engel et al. (2004)
[b]Engel and Wang (2005)
[c]Duan et al. (2015)

2.3 PMAT Inhibitors

Most of the known PMAT inhibitors are also positively charged organic molecules. However, while PMAT substrates are mostly smaller and more polar OCs (type I cations), high affinity PMAT inhibitors are generally bulkier and more hydrophobic (type II cations) (Table 2). Similar to what was observed for PMAT substrate profile, there is also a large overlap between OCT and PMAT inhibitors. A wide range of known OCT inhibitors were tested and most of them cross-inhibited PMAT (Engel

and Wang 2005). Several compounds such as decynium-22 (D22), quinidine, and rhodamine123 potently inhibited PMAT, but none of them show selectivity towards PMAT. For instance, D22, a cationic derivate of quinoline and a commonly used inhibitor of PMAT is able to inhibit OCT3 with the same potency ($K_i \sim 100$ nM) and is also able to inhibit OCT1 and 2 with K_i equal to 0.98 and 1.13 μM, respectively (Hayer-Zillgen et al. 2002; Engel and Wang 2005).

One strategy used in many studies for differentiating PMAT from OCT activity is the use of corticosterone. Corticosterone is an uncharged steroid hormone which shows much greater potency towards OCTs over PMAT (Ki = 450 μM for PMAT versus $IC_{50} < 35$ μM for OCT1-3), being especially selective towards OCT3 ($IC_{50} = 0.29$ μM) (Hayer-Zillgen et al. 2002; Engel and Wang 2005). Due to the selectivity of corticosterone towards OCTs, sensitivity towards D22 but not towards corticosterone is often used in literature for discerning PMAT and OCTs activity (Baganz et al. 2008; Duan and Wang 2010, 2013; Naganuma et al. 2014; Matthaeus et al. 2015). This approach however is not ideal for in vivo studies since corticosterone elicits a variety of genomic and nongenomic effects, which could affect many physiological processes and complicate the interpretation of results. Selective inhibitors of uptake$_1$ monoamine transporters (i.e., SLC6A family – serotonin, norepinephrine, and dopamine transporters, SERT, NET, and DAT) such as fluoxetine, desipramine, and GBR12935 were also found to inhibit PMAT (Engel et al. 2004). However, the K_i values obtained for PMAT were much higher (2–3 orders of magnitude) than observed towards SERT, NET, and DAT, indicating a strong preference of those inhibitors towards transporters from the SLC6A family.

In order to streamline the identification and characterization of potential PMAT inhibitors, a fluorescence assay was recently developed using IDT307, an MPP^+ fluorescent analog transported by PMAT (Duan et al. 2015). Using this assay, the HIV protease inhibitors were identified as inhibitors of PMAT, showing higher sensitivity towards PMAT in comparison with OCTs. In particular, lopinavir is a potent PMAT inhibitor and exhibited more than 120-fold selectivity toward PMAT over OCT1 ($IC_{50} = 1.4$ μM and 174 μM respectively), with no significant inhibitory effect for OCT2 or OCT3 at the highest tested concentration. While lopinavir is selective towards PMAT and can be a useful tool to differentiate PMAT activity from those of OCTs in in vitro and ex vivo studies, its use as a PMAT-selective inhibitor in vivo can be limited by several factors. Lopinavir is highly bound to plasma proteins and is a substrate of the efflux transporter P-gp (Van Waterschoot et al. 2010). The presence of P-gp at the blood–brain barrier may limit the entry of lopinavir into the brain and thus its inhibition efficiency towards PMAT in vivo in the brain. Therefore, the search for highly specific PMAT inhibitors suitable for in vivo studies continues.

The structure-activity relationship of OCs interacting with PMAT as substrates and inhibitors was previously investigated (Engel and Wang 2005; Ho et al. 2011). A positively charged nitrogen atom and a hydrophobic mass are the two most prominent features of PMAT substrates and inhibitors. A planar aromatic mass is often associated with high affinity interaction with PMAT (Engel and Wang 2005). Using a variety of phenylalkylamine analogs, the optimal distance between the

positively charged nitrogen and the aromatic ring was determined to be between 5.2 and 7.7 Å, which is consistent with a spacer chain length of 2–3 carbons (Ho et al. 2011). Several 3D pharmacophore models were also generated through molecular modeling based on the analysis of molecules known to interact with PMAT (Ho et al. 2011). These models are described by the presence of a hydrogen bond donor (representative of the positively charged nitrogen) and 2–3 hydrophobic features. In agreement to what was observed using the phenylalkylamine analogs, the distance between the hydrogen bond donor and the hydrophobic mass in these models ranges from 5.20 to 7.02 Å. As expected from the large substrate and inhibitor overlaps with the OCTs, the pharmacophore models of PMAT showed remarkable similarities to those developed for the OCTs (Bednarczyk et al. 2003; Zolk et al. 2009).

2.4 Mechanism of Transport

Solute carrier transporters (SLC) can mediate transmembrane flux of their substrates through both passive and active mechanisms. Ion coupled transporters, such as the high-affinity monoamine transporters (i.e., SERT, DAT, NET), mediate uphill transport of their substrates by coupling to the downhill flow of inorganic ions (e.g., Na^+, Cl^-). The inside-negative membrane potential universally present in animal cells can also influence membrane transport process. For instance, it is used by the OCTs as a driving force to power cellular uptake of positively charged molecules (Wagner et al. 2016). Radiotracer uptake studies in PMAT-expressing MDCK cells and *Xenopus laevis* oocytes showed that different from the high affinity biogenic amine transporters, Na^+ and Cl^- do not affect PMAT-mediated transport (Engel et al. 2004). In contrast, PMAT activity is highly sensitive to membrane potential changes (Engel et al. 2004; Zhou et al. 2007c). In agreement with these observations, two-microelectrode voltage-clamp studies in *Xenopus laevis* oocytes explicitly demonstrated that PMAT-mediated histamine uptake is associated with substrate-evoked, inwardly directed currents under voltage-clamp conditions (Itagaki et al. 2012). Kinetic analysis further showed that the inside-negative membrane potential enhances PMAT-mediated histamine transport by increasing maximal transport velocity with little effect on apparent binding affinity. These studies unequivocally demonstrated PMAT as an electrogenic transporter that uses the physiological inside-negative membrane potential to drive its uptake of OCs.

Protons also exert a strong stimulatory effect on PMAT activity. Extracellular pH has been shown to impact PMAT-mediated uptake of many substrates (Barnes et al. 2006; Xia et al. 2007; Zhou et al. 2007c, 2010; Itagaki et al. 2012). In general, an acidic extracellular pH is associated with an increase in PMAT-mediated transport whereas an alkaline pH is associated with reduced PMAT activity (Wang 2016). This stimulatory effect is not substrate-specific, but observed for nearly all tested substrates, including MPP^+, 5-HT, metformin, adenosine, and histamine (Xia et al. 2007; Zhou et al. 2007c, 2010; Itagaki et al. 2012). The proton ionophore FCCP (carbonyl cyanide p-trifluoromethoxyphenylhydrazone) greatly reduces the uptake of MPP^+ by PMAT (Xia et al. 2007; Itagaki et al. 2012). Two-microelectrode

voltage-clamp analysis in oocytes further demonstrated that the pH effect is present under voltage-clamp conditions, providing strong evidence that the effect of pH on PMAT uptake is unlikely to be due to an indirect effect of proton on membrane potential (Itagaki et al. 2012). Currently this effect is hypothesized to occur either via a direct coupling mechanism of OCs with protons or through a pH-induced change in protein ionization and folding state, which could provoke a change in the intrinsic catalytic activity of PMAT (Wang 2016). More studies are needed to understand the precise mechanism underlying the effect of protons on PMAT-mediated transport.

3 Expression and Distribution in the CNS

3.1 Regional Expression in Rodent and Human Brains

While PMAT mRNA/protein has been reported to be expressed in multiple tissues including brain, heart, small intestine, kidney, and liver (Engel et al. 2004; Barnes et al. 2006; Dahlin et al. 2007; Zhou et al. 2007c; Xia et al. 2009; Duan and Wang 2010), the brain has consistently been shown to be the major site of PMAT expression. Multiple studies, including northern blot, RT-PCR, in situ hybridization (ISH), and immunostaining analysis, have consistently demonstrated high expression of PMAT mRNA and protein in human and rodent brains (Engel et al. 2004; Dahlin et al. 2007, 2009; Vialou et al. 2007; Duan and Wang 2010, 2013). Using non-radioactive ISH, Dahlin et al. showed that mPmat mRNA is widely expressed in the mouse brain. The highest expression levels are observed in forebrain cortex, olfactory tubercle, dentate gyrus of hippocampus, cerebellum, and choroid plexus (Dahlin et al. 2007, 2009). This mRNA distribution pattern is in good agreement with the ISH data for *Slc29a4* from the Allen Mouse Brain Atlas (http://brainatlas.com/aba/) (Dahlin et al. 2009). A similar mRNA expression pattern is also observed for rat Pmat by ISH studies in rat brain sections (Vialou et al. 2007). Using an anti-PMAT polyclonal antibody, immunohistochemical staining further confirmed the widespread expression of mPmat protein across the mouse brain, consistently showing high protein expression in mouse forebrain cortex, hippocampus, olfactory areas, cerebellum, and choroid plexus (Dahlin et al. 2007).

The expression of PMAT mRNA in the human brain was initially analyzed by northern blot. Widespread expression was found in all tested regions including cerebellum, cerebral cortex, medulla, occipital pole, frontal and temporal lobes, putamen, and spinal cord (Engel et al. 2004). Using quantitative real-time PCR, Duan et al. quantified the copy numbers of PMAT in several human brain regions and choroid plexus (Duan and Wang 2010, 2013). While the PMAT transcript was found in all areas, it is particularly abundant in the cerebral cortex, hippocampus, substantia nigra, medulla oblongata, cerebellum, and choroid plexus (Engel et al. 2004; Duan and Wang 2010, 2013). The expression of PMAT protein in human brain was confirmed by immunoblotting and immunostaining studies in human cerebellum and choroid plexus tissue samples (Dahlin et al. 2007; Duan and Wang 2013).

3.2 Cell Type-Specific Expression in the Brain

In the mouse brain, ISH and immunohistochemistry detected mPmat expression in diverse groups of neurons including pyramidal neurons, interneurons, granular neurons, and Purkinje cells (Dahlin et al. 2007). Co-localization studies with the neuronal marker MAP2 further demonstrated that mPmat co-localizes extensively with MAP2. Consistently, immunostaining studies in human cerebellum tissue sections revealed intense PMAT immunoreactivity in axons and dendrites of neuronal cells (Dahlin et al. 2007). The expression and distribution of rPmat mRNA in the rat brain was also determined by Vialou et al. using in situ hybridization/immunohistochemistry co-labeling (Vialou et al. 2007). The rPmat mRNA was found in various neuron subtypes throughout the brain. Interestingly, rPmat was expressed in some, but not all, aminergic pathways. On the other hand, high expression was observed in cholinergic, glutamatergic, and GABAergic neurons in several discrete brain areas (Vialou et al. 2007).

Beside neurons, very high PMAT expression is found in the epithelial cells of the choroid plexus, which form the blood–cerebral spinal fluid (CSF) barrier. ISH and immunohistochemical studies in mouse and rat brains revealed choroid plexus as one of the brain regions with highest Pmat mRNA and protein expression (Dahlin et al. 2007; Vialou et al. 2007). Western blot analysis of human choroid plexus homogenate also detected a strong band with expected molecular mass of human PMAT (Duan and Wang 2013). Immunofluorescence staining of human choroid plexus sections showed that PMAT is predominantly expressed on the surface of choroid plexus epithelial cells and co-localizes strongly with the apical membrane marker Na^+/K^+-ATPase (Duan and Wang 2013).

With regard to astrocytes, initial co-localization studies in mouse brain revealed very little overlap of mPmat immunoreactivity with that of the astrocyte marker glial fibrillary acidic protein (GFAP) (Dahlin et al. 2007). Nevertheless, the high stringency used in these immunostaining studies may not be able to detect lower level of mPmat protein expression. In addition, there is also evidence that GFAP may not be present in all astrocytes (Walz and Lang 1998). More sensitive RT-PCR analyses have shown the expression of human PMAT mRNA in primary human astrocytes and human astrocytoma-derived cell lines (e.g., 1321N1, A172) (Engel et al. 2004; Yoshikawa et al. 2013; Naganuma et al. 2014).

4 Mouse Model for PMAT

Functional analyses of mouse and rat Pmat transporters in expression systems showed that the rodent Pmat transporters share substrate specificity and transport kinetics similar to those of human PMAT (Shirasaka et al. 2017). To elucidate the in vivo and physiological function of PMAT, our laboratory developed a knockout mouse model for Pmat (Duan and Wang 2013). The targeting vector was designed to replace exons 3–7 of the murine *Slc29a4* gene upon homologous recombination, producing an altered allele in which exon 2 is joined with exon 8. This would result

in a defective mRNA transcript with multiple premature stop codons after the first 72 amino acids, generating a nonfunctional short peptide with only one partial transmembrane domain. The targeting vector was transfected into G4 embryonic stem cells and a homologous recombinant clone was identified and used for blastocyst injection into C57BL/6J embryos to generate chimeric mice. The chimeric males were crossed with C57BL/6J females, and germ line transmission was identified by PCR genotyping. The F0 heterozygotes were then interbred to generate wild-type ($Pmat^{+/+}$) and knockout ($Pmat^{-/-}$) mice. The knockout mice have been continuously backcrossed with wild-type C57BL/6J mice to achieve a genetic background of more than 99% C57BL/6J. The disruption of the mPmat function in the gene product was also confirmed by isolating the defective mRNA species from $Pmat^{-/-}$ mice followed by expression and functional analysis in Flp-in HEK293 cells (Duan and Wang 2013).

$Pmat^{-/-}$ mice are viable, fertile with no overt physiological abnormalities (Duan and Wang 2013). Initial blood chemistry showed no significant differences in baseline values of various serum biomarkers from wild-type mice. Quantitative real-time PCR revealed that whole brain expression of functionally related transporters such as the high affinity monoamine transporters (*Sert, Dat, Net*) and *Oct3* are not significantly changed in knockout mice. The generation of the *Slc29a4* global knockout model provided an invaluable model to study the role of PMAT in vivo. This model has been used to analyze the function of Pmat in several studies (Duan and Wang 2013; Gilman et al. 2018; Bowman et al. 2020; Wei et al. 2020).

5 Physiological Roles in Monoamine Neurotransmission

5.1 $Uptake_1$ and $Uptake_2$

In the CNS, the monoamine neurotransmitters such as serotonin (5-HT), norepinephrine, and dopamine, are important chemical messengers that participate in a myriad of physiological and cognitive processes, including behavior, emotion, learning, and memory (Carlsson 1987; Greengard 2001). Like other chemical transmitters, monoamine neurotransmitters are synthesized in specific monoaminergic neurons and are stored in secretory vesicles at the axon terminal of the presynaptic neuron. During neurotransmission, the neurotransmitters are released into the synaptic cleft. They diffuse across the cleft and bind to specific receptors on postsynaptic neurons to elicit a variety of responses (Fig. 2). The signaling process is terminated by the removal of the released monoamines from the synaptic space. This process is carried out by membrane proteins capable of transporting monoamines into presynaptic neurons or adjacent cells, where the transmitter can either be recycled by repackaging into secretory vesicles or be degraded by intracellular enzymes such as the monoamine oxidases (MAOs) or the catechol-O-methyltransferase (COMT) (Torres et al. 2003).

The uptake of released monoamines into presynaptic neurons is mainly carried out by a family of Na^+- and Cl^--dependent, high affinity, low capacity transporters

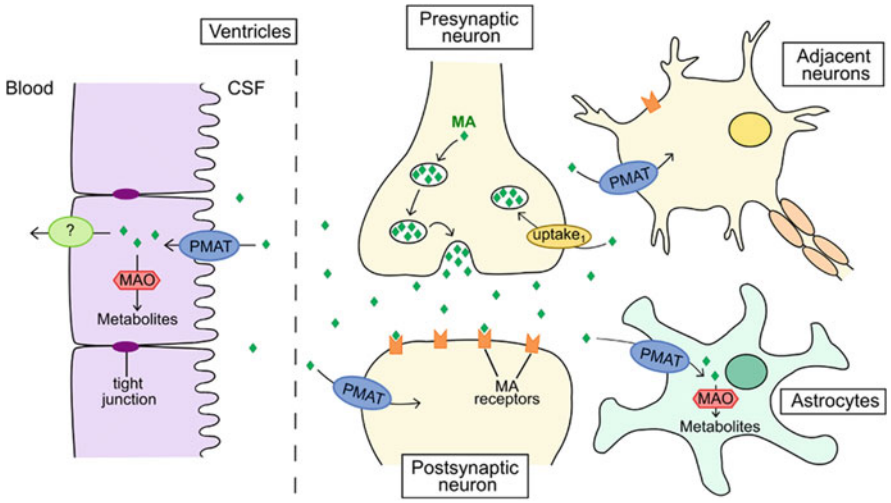


Fig. 2 Proposed roles of PMAT in monoamine (MA) clearance in the brain. PMAT is present in neuronal (yellow) and non-neuronal (purple and green) cells. It acts as an uptake$_2$ transporter and mediates the uptake of MA that has diffused out of the synaptic cleft into the interstitial fluid or CSF. MA can be metabolized by intracellular enzymes (e.g., MAO) or in the case of choroid plexus epithelial cells (purple) may be further exported into the blood

collectively known as "uptake$_1$". Uptake$_1$ include DAT, SERT, and NET (Blakely et al. 1994; Torres et al. 2003; Kristensen et al. 2011). These transporters share high sequence similarity and belong to the solute carrier 6 (SLC6) family. The reported apparent affinities (K_m) of uptake$_1$ transporters are typically in nanomolar to low micromolar range. In the CNS, DAT, SERT, and NET are expressed almost exclusively in the nerve terminals of dopaminergic, serotonergic, or noradrenergic neurons. Due to their major role in the reuptake of monoamines from the synaptic cleft, uptake$_1$ transporters are the targets of many psychostimulants (e.g., cocaine and amphetamines), neurotoxins (e.g., MPP^+) and antidepressants (e.g., selective serotonin reuptake inhibitors, tricyclic antidepressants) (Blakely et al. 1994; Torres et al. 2003; Kristensen et al. 2011).

Besides uptake$_1$, a different monoamine uptake system, termed uptake$_2$, was first described by Iversen (1965) in peripheral tissues with sympathetic innervation. Similar uptake$_2$ activities were also detected in brain areas such as striatum and cortex (Hendley et al. 1970; Wilson et al. 1988). Different from the uptake$_1$ system, uptake$_2$ transport is broadly selective, Na^+ and Cl^- independent and of low-affinity and high-capacity (Eisenhofer 2001; Daws 2009). The role of uptake$_2$ was initially considered to be related to facilitating the uptake of monoamines for their metabolism by intracellular enzymes such as MAOs and COMT (Lightman and Iversen 1969; Trendelenburg et al. 1987). However, the presence of uptake$_2$ activity in the brain suggested the possibility of uptake$_2$ to function as a secondary monoamine uptake system for the clearance of monoamine neurotransmitters in the CNS

(Gründemann et al. 1998; Eisenhofer 2001; Engel et al. 2004; Daws 2009; Matthaeus et al. 2015).

5.2 PMAT as a Brain Uptake$_2$ Transporter

In 1998, two research groups reported the cloning and characterization of OCT3 as the extraneuronal monoamine transporter (Gründemann et al. 1998; Wu et al. 1998). OCT3 showed classic features of the uptake$_2$ activity characterized in extraneuronal tissues, broadly transporting catecholamines with low affinity and high capacity. OCT3 is Na^+ and Cl^- independent but highly sensitive to corticosterone, and was thus considered as the uptake$_2$ transporter in sympathetically innervated tissues and in brain cells (Gründemann et al. 1998; Wu et al. 1998). Besides OCT3, the closely related OCT1 and OCT2 are also capable of mediating uptake$_2$-like transport of monoamine neurotransmitters (Gründemann et al. 1999; Koepsell et al. 2007).

In 2004, we reported the identification and characterization of PMAT as a novel monoamine transporter in the human brain. PMAT-mediated monoamine transport also shows classic uptake$_2$ characteristics, such as Na^+ and Cl^- independency and low substrate affinity but high transport capacity (Engel et al. 2004). With the identification and functional characterization of PMAT and OCTs, it is now clear that uptake$_2$ consists of a group of organic cation transporters with broad monoamine selectivity. Although all these transporters are capable of mediating Na^+- and Cl^--independent, low-affinity and high-capacity monoamine uptake, PMAT and OCT3 are likely to represent the major uptake$_2$ transporters in the brain and in sympathetically innervated tissues due to their marked expression in these tissues (Duan and Wang 2010).

The functional properties and brain distribution of PMAT appear to be complementary to uptake$_1$ monoamine transporters. SERT, DAT, and NET function as Na^+ and Cl^- dependent, high affinity, low capacity transporters. In contrast, PMAT-mediated transport is Na^+ and Cl^- independent and is of low affinity and high capacity in nature. For instance, the apparent affinities (K_m) of PMAT for 5-HT and dopamine are 2–3 orders of magnitude lower than those of SERT and DAT. Meanwhile, the V_{max} values of PMAT-mediated 5-HT and dopamine transport are also 2–3 orders higher than the V_{max} values of SERT or DAT (Engel et al. 2004). Furthermore, while uptake$_1$ transporters are exclusively expressed in monoaminergic neurons, PMAT is broadly expressed in many brain areas that may or may not express the uptake$_1$ (Engel et al. 2004; Dahlin et al. 2007). Lastly, PMAT is generally resistant to inhibitors of DAT, SERT, and NET. The functional characteristics of PMAT and its strategically broad localization in the brain suggest that this uptake$_2$ transporter may supplement the roles of the high affinity transporters under certain conditions. Based on these observations, we first hypothesized that PMAT is a brain uptake$_2$ transporter responsible for clearing released neurotransmitters that have escaped reuptake by the presynaptic uptake$_1$ transporters (Engel et al. 2004; Zhou et al. 2007a) (Fig. 2). PMAT may also play an

active role in monoamine uptake in brain areas that do not express uptake$_1$ (Dahlin et al. 2007).

The in vivo concentrations of monoamine neurotransmitters vary significantly in different brain areas. The extracellular concentrations of the monoamine are highest at its site of release and decline as the transmitter diffuses away (Bunin and Wightman 1998, 1999). It has been shown that synaptic concentrations of neurotransmitters can transiently reach high concentrations in the millimolar range (Bunin and Wightman 1998), where the high affinity transporters would be saturated. Although the precise localization of PMAT in neurons is still unknown, it is possible that the transporter is present in postsynaptic membranes and participates in the clearance of monoamines at these high concentration areas (Fig. 2). Furthermore, monoamine neurotransmitters are known to diffuse away from the synaptic cleft and exert neuromodulatory effects on other cells at distances well beyond their sites of release (i.e., volume transmission) (Agnati et al. 2010). The widespread expression of PMAT in the CNS makes it a perfect candidate to modulate monoamine signaling in volume transmission by taking up diffused neurotransmitters in non-monoaminergic neurons and in astroglial cells (Fig. 2).

5.3 Comparison Between PMAT and OCT3

The pronounced expression of PMAT and OCT3 in the CNS and sympathetically innervated tissues indicates that they are the two most prominent uptake$_2$ transporters for endogenous monoamines. PMAT is widely expressed in the human and rodent brains, including areas that do not express uptake$_1$ transporters (Dahlin et al. 2007; Duan and Wang 2010). Although PMAT is mostly found in neurons (Dahlin et al. 2007; Vialou et al. 2007), some studies have also reported expression in astrocytes (Yoshikawa et al. 2013). OCT3 was also found to be expressed in multiple brain regions in both astroglial and neural cells (Cui et al. 2009; Gasser et al. 2009).

The large overlaps in substrate specificity and broad brain distribution of PMAT and OCT3 raised important questions regarding their specific contribution to uptake$_2$ monoamine clearance in the brain. To explore the kinetic difference of PMAT and OCT3 in transporting endogenous monoamines, our laboratory developed stably transfected HEK293 cell lines that express hPMAT and hOCT3 at comparable levels (Duan and Wang 2010). Parallel and detailed kinetics analysis in these cell lines revealed distinct and seemingly complementary patterns for the two transporters in transporting monoamine neurotransmitters. hPMAT is more selective toward serotonin (5-HT) and dopamine, with the rank order of transport efficiency (V_{max}/K_m) being dopamine, 5-HT $\gg$ histamine, norepinephrine, epinephrine (Table 3). The substrate preference of hPMAT toward these amines is mainly driven by large differences in K_m (Duan and Wang 2010). In contrast, hOCT3 is less selective toward the monoamines with a rank order of V_{max}/K_m being histamine $>$ norepinephrine, epinephrine $>$ dopamine $>$5-HT (Table 3). Interestingly, hOCT3 showed

Table 3 Relative transport efficiency of hPMAT and hOCT3 towards monoamine neurotransmitters

Relative transport efficiency to MPP^+		
Substrate	hPMAT (%)	hOCT3 (%)
MPP^+	100	100
Dopamine	93	23
Serotonin (5-HT)	85	12
Histamine	16	57
Norepinephrine	14	35
Epinephrine	13	30

Values obtained from Duan and Wang (2010). Percentage values indicate transport efficiency (V_{max}/K_m) relative to the classical substrate MPP^+

comparable K_m, but significantly different V_{max} values, for the endogenous monoamines.

Using real-time PCR assay, the copy numbers of hPMAT and hOCT3 transcripts were quantified in several human tissue (Duan and Wang 2010). In spinal cord and eight brain regions, hPMAT expression is much higher than hOCT3, while hOCT3 is more abundant than hPMAT in adrenal glands and skeletal muscle. Other groups have also reported high OCT3 expression in circumventricular organs and broad expression throughout the rodent brain (Vialou et al. 2004; Amphoux et al. 2006; Gasser et al. 2006). Taken together, these data suggest that PMAT likely represents the major uptake$_2$ transporter for 5-HT and dopamine (two major centrally active neurotransmitters) in the CNS. OCT3, on the other hand, may be the major uptake$_2$ transporter for histamine, norepinephrine, and epinephrine, especially in peripheral tissues with sympathetic innervation.

5.4 PMAT in Brain 5-HT Clearance

Among the monoamine neurotransmitters, 5-HT is robustly transported by PMAT with highest affinity (Engel et al. 2004; Duan and Wang 2010) (Table 1). 5-HT is a major brain monoamine neurotransmitter involved in complex pathways associated with affection, emotion, learning, and mood modulation (Frazer and Hensler 1999; Meneses and Liy-Salmeron 2012). The major pathway for presynaptic clearance of 5-HT is mediated by SERT expressed in serotoninergic neurons (Blakely et al. 1994; Torres et al. 2003). Several studies have provided evidence of significant involvement of PMAT in 5-HT clearance in the brain (Zhou et al. 2007a; Duan and Wang 2010; Horton et al. 2013; Hosford et al. 2015).

Our laboratory first used an antisense hybrid depletion approach to assess PMAT's contribution to total brain 5-HT uptake in vitro (Zhou et al. 2007a). *Xenopus laevis* oocytes were injected with total human brain poly(A)$^+$ mRNA to allow the expression of the full spectrum of genes in the brain. Radiotracer uptake studies showed a ~2.5-fold increase in 5-HT uptake compared to control oocytes injected with water. Pre-hybridization of poly(A)$^+$ mRNA with PMAT or SERT antisense oligonucleotides, respectively, reduced mRNA-induced 5-HT uptake by

40–60% and 60–70% (Zhou et al. 2007a). These data suggest that functional transcripts of PMAT are present in the human brain and that PMAT may be significantly involved in brain uptake of 5-HT.

In vitro analysis showed that PMAT is resistant to selective serotonin reuptake inhibitors (SSRIs) that are highly potent and selective towards SERT (Zhou et al. 2007a; Haenisch and Bönisch 2010). We have taken the advantage that PMAT is highly sensitive to D22, but resistant to SSRIs and the OCT inhibitor corticosterone to assess potential contribution of Pmat in synaptosomes prepared from whole mouse brain (Duan and Wang 2010). Using this approach, we provided the evidence that PMAT may contribute to approximately 20% of total 5-HT uptake in mouse whole brain synaptosomes (Duan and Wang 2010). Corroborating with our findings, an in vivo study by Horton et al. (2013) was able to show that fluvoxamine (an SSRI) inhibition of 5-HT clearance in mouse hippocampus was potentiated when in combination with D22 – a potent inhibitor of PMAT and OCT3 – in both wild-type and Oct3 knockout mice, suggesting an involvement of mouse Pmat in 5-HT clearance. In addition, fast-cyclic voltammetry studies suggest that 5-HT clearance in the nucleus tractus solitarii of anesthetized rats is possibly under the regulation of PMAT, but not OCT3 or SERT (Hosford et al. 2015).

5.5 PMAT as a Clearance Mechanism at the Blood–CSF Barrier

Beside neurons, very high PMAT expression is found in the epithelial cells of the choroid plexus (Dahlin et al. 2007; Duan and Wang 2013). Located in the lateral, third, and fourth brain ventricles, the primary function of the choroid plexus is to produce and secrete CSF that baths and protects the brain (Ho et al. 2012a). The choroid plexus epithelial cells are joined by tight junctions and form the blood–CSF barrier. These epithelial cells are polarized with their apical membranes facing the CSF and the basolateral membrane facing the blood circulation. The blood–CSF barrier expresses numerous membrane transporters to facilitate solute exchange between the CSF and the systemic circulation (Keep and Smith 2011; Ho et al. 2012a).

Expression profiling analysis of ISH data from the Allen Brain Atlas showed that Pmat is one of the highest expressed *Slc* genes in the mouse choroid plexus (Dahlin et al. 2009; Ho et al. 2012a). Real-time PCR, western blot, and proteomic analyses also confirmed the abundant expression of PMAT mRNA and protein in human choroid plexus (Duan and Wang 2013). Importantly, other organic cations transporters (OCT1-3 and MATE1/2K) and uptake$_1$ monoamine transporters (SERT, DAT, NET) are minimally expressed in choroid plexus. The PMAT protein is localized to the apical (CSF-facing) membrane of the choroid plexus epithelial cells, consistent with a role in mediating active uptake of monoamines from the CSF into choroid plexus cells. Once inside those cells, monoamine neurotransmitters can be metabolized by the intracellular MAOs expressed within choroid plexus cells (Kaplan et al. 1981; Vitalis et al. 2002) or may be further exported across the basolateral membrane into the blood (Fig. 2).

The generation of the Pmat knockout mouse model provided a new tool for assessing the contribution of PMAT in the uptake of monoamines and other organic cations from the CSF (Duan and Wang 2013). Ex vivo uptake studies with live choroid plexus tissues revealed that the uptake of MPP^+, 5-HT, and dopamine is impaired in the choroid plexus of Pmat knockout mice. Furthermore, D22 reduced the uptake of 5-HT and dopamine into the choroid plexus of wild type but not Pmat knockout mice. In contrast, neither corticosterone (an OCT1-3 inhibitor) nor RTI-55 (a potent inhibitor of $uptake_1$ transporters) showed an effect in the uptake of 5-HT or dopamine in choroid plexus (Duan and Wang 2013). Together, these data demonstrated that PMAT is the major monoamine uptake transporter at the blood–CSF barrier. Consistent with these findings, CSF clearance studies in anesthetized rats showed that intracerebroventricular administered MPP^+ is quickly eliminated from the CSF, and the addition of dopamine and 5-HT inhibited this process, indicating a significant role of PMAT in regulating CSF organic cation concentrations in vivo (Okura et al. 2011). A similar study by Usui et al. (2016) using lopinavir as an inhibitor also pointed towards a significant role of PMAT in the clearance of histamine from the CSF.

6 Potential Involvement in Brain Pathophysiological Processes

In the CNS, aberrant levels of monoamine neurotransmitters are associated with a number of brain disorders such as depression, autism, schizophrenia, Parkinson's disease, and drug addiction. Due to their major roles in regulating extracellular monoamine levels, altered function of $uptake_1$ transporters has been implicated in several neuropsychiatric conditions (Kristensen et al. 2011; Pramod et al. 2013). For instance, reduced transporter expression and function due to the SERT promoter region polymorphism (e.g., 5-HTTLPR) has been associated with many neuropsychiatric conditions such as bipolar disorder, depression, obsessive–compulsive disorder, suicide, eating disorders, substance-abuse disorders, autism, and attention-deficit/hyperactivity disorder (Murphy and Moya 2011). In addition, therapeutic responses and side effects following treatment with SSRIs have been found to be associated with SLC6A4 variants.

To date, no study has systematically analyzed the allele frequency and functional consequence of single nucleotide polymorphisms (SNPs) of *SLC29A4* in the human populations. Our laboratory first suggested that PMAT may play a role in intestinal absorption of the antidiabetic drug metformin based on its expression in the gastrointestinal tract and its robust in vitro transport activity towards metformin (Zhou et al. 2007c). Several pharmacogenomics studies have explored the relationship between *SLC29A4* intronic variations and metformin pharmacokinetics and pharmacodynamics (Christensen et al. 2011; Duong et al. 2013; Dawed et al. 2019). A recent study in 286 severe metformin-intolerant and 1,128 metformin-tolerant individuals showed a significant association of the G allele at rs3889348 with gastrointestinal intolerance of metformin (Dawed et al. 2019).

Little is currently known regarding the involvement of PMAT in monoamine-related brain disorders. The impact of genetic polymorphisms or mutations in the human *SLC29A4* gene on neuropsychiatric and neurodegenerative disorders has not been explored. Nevertheless, a number of in vitro and in vivo studies suggest that PMAT should be considered as a candidate gene potentially involved in several monoamine-related brain disorders. Below we discuss these possibilities in the context of monoamine pathophysiology and speculate the potential involvement of PMAT under these scenarios.

6.1 Autism

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition that impacts social interaction, verbal and nonverbal communication, and repetitive behavior. ASD manifestation and severity varies broadly among individuals and includes an umbrella of conditions that used to be diagnosed separately, such as autism disorder, Asperger's syndrome, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified. Despite strong evidence pointing towards genetic heritability of autism, only few genes associated with ADS have been identified and they do not explain the majority of cases (Muhle et al. 2004; Sandin et al. 2017; Waye and Cheng 2018).

Serotonin (5-HT) is known to be an important neurotransmitter involved in the development and regulation of the CNS, playing a role in many behavior and psychological processes (Whitaker-Azmitia 2001). The study of this neurotransmitter is especially interesting in the context of ASD, considering that many serotonin-related abnormalities have been identified in ASD patients. For instance, close to one-third of autistic patients and their first-degree relatives present hyperserotonemia (increase in whole-blood and platelet serotonin), which is believed to be associated with atypical maturation of the serotoninergic system (Cook and Leventhal 1996; Leboyer et al. 1999; Whitaker-Azmitia 2001; Cross et al. 2008). In addition, low CSF levels of 5-hydroxyindolacetic acid (5-HIAA – a 5-HT metabolite) were found in up to 20% of patients with neurological disorders, including ASD patients (De Grandis et al. 2010). This evidence indicates that abnormal serotonin neurotransmission may be important in autism manifestation, and therefore genes of proteins involved in serotonin homeostasis (such as SERT and PMAT) may be of special interest.

Using a genetic candidate gene approach, Adamsen et al. (2014) identified a high prevalence of three rare non-synonymous mutations within the PMAT gene in ASD patients versus unaffected individuals. Genome analysis confirmed that these mutations were inherited and not de novo mutations. When transfected into MDCK cells, all mutants were localized to the plasma membrane at comparable levels to wild-type PMAT; however, two of them (A138T and D326E) showed reduced uptake of 5-HT. At least two ASD subjects with either the A138T or the D326E mutation had low 5-HIAA levels in the CSF and elevated serotonin levels in blood and platelets. As stated earlier, PMAT is widely expressed in the brain

including regions that lack significant SERT expression (Dahlin et al. 2007). It was speculated that PMAT dysfunction may play a significant role in the neurodevelopment of ASD patients (Adamsen et al. 2014). It was hypothesized that the reduced functionality of PMAT leads to a compromised serotonin clearance – especially in areas with low SERT expression – and may promote high prenatal exposure to serotonin. Consequently, high serotonin level leads to negative feedback in auto-receptors, perturbing the development of serotonin network and its local synthesis and culminating in low levels of serotonin and metabolites in the brain. In the blood, 5-HT is mainly stored in platelets through the uptake by SERT that is abundantly expressed in these cells (Jedlitschky et al. 2012). It is presently unknown if PMAT is expressed in platelets. However, the elevated platelet level of 5-HT in autism patients with the A138T or D326E mutation, corresponding to a reduced function of PMAT, indicates that PMAT may not play a significant role in 5-HT uptake and storage in platelets. Nevertheless, given the relatively small size of the ASD patient samples carrying the *SLC29A4* mutations, more studies are necessary to confirm these findings and elucidate the role of PMAT in serotonergic abnormalities and ASD development.

6.2 Depression

Depressive disorders are complex and have diverse manifestation in patients, with severity of symptoms varying from mild to severe. Tricyclic antidepressants (TCA), MAO inhibitors, serotonin and norepinephrine reuptake inhibitor (SNRIs), and selective serotonin reuptake inhibitors (SSRIs) are some of the major classes of antidepressants currently used for depression treatment. Most of them exert their effect by affecting the uptake and/or metabolism of serotonin in the brain, which is known to be involved in depression pathophysiology (Cowen and Browning 2015). The SSRIs, such as fluoxetine, citalopram, and sertraline, are some of the most widely prescribed medications in the treatment of depression, increasing extracellular levels of serotonin in the brain through inhibition of its reuptake by SERT. While the uptake$_1$ transporter SERT plays an essential role in serotonin homeostasis in the CNS, a significant portion of individuals undergoing SSRI treatment have poor or delayed response, with 30–40% of patients not responding satisfactorily to treatment (Steffens et al. 1997; Al-Harbi 2012).

Due to their complementary role in brain 5-HT uptake, uptake$_2$ transporters, such as PMAT and OCT3, have been hypothesized as potential targets to improve the response and reduce latency to antidepressant treatment (Schildkraut and Mooney 2004; Zhou et al. 2007a; Daws 2009). PMAT is highly expressed in human brain and transports 5-HT efficiently (Engel et al. 2004; Dahlin et al. 2007). We and others previously showed that many antidepressants including SSRIs and TCAs do not affect PMAT transport activity at clinically relevant concentrations (Zhou et al. 2007a; Haenisch and Bönisch 2010), indicating that PMAT may play a compensatory role in serotonin uptake, buffering the effect of these antidepressants and

therefore contributing to the clinical presentation of treatment resistance (Zhou et al. 2007a; Daws 2009).

Current evidence supporting this hypothesis comes mostly from pharmacological inhibition studies associating the use of uptake$_1$ inhibitors (i.e., SSRIs) and PMAT inhibitors (i.e., D22), while using knockout or selective inhibition (i.e., corticosterone) to rule out the impact of OCT3 in serotonin uptake. Both in vivo experiments with rodents and in vitro studies using mouse brain synaptosome have demonstrated that D22 potentiates the effect of SSRIs, decreasing uptake and clearance of serotonin and improving antidepressant-like effect (Duan and Wang 2010; Horton et al. 2013). The recent development of Pmat knockout mouse model provides a new tool for investigating the psychological and neurological function of Pmat in the CNS. While Pmat deficient mice do not have an overt phenotype (Duan and Wang 2013), they display subtle differences in anxiety and coping behaviors (Gilman et al. 2018). In addition, a recent study measuring real-time clearance of 5-HT in mouse hippocampus showed that ketamine was able to significantly inhibit serotonin clearance in wild-type but not in Sert$^{-/-}$ or Pmat$^{-/-}$ mice; pointing towards PMAT as a potential contributing factor in ketamine's antidepressant-like effects (Bowman et al. 2020). Further neurochemistry and behavioral studies of these animals, as well as comparative studies between SSRI treatment in Pmat$^{-/-}$ and wild-type mice may provide a direct link between PMAT and the treatment of depressive disorders. If proven, PMAT might represent a promising target in the development of new and improved antidepressant drugs.

6.3 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of nigrostriatal dopaminergic neurons. PD is a multifactorial disease with both genetic and environmental factors playing roles. CNS exposure to toxic organic cations can damage nigrostriatal dopaminergic pathways and produce parkinsonian syndromes, as seen with the classic PD protoxin MPTP (Sian et al. 1999). MPTP is a lipophilic compound that can readily pass through the blood–brain barrier. Once inside in the brain, MPTP is rapidly converted to the toxic metabolite MPP$^+$ in astrocytes by MAO-B. After releasing from the astrocytes through OCT3 (Cui et al. 2009), MPP$^+$ is selectively transported into the dopaminergic neurons through DAT, where it impairs mitochondrial respiration chain and precipitates cell death (Sian et al. 1999).

CNS exposure to environmental or endogenously produced MPP$^+$-like toxins, such as paraquat, certain β-carbolines, and tetrahydroisoquinoline derivatives, has long been implicated in the etiology of PD (Nagatsu 1997; Collins and Neafsey 2000). β-carbolines are naturally found structural analogs of MPP$^+$, and 1-benzyl-1,2,3,4-tetrahydroisoquinoline is an example of an endogenously produced neurotoxin found to be elevated in the CSF of PD patients (Kotake et al. 1995). Interestingly, many of those cationic neurotoxins were found to be substrates of PMAT. MPP$^+$ is efficiently transported by cell lines expressing PMAT (Engel et al. 2004). Cytotoxicity assays demonstrated that harmalan and norharmanium (two

β-carbolines) are also transportable substrates of PMAT (Ho et al. 2011). Similarly, 1-benzyl-1,2,3,4-tetrahydroisoquinoline was also reported to be a PMAT substrate (Wu et al. 2015). Cells expressing PMAT are ~15-fold more sensitive to the toxic effect of those compounds (Ho et al. 2011).

The choroid plexus in the brain plays a major role in maintaining CSF homeostasis and clearing potentially harmful compounds from the CNS. PMAT is highly expressed in human and mouse choroid plexuses and is localized to the apical membrane (CSF-facing) of the choroid plexus epithelial cells (Duan and Wang 2013). By clearing cationic neurotoxins from the CSF and preventing its accumulation in the brain, PMAT may play a protective role in the CNS, potentially lowering the risk of PD. Further investigation of this hypothesis is needed to elucidate the functional and toxicological significance of PMAT in PD etiology.

7 Summary

Since the discovery of PMAT in 2004, great progress has been made in our understanding of this transporter at the molecular and functional levels. Work conducted in the past 15 years has clearly established that PMAT functions as a polyspecific organic cation transporter that transports biogenic amines and xenobiotic organic cations. PMAT-mediated substrate-uptake is Na^+-independent and driven by inside-negative membrane potential. Broadly expressed in the brain in both neural and non-neural cells, PMAT represents a major brain $uptake_2$ transporter for centrally acting monoamine neurotransmitters such as 5-HT and dopamine. By serving as a complementary clearance mechanism for released monoamine neurotransmitters, PMAT may play a regulatory role in monoamine neurotransmission and neuromodulation. Nevertheless, we are still at the beginning of understanding the in vivo significance of this transporter in brain monoamine physiology and pathophysiology. Elucidating the roles of PMAT in monoamine neurotransmission and its involvement in neuropsychiatric and neurodegenerative diseases would be important and exciting directions for the time ahead.

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