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The Plasma Membrane Monoamine Transporter (PMAT): Structure, Function, and Role in Organic Cation Disposition

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Abstract

Plasma membrane monoamine transporter (PMAT) is a new polyspecific organic cation transporter that transports a variety of biogenic amines and xenobiotic cations. Highly expressed in the brain, PMAT represents a major uptake$_2$ transporter for monoamine neurotransmitters. At the blood–cerebrospinal fluid (CSF) barrier, PMAT is the principal organic cation transporter for removing neurotoxins and drugs from the CSF. Here I summarize our latest understanding of PMAT and its roles in monoamine uptake and xenobiotic disposition.

ORGANIC CATION TRANSPORT AND THE DISCOVERY OF PMAT

Numerous endogenous compounds (e.g., biogenic amines) and xenobiotics (e.g., drugs, environmental toxins) carry a primary, secondary, or tertiary amine moiety that is protonated at physiological pH. Compounds containing a quaternary ammonium entity are permanently charged cations. These molecules are collectively termed organic cations (OCs). OCs are diverse in chemical structures and display a wide spectrum of physiological, pharmacological, and toxicological activities *in vitro* and *in vivo*. Due to their charge and hydrophilicity, many OCs cannot readily cross cell membranes by passive diffusion. To facilitate OC entry to or exit from cells, mammalian cells have evolved complex OC transport systems, including the classic organic cation transporters 1–3 (OCT1-3) from the solute carrier 22 (*SLC22*) family and the multidrug and toxin extrusion proteins 1 and 2-K (MATE1/2-K) encoded by the *SLC47* family.[1,2] OCT1-3 mediate Na^+-independent, electrogenic OC uptake, and utilize the inside-negative membrane potential as a driving force. MATE1/2-K, on the other hand, function as proton/OC exchangers that couple OC efflux with a physiologic inwardly directed proton gradient. In excretory organs such as the kidney and liver, OCTs and MATEs are respectively expressed at the basolateral and apical membranes of the secretory epithelium to sequentially mediate transepithelial secretion of OCs into the urine or bile.[1,2] OCTs and MATEs are considered “polyspecific” or “multispecific,” as they interact with a wide array of cationic compounds with diverse chemical structures. A few neutral compounds (e.g., steroid hormones) also interact with the OCTs.[1]

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CONFLICT OF INTEREST

I have no conflicts of interest to declare.

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In 2004 we reported the cloning and functional characterization of a novel brain monoamine transporter—PMAT.[3] PMAT (*SLC29A4*) was first identified from public genomic databases as the fourth member in the mammalian *SLC29* gene family, which encodes equilibrative nucleoside transporters (ENTs) that are molecularly and functionally distinct from the OCTs.[4] Except PMAT, all other members of the *SLC29* family, namely, ENT1-3, function as nucleoside transporters that specifically transport purine and pyrimidine nucleosides (e.g., uridine, adenosine, cytidine) and their structural analogs (Table 1). ENT1 and 2 are classic nucleoside transporters that play important roles in cellular uptake of physiologic nucleosides and therapeutic nucleoside analogs (e.g., cytarabine, fludarabine).[5,6] ENT3 is an intracellular transporter crucial for lysosomal and mitochondrial nucleoside transport.[7,8] Alternatively named ENT4, PMAT was initially hypothesized to transport nucleosides or related compounds. However, a series of studies carried out in our laboratory demonstrated that other than a moderate activity for adenosine, PMAT does not interact with nucleosides, nucleobases, or nucleotides.[3,9,10] Instead, it robustly transports serotonin (or 5-hydroxytrptamine, 5-HT), dopamine (DA), and other monoamine neurotransmitters. We thus named the transporter the “plasma membrane monoamine transporter (PMAT)” to reflect its cellular localization and physiologic substrate profile.[3] We subsequently found that, besides monoamine neurotransmitters, PMAT also transports a variety of structurally diverse OCs and shares a striking functional similarity to the OCTs.[9] The discovery of PMAT as a previously unknown monoamine and OC transporter has since introduced a new player to the arenas of monoamine physiology as well as disposition of cationic drugs and toxins.

MOLECULAR AND FUNCTIONAL CHARACTERISTICS OF PMAT

Molecular features

The full-length human PMAT cDNA was first cloned from a human kidney cDNA library.[3] Subsequently, cDNAs encoding the rodent homologs were also isolated from the mouse and rat.[11,12] The human PMAT cDNA encodes a membrane protein of 530 amino acid residues with a predicted molecular mass of 58 kDa. Human PMAT protein is predicted to possess 11 membrane-spanning domains with a long intracellular N-terminus and a short extracellular C-terminus (Figure 1). There is one potential N-linked glycosylation site (Asn-523) and several putative protein kinase C and cAMP-dependent kinase phosphorylation sites. Mouse and rat Pmat proteins share 86–87% sequence identity with human PMAT and are predicted to possess a similar membrane topology.

Tissue distribution and subcellular localization

Northern blot, real-time reverse-transcription polymerase chain reaction (RT-PCR), and multiple tissue expression array analyses showed that PMAT mRNA is expressed in multiple human tissues including brain, heart, small intestine, pancreas, kidney, skeletal muscle, and liver.[3,13,14] Among these analyzed tissues, the brain consistently showed the highest expression of PMAT. In the human brain, PMAT transcripts are broadly distributed in many brain regions, with high levels of expression found in cerebral cortex, hippocampus, substantia nigra, medulla oblongata, cerebellum, and choroid plexus.[3,14,15] Immunoblotting and immunostaining studies with specific antibodies have confirmed the expression of PMAT protein in human choroid plexus, cerebellum, intestine, kidney, and heart.[11,13,15–17]

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Similar to human PMAT, mouse and rat Pmats were also found in multiple tissues, with brain being a major site of expression.[11,18] In the mouse brain, Pmat mRNA and protein are widely distributed in numerous anatomical areas, with high levels of expression found in fore-brain cortex, olfactory tubercle, dentate gyrus of the hippocampus, cerebellum, and epithelial cells of the choroid plexus.[11] There may be some difference in PMAT expression between species. For instance, PMAT/Pmat mRNA and protein are expressed in human and rat kidneys[16]; but mouse kidney appears to lack significant expression of Pmat.[11,16] Interestingly, in human and rat kidneys PMAT/Pmat proteins were not found in renal tubular cells but were predominantly localized to glomerular podocytes, which are highly specialized visceral epithelial cells crucial for maintaining the integrity of the glomerular filtration barrier.[16]

When heterologously expressed in Madin-Darby Canine Kidney (MDCK) and Human Embronic Kidney (HEK) cells, PMAT protein is localized to the cell surface membrane, gaining its name as a plasma membrane transporter.[3,14] In polarized MDCK monolayer culture, PMAT is specifically targeted to the apical membrane.[19] Consistent with these findings, endogenous PMAT/Pmat proteins have been localized to the apical membranes of human and mouse choroid plexus epithelial cells.[15] In human intestine, PMAT protein was found to concentrate on the apical surface of the mucosal epithelial layer, although punctate intracellular staining was also observed.[17] Similarly, in rat primary cardiomyocytes both cell surface and punctate intracellular staining were overserved.[13] The nature and functional significance of the intracellularly expressed PMAT in these cells are currently unknown.

Substrate and inhibitor specificity

Numerous OCs with diverse chemical structures interact with PMAT either as substrates or as inhibitors.[9,20] Most PMAT substrates identified to date are small and polar OCs (i.e., type I cations), which include biogenic amines, cationic drugs, and neurotoxins (Figure 2). While many PMAT OC substrates contain one or more aromatic rings, aliphatic OCs, such as tetraethylammonium (TEA) and metformin, are also transported by PMAT.[9,17] Most PMAT substrates identified to date are also known substrates of the OCTs. For example, 1-methyl-4-phenylpyridinium (MPP^+) and metformin, two prototype OCT substrates, are efficiently transported by PMAT with apparent binding affinities (K_m) similar to those of the OCTs.[3,17] The monoamine neurotransmitters, which are endogenous PMAT substrates, are all known to be transported by OCT1-3.[21] Nevertheless, there are notable differences in substrate specificity and transport kinetics among these transporters. For instance, TEA is a very good substrate for OCT1 and OCT2, but only a moderate substrate for OCT3 and PMAT.[9,21] In Flp-in HEK293 cells expressing human PMAT and OCT3 at comparable levels, PMAT and OCT3 showed substantial kinetic differences towards the monoamine neurotransmitters.[14] PMAT showed a strong kinetic preference for 5-HT and DA over other mono-amines (i.e., histamine, norepinephrine, and epinephrine), whereas a seemingly opposite preference was observed for OCT3. Another interesting feature is that while PMAT does not typically interact with nucleosides or nucleoside analogs, it does exhibit a moderate transport activity towards the purine nucleoside adenosine.[10,13] Nevertheless, under both physiologic (pH 7.4) and acidic (pH 6.6) conditions, PMAT demonstrated a strong

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preference for OC substrates over adenosine, only transporting adenosine at an efficiency (defined as V_{max}/K_m) less than one-tenth of that of MPP^+ or 5-HT.[10]

Many bulkier and more hydrophobic cations (i.e., type II cations) interact with PMAT as inhibitors. A range of known OCT inhibitors have been tested for their interactions with PMAT, and most of them cross-inhibited PMAT.[9] Several compounds, including decynium-22 (D22), quinidine, and rhodamine 123 potently inhibited PMAT, but none of them are selective toward PMAT. For example, D22, a cation derivative of quinoline, inhibits PMAT and OCT3 with equal potency (K_i ~0.1 μM); and D22 also inhibits OCT1 and 2 at low micromolar concentrations (Table 2).[9,22] Among the tested OCT inhibitors, corticosterone, an uncharged steroid hormone, showed more selectivity toward the OCTs over PMAT. While corticosterone inhibits human OCT3 with greater potency than OCT1 and OCT2, it is much less potent for PMAT than for OCT1-3 (Table 2).[9,22] Due to the lack of a PMAT-specific inhibitor, sensitivity to D22 but not to corticosterone has been used as an indirect approach in many cell and tissue studies to discern PMAT activity from those of the OCTs.[14,23–26] Recently, we identified the fluorescent MPP^+ analog, IDT307, as a transportable substrate for PMAT and developed a fluorescence assay for rapid identification and characterization of PMAT inhibitors.[27] Using this assay, we found that human PMAT and OCTs exhibit distinct sensitivity toward HIV protease inhibitors. PMAT is highly sensitive to HIV protease inhibitors, whereas OCT2 and 3 are resistant. OCT1 showed an intermediate sensitivity and a distinct inhibition profile from PMAT. Furthermore, we found that lopinavir is a potent and selective PMAT inhibitor, exhibiting more than 120-fold selectivity toward PMAT over OCT1, with no significant interaction with OCT2 and 3 (Table 2). Although lopinavir also interacts with other drug transporters (e.g., P-gp) and has limited permeability across the blood–brain barrier,[28] it may represent a useful chemical tool to differentiate PMAT activity from those of OCTs in *in vitro* and *ex vivo* studies.

Two studies have explored the structure–activity relationship of PMAT in interacting with OC substrates and inhibitors.[9,20] A positively charged nitrogen atom and a hydrophobic mass are the two common features of PMAT substrates and inhibitors. A planar aromatic mass is associated with high-affinity interaction with PMAT.[9] Using a series of phenylalkylamine analogs, the optimal distance between the positively charged nitrogen atom and the aromatic ring was determined to be in the range of 5.2–7.7 Å, which corresponds to a spacer chain length of 2–3 carbons.[20] Using molecular modeling, several 3D pharmacophore models have been developed based on the analysis of known PMAT substrates and inhibitors.[20] These models are characterized by a hydrogen bond donor, representing the positively charged nitrogen atom, and 2–3 hydrophobic features. The distance between the hydrogen bond donor and the hydrophobic features ranges between 5.20 and 7.02 Å, which is in good agreement with results obtained with the phenylalkylamine analogs. Not surprisingly, these PMAT pharmacophore models share similar features with those previously developed for the OCTs. The 2–3 hydrophobic features and their defined distance to the positively charged nitrogen center are molecular descriptors known to be important for high affinity interactions with the OCTs.[29,30]

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Mechanism of transport

Effect of membrane potential—In virtually all animal cells, there is a baseline electric potential difference across the plasma membrane, which typically ranges from −40 to −90 mV. This inside-negative membrane potential, rising largely from the diffusional property of the K^+ ion across the cell membrane, influences multiple cellular and physiological processes. It is used by the OCTs as a driving force to power cellular uptake of OCs, allowing these transporters to accumulate OCs at intracellular concentrations 10–15 times their extracellular concentrations.[31] Similar to the OCTs, radiotracer uptake studies in PMAT-expressing MDCK cells or *Xenopus laevis* oocytes showed that PMAT-mediated OC transport is Na^+- and Cl^--independent, but is sensitive to membrane potential.[3,17,32] Depolarization of cell membranes, either by increasing extracellular K^+ concentrations or pharmacological blockade of K^+ channels, significantly reduced PMAT-mediated uptake of MPP^+ and metformin.[3,17] On the other hand, membrane hyperpolarization increased PMAT uptake activities. Two-microelectrode voltage-clamp studies in oocytes further showed that PMAT-mediated histamine uptake is associated with substrate-evoked, inwardly directed currents under voltage-clamp conditions.[32] Substrate-induced currents were independent of Na^+ but increased proportionally as the membrane potential became more negative. Detailed kinetic analysis revealed that this energizing effect of negative membrane potential on PMAT was due to an increase in maximal transport velocity, with little effect on apparent binding affinity.[32] Together, these studies established that PMAT is an electrogenic transporter that utilizes the physiologic inside-negative membrane potential as a driving force to facilitate cellular uptake of OCs.

Effect of pH—Extracellular pH may influence carrier-mediated transport through multiple mechanisms. Protons may be directly used as coupling ions to drive substrate transport, as in the case of H^+-coupled oligopeptide cotransporters (e.g., PepTs) or the H^+/OC antiporters MATEs. Alternatively, protons may exert an effect on the folding or ionization state of the transporter protein, leading to a change in transport activity. Protons may also uniquely influence OC transport either by changing the degree of ionization of the substrate or by modulation of membrane potential. The activities of OCTs have been reported to be sensitive to extracellular pH.[33–35] However, further analyses suggest that the observed pH effects were nonspecific and likely due to indirect effects of protons on substrate ionization or alteration of membrane potential.[33,35]

Different from the OCTs, protons appear to exert a specific stimulatory effect on PMAT-mediated transport. Barnes *et al.* first reported that PMAT-mediated adenosine uptake was simulated by an acidic extracellular pH, whereas 5-HT transport by PMAT was relatively insensitive to pH changes.[13] We subsequently demonstrated that the stimulatory effect of acidic pH is not specific to adenosine, but occurs with all other PMAT substrates, such as MPP^+, 5-HT, metformin, and histamine.[10,17,19,32] Lowering extracellular pH from 7.4 to 6.6 stimulated PMAT-mediated uptake of by 3–4-fold, whereas elevating extracellular pH to 8.2 abolished transport activity. For PMAT-mediated MPP^+ uptake, the V_{max} increased by 4-fold at pH 6.6, whereas there was no change in K_m.[19] Because MPP^+ is a permanently charged cation, this stimulatory effect of protons is unlikely due to an increase in substrate ionization. Similar effects of protons were observed in two-microelectrode voltage-clamp

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analysis.[32] Histamine-induced currents exhibited great sensitivity to the pH of the perfusate. Consistent with results from radiotracer uptake studies, an acidic condition (pH 6.0) increased the maximal rate of histamine-induced current by 3–4-fold without affecting the apparent binding affinity.[32] Because these studies were conducted under voltage-clamp conditions with fixed membrane holding potentials, the effect of protons is unlikely due to an effect on membrane potential. Instead, these data support a more specific effect of protons on PMAT, which may occur via a direct H^+/OC coupling mechanism or through a proton-induced change in the intrinsic transport activity of PMAT. More studies are needed to definitively establish the role of protons in PMAT-mediated transport.

Structure–function relationship

The unique substrate selectivity of PMAT in the *SLC29* family has brought up some intriguing questions: why does PMAT behave so differently from the ENTs? Is it structurally more related to the ENTs or to the OCTs? At the protein level, the sequence similarity of PMAT to the OCTs is insignificant. While human and rodent PMATs only exhibit an overall low sequence identity (~20%) to the ENTs, sequence identity between PMAT and ENTs increases significantly up to 35–40% in the transmembrane (TM) region. Both PMAT and ENTs are predicted to possess an 11-TM topology, whereas the OCTs are predicted to have 12 membrane-spanning domains.[1,3,5] Moreover, PMAT appears to retain several features reminiscent of the ENTs. It transports the purine nucleoside adenosine and preserves low-affinity binding to the classic ENT inhibitors NBMPR, dipyridamole, and dilazep.[10,36] Based on these observations, we hypothesized that PMAT maintains the overall protein architecture of the ENTs but has diverged from the ENT lineage to handle structurally diverse OCs via changes of key amino acid residues in its substrate binding pocket. Indeed, a chimeric transporter consisting of the N-terminal (TM1-6) half of PMAT and the C-terminal half (TM7-11) of ENT1 is functional and behaved like PMAT, transporting MPP^+ but not uridine.[37] These data suggest that, although the C-terminal half of PMAT may participate in the formation of the substrate permeation pathway, its major OC recognition sites are located within the N-terminal half (Figure 1), a region also known to be important for the ENTs in their interaction with nucleoside substrates. Within TM1-6, site-directed mutagenesis analyses further identified a number of key residues involved in substrate recognition and interaction.[36–38] A negatively charged glutamate residue (E206) on TM5 is critical for the cation selectivity of PMAT.[37] Replacement of this residue by charge reversal (E206R) or neutralization (E206Q) abolished OC transport activity, whereas conserving the negative charge (E206D) restored transporter function. Interestingly, mutant E206Q, which possessed the equivalent residue in ENT1, gained a transport activity for uridine,[37] further supporting an intrinsic relationship between PMAT and the ENTs. Another residue in TM5, T220, also showed a direct influence on PMAT transport activity towards OCs. Helical wheel analysis revealed a distinct amphipathic pattern of residue distribution on TM5 with E206 and T220 clustered in the center of a hydrophilic face, suggesting a critical role of TM5 in forming part of the substrate permeation pathway.[37] Several additional residues (Y85, Y112, I89) on TM1 and TM2 were also identified to be important for PMAT to interact with its substrates or inhibitors.[36,38]

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ROLE IN MONOAMINE NEUROTRANSMITTER REGULATION

The main physiological substrates of PMAT are the bioactive amines including 5-HT, DA, norepinephrine, histamine, and epinephrine (Figure 2). Synthesized and released by specific mono-aminergic neurons and adrenal glands, these neurotransmitters and neurohormones regulate a myriad of physiological, endocrine, behavioral, and cognitive processes.[39,40] 5-HT is a key mediator of mood, vascular function, and gastrointestinal motility. DA plays an important role in higher-order brain functions such as cognitive control, arousal, reward, and motivation. Norepinephrine, also known as noradrenaline, is a main neurotransmitter of the sympathetic nervous system and a major mediator of the "fight-or-flight" response.[41] Numerous drugs act on these monoamine signaling systems. Norepinephrine, DA, and epinephrine themselves are used as injectable drugs for the treatment of several clinical conditions such as critically low blood pressure, cardiac arrest, and anaphylaxis.

Cellular uptake of released monoamine neurotransmitters by membrane transporters is a major mechanism to inactivate these chemical messengers. By removing the transmitter from its receptor-binding site, transporter-mediated uptake determines the intensity and duration of the signaling process. Two distinct systems, termed uptake$_1$ and uptake$_2$, are responsible for cellular uptake of monoamine neurotransmitters.[42,43] Uptake$_1$ consists of highly specific neurotransmitter transporters from the *SLC6* family and includes the serotonin transporter (SERT), the dopamine transporter (DAT), and the norepinephrine transporter (NET).[44,45] Predominantly expressed on serotonergic, dopaminergic, or adrenergic neurons, SERT, DAT, and NET mediate Na^+- and Cl^--dependent, high-affinity and low-capacity uptake of released neurotransmitters. These uptake$_1$ transporters play a major role in clearing released monoamines from the synaptic cleft and are the targets for numerous clinically used antidepressants (e.g., selective serotonin reuptake inhibitors or SSRIs), psychostimulants (e.g., amphetamines, cocaine), and neurotoxins (e.g., MPP^+).[44,45] Different from uptake$_1$, monoamine transport by uptake$_2$ is Na^+- and Cl^--independent, broadly selective, and of low-affinity and high-capacity.[42,43] Originally found in sympathetically innervated tissues such as heart and smooth muscle cells, uptake$_2$ was initially thought to facilitate monoamine uptake for their metabolism by intracellular monoamine oxidases and catechol-O-methyltransferase.[46,47] Uptake$_2$ activities are also present in the brain and these transporters are thought to play a backup role for uptake$_1$ in brain monoamine clearance.[3,35,42,43,48]

The molecular identity of uptake$_2$ monoamine transporters was unclear for a long time. In 1998, two groups reported the cloning of OCT3 as the extraneuronal monoamine transporter or uptake$_2$.[35,48] With the identification and functional characterization of PMAT, it is now increasingly being recognized that uptake$_2$ consists of multiple OC transporters with broad mono-amine selectivity. While OCT1-3 and PMAT are all capable of mediating Na^+- and Cl^--independent, low-affinity and high-capacity monoamine uptake, PMAT and OCT3 are likely to represent the most prominent uptake$_2$ transporters due to their marked expression in the central nervous system (CNS) and sympathetically innervated tissues. In the brain, PMAT is broadly expressed in many areas that may or may not express uptake$_1$. PMAT is predominantly found in neurons,[11,18] although some studies also reported expression in astrocytes.[49] OCT3 is also broadly expressed in multiple brain regions in both neural and

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astroglial cells.[50,51] In human and rodent brains, the expression of PMAT in most tested brain regions is much higher than that of OCT3, suggesting a critical role of PMAT in monoamine regulation in the CNS.[14] In contrast, OCT3 is highly expressed in adrenal glands and skeletal muscle, where norepinephrine and epinephrine release regulates several physiological responses. Interestingly, the tissue distribution of these two transporters appears to coincide with their preference for individual mono-amines.[14] While PMAT and OCT3 both broadly transport biogenic amines, they exhibit significant kinetic differences in their transport efficiency towards the monoamine neurotransmitters. PMAT shows a strong preference for 5-HT and DA, two major centrally acting neurotransmitters. In contrast, OCT3 favors histamine, norepinephrine, and epinephrine, which act on both central and peripheral systems. Therefore, PMAT may represent the primary uptake$_2$ transporter for 5-HT and DA in the CNS, whereas OCT3 is likely to be the major uptake$_2$ transporter in histaminergic and adrenergic systems.

Analyses of PMAT interaction with a number of clinically used uptake$_1$ inhibitors, including SSRIs and tricyclic antidepressants, showed that PMAT is generally resistant to uptake$_1$ inhibitors at clinically used concentrations.[3,52,53] Based on its strong brain expression and robust *in vitro* transport activity for mono-amines, we hypothesized that PMAT is responsible for clearing released neurotransmitters that have escaped neuronal reuptake by the uptake$_1$ transporters.[3,53] Furthermore, the transporter may play an active role in monoamine uptake in brain areas lacking significant expression of uptake$_1$ or when uptake$_1$ function is compromised by pharmacological inhibition (e.g., chronic use of SSRIs) or by genetic polymorphisms. To date, evidence supporting this hypothesis primarily came from pharmacological studies taking the advantage that PMAT is highly sensitive to D22, but resistant to uptake$_1$ inhibitors (e.g., SSRIs) and the OCT inhibitor corticosterone. Using this approach, we found that PMAT may contribute up to 20–35% of total uptake of 5-HT and DA in mouse brain synaptosomes.[14] In the nucleus tractus solitarii of anaesthetized rats, fast-cyclic voltammetry studies suggested that 5-HT clearance is under the regulation of the PMAT, but not SERT or OCT3.[54] In cultured human astrocytes, which coexpress PMAT and OCT3 but not uptake$_1$ transporters, pharmacological inhibition and gene knockdown studies suggested a major contribution of PMAT to monoamine transport.[26,49] Despite these encouraging results, a direct link of PMAT with monoamine neurotransmission *in vivo* is still missing. With the recent development of a mouse knockout model of Pmat,[15] detailed neurochemistry studies combined with comprehensive behavioral analysis in these animals may hold the key for further understanding the physiological and neurological function of PMAT *in vivo*.

In the CNS, dysregulation of monoamine neurotransmission is critically involved in a number of brain disorders, such as depression, autism, schizophrenia, Parkinson's disease, and drug addiction. Although a recent study in patients with autism spectrum disorder with altered CSF levels of 5-HT suggested a potential association with rare mutations in the PMAT (*SLC29A4*) gene,[55] little is currently known regarding the involvement of PMAT in monoamine-related brain disorders. If PMAT is proven to indeed regulate monoamine neurotransmission *in vivo*, there could be enormous clinical and therapeutic implications for this transporter. Up to 30–40% patients do not satisfactorily respond to currently marketed SSRIs, which act by specifically inhibiting SERT-mediated 5-HT uptake.[56] Compensatory 5-

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HT uptake by PMAT may buffer the effects of these frontline antidepressants, contributing to clinical resistance to these drugs.[42,53] If proven true, PMAT may represent a promising drug target for the development of more effective treatment for depression.

ROLE IN XENOBIOTIC CATION DISPOSITION

Besides endogenous OCs (e.g., biogenic amines), PMAT transports a broad spectrum of structurally diverse xenobiotic cations including clinically used drugs and environmental toxins (Figure 2).). To date, limited studies have been performed to comprehensively characterize the substrate profile of PMAT towards therapeutic drugs. However, given its large substrate overlap with the OCTs, it is reasonable to suspect that many of the OCT substrate drugs are likely to be PMAT substrates. Indeed, metformin, an oral antihyperglycemic and a prototype OCT substrate, is an excellent PMAT substrate.[17] Unpublished data from our laboratory also showed that atenolol, a widely used β-blocker and a substrate of OCT1/2,[57] is also a transportable substrate of PMAT. Besides therapeutic drugs, a number of MPP^+-like neurotoxins, including certain β-carbolines and isoquinolines, have been shown to be transported by PMATs.[20,58] Thus, PMAT may also play a role in the disposition of cationic drugs and toxins in epithelial barrier tissues such as the blood–CSF barrier and the intestinal barrier, where significant expression of PMAT has been demonstrated.

Role at the blood–CSF barrier

The mammalian brain is protected from circulating drugs, toxins, and blood-borne pathogens by the blood–brain barrier formed by the tightly sealed brain capillaries. However, in the lateral, third and fourth brain ventricles, the capillaries are fenestrated, shifting the barrier function to the blood–CSF barrier formed by the tightly jointed choroid plexus epithelial cells.[59] Besides CSF production, the choroid plexus epithelial cells express numerous membrane transporters to actively remove metabolic waste, foreign substances, and excess neurotransmitters from the CSF.[60,61] Earlier studies showed that choroid plexus is able to accumulate monoamine neurotransmitters and possesses uptake activities for xenobiotic OCs.[62,63] This OC transport system may play a significant role in clearing monoamine neurotransmitters, cationic neurotoxins, drugs, and drug metabolites from the brain. Although OCTs have been postulated to play a role in OC transport at the blood–CSF barrier, there is essentially no expression or functional data to support their roles in OC transport in choroid plexus.

In situ hybridization and immunohistochemical studies in rodent brain revealed choroid plexus as one of the brain regions with highest Pmat mRNA and protein expression (Figure 3).[11,18] Expression profiling analysis of *in situ* hybridization data from the Allen Brain Atlas further revealed that Pmat is among the top 10% highly expressed *Slc* genes in the mouse choroid plexus.[60,64] Real-time PCR, western blot, and proteomic analyses also confirmed the abundant expression of PMAT mRNA and protein in human choroid plexus.[15] In contrast, other OC and monoamine transporters, including OCT1-3, MATE1/2-K, and the $uptake_1$ monoamine transporters (SERT, NET, DAT), are minimally expressed in human and mouse choroid plexuses (Figure 3).[15] Immunolocalization studies further demonstrated that

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PMAT protein is localized to the apical CSF-facing membrane of the choroid plexus epithelium.[15] These data strongly suggest a role of PMAT in transporting endogenous monoamines as well as xenobiotic OCs from the CSF into choroid plexus epithelial cells (Figure 3). Once inside the cells, monoamine neurotransmitters (e.g., 5-HT, DA) can be metabolized by monoamine oxidases or catechol-O-methyltransferase that are known to be expressed in the choroid plexus.[65,66] Nonmetabolizable OCs may be further exported into the blood at the basolateral membrane by a mechanism yet to be clarified.

By conducting CSF clearance studies in anesthetized rats, Okura *et al.* first showed that [^{3}H]MPP^+ administered to the left ventricle was quickly eliminated from the CSF, likely through PMAT-mediated CSF-to-blood transport.[12] However, nonselective inhibitors were used in that study. To further evaluate the role of PMAT *in vivo*, we generated a mouse model with targeted deletion of exons 3–7 of the murine *Slc29a4* gene.[15] This resulted in a defective mRNA transcript encoding a short peptide with no transport activity. While the Pmat knockout mice are viable, fertile with no overt physiological abnormalities, the uptake of MPP^+, 5-HT, and DA was severely impaired in choroid plexus from the knockout mice.[15] Furthermore, D22 had no effect on choroid plexus OC uptake in Pmat null mice but reduced the uptake in wildtype mice to the same level seen in knockout mice. In contrast, corticosterone, which strongly inhibits OCT1-3 but not PMAT (Table 2), had no effect on OC uptake in choroid plexus from both wildtype and knockout mice. Similarly, RTI-55, a potent inhibitor of $uptake_1$ monoamine transporters, also showed no effect on 5-HT or DA uptake in choroid plexus.[15] Together, these data demonstrated that PMAT is the principal OC uptake transporter at the blood–CSF barrier responsible for transporting bioactive amines and xenobiotic OCs from the CSF into choroid plexus. OCTs and $uptake_1$ transporters, on the other hand, do not significantly contribute to OC and monoamine transport at the blood–CSF barrier.

Derived from the protoxin MPTP, the PMAT substrate MPP^+ is a dopaminergic neurotoxin that produces Parkinson's syndrome in humans and animal models. CNS exposure to environmental or endogenously produced MPP^+-like toxins, such as paraquat, certain β-carboline and tetrahydroisoquinoline metabolites, has long been implicated in the etiology of Parkinson's disease.[67,68] Using cytotoxicity assays, we showed that two β-carbolines, harmalan and norharmanium, are transportable substrates of PMAT.[20] HEK293 cells expressing PMAT are 14–15-fold more sensitive to the toxicity of harmalan and norharmanium. 1-Benzyl-1,2,3,4-tetrahydroisoquinoline, an endogenously produced neurotoxin with reportedly higher CSF levels in patients with Parkinson's disease,[67] was also reported to be a PMAT substrate.[58] By removing MPP^+-like cationic neurotoxins from the CSF and preventing brain accumulation of cationic neurotoxins, PMAT may play a protective role against Parkinson's disease.

With regard to PMAT expression at the blood–brain barrier, conflicting data have been reported. While we and another group found no significant expression of Pmat in mouse brain endothelial cells,[11,69] two other groups reported its expression in rat and mouse brain capillaries.[12,58] The reason for this discrepancy is unclear, but likely due to the use of more sensitive PCR analysis, potential contamination of brain microvessels by other types of brain cells, or the use of nonspecific antibodies. Nevertheless, the expression of PMAT in brain

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endothelial cells is unlikely to be high, consistent with a low, nonsaturable transport of MPP^+ at the blood–brain barrier as revealed by *in situ* carotid perfusion studies in mice.[69]

Role in metformin absorption

We previously showed that the 58 kDa PMAT protein is expressed in human small intestine and concentrated on the tips of the mucosal epithelial layer.[17] PMAT robustly transports metformin and thus may be involved in intestinal absorption of this widely used antidiabetic drug.[17] PMAT-mediated metformin transport can be further stimulated by an acidic pH, making it an attractive candidate for metformin absorption in the acidic environment of the gut lumen. However, currently there is no *in vivo* data to suggest an impact of PMAT on metformin pharmacokinetics. Two pharmacogenetics studies have examined the impact of single nucleotide polymorphisms (SNPs) of metformin transporters (OCT1, OCT2, OCT3, MATE1, and PMAT) on metformin pharmacokinetics in healthy subjects and diabetic patients treated with metformin.[70,71] No significant association was found between PMAT SNPs and metformin pharmacokinetics and pharmacodynamics. However, these two studies used small sample size and also failed to detect an association with OCT2 and MATE1, which are known to be important determinants of metformin pharmacokinetics. Furthermore, the functional consequence of the studied PMAT SNPs is unknown, as no study has systematically characterized the PMAT SNPs and their allele frequencies in healthy and diabetic populations. Lastly, multiple metformin transporters, including OCT1 and OCT3, are expressed in the intestinal enterocytes, which may compensate and diminish the impact of PMAT.[72] Clearly, more studies are necessary to clarify the role of PMAT in the disposition and clinical response of metformin and other OC drugs.

CONCLUSION

Molecular and biochemical work performed over the past decade has greatly advanced our understanding of PMAT from a previously unknown transporter to an important player in membrane transport of monoamine neurotransmitters and xenobiotic cations. We now know that PMAT is highly and broadly expressed in the brain, and represents a major brain $uptake_2$ transporter for centrally acting monoamine neurotransmitters. By serving as a complementary clearance mechanism for released monoamine neuro-transmitters, PMAT may play a regulatory role in monoamine neurotransmission, and may thus likely be involved in a number of monoamine-related brain disorders. We now also know that PMAT is abundantly expressed at the blood–CSF barrier, specifically localized to the CSF-facing apical membrane, and facilitates OC uptake from the CSF. By doing so, PMAT may be an important determinant of brain exposure to cationic neurotoxins of both endogenous and environmental origins. Nevertheless, despite this progress, we are still at the beginning of appreciating the *in vivo* function and significance of this transporter in health and disease. Elucidating the roles of PMAT in monoamine neurotransmission and exploring its potential as a novel drug target are exciting research directions for this recently identified transporter. Understanding the clinical significance of PMAT in brain and tissue-specific disposition of OC drugs and toxins and exploring its linkage as a risk factor for Parkinson’s disease are equally important research areas for the next decades to come.

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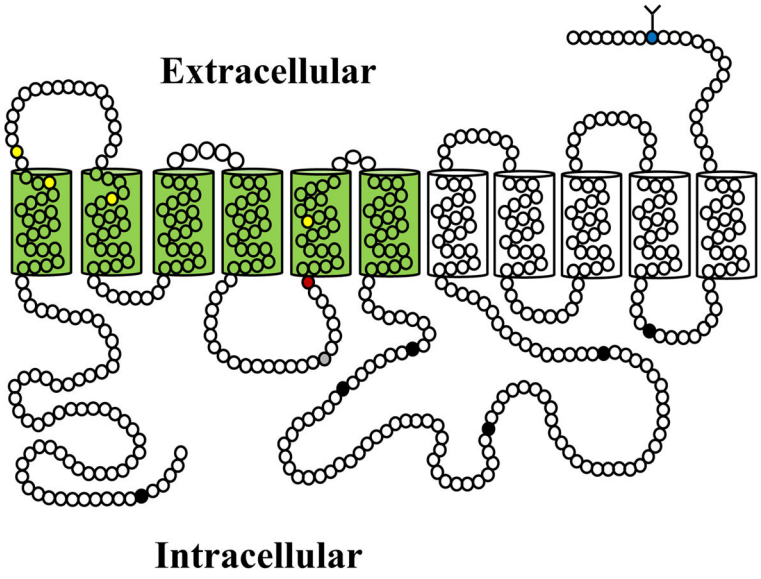

Figure 1.

Proposed topology of human PMAT depicting 11 transmembrane domains. Circles represent individual amino acid residues. Blue circle with branched lines indicates the potential N-linked glycosylation site. PMAT has six consensus sites for protein kinase C phosphorylation (filled black circles) and one cAMP-dependent protein kinase phosphorylation site (filled gray circle). TM1-6 important for substrate recognition are shaded in green. Residues involved in charge recognition (E206, red) and substrate interaction (Y85, Y112, I89, T220; yellow) are indicated.

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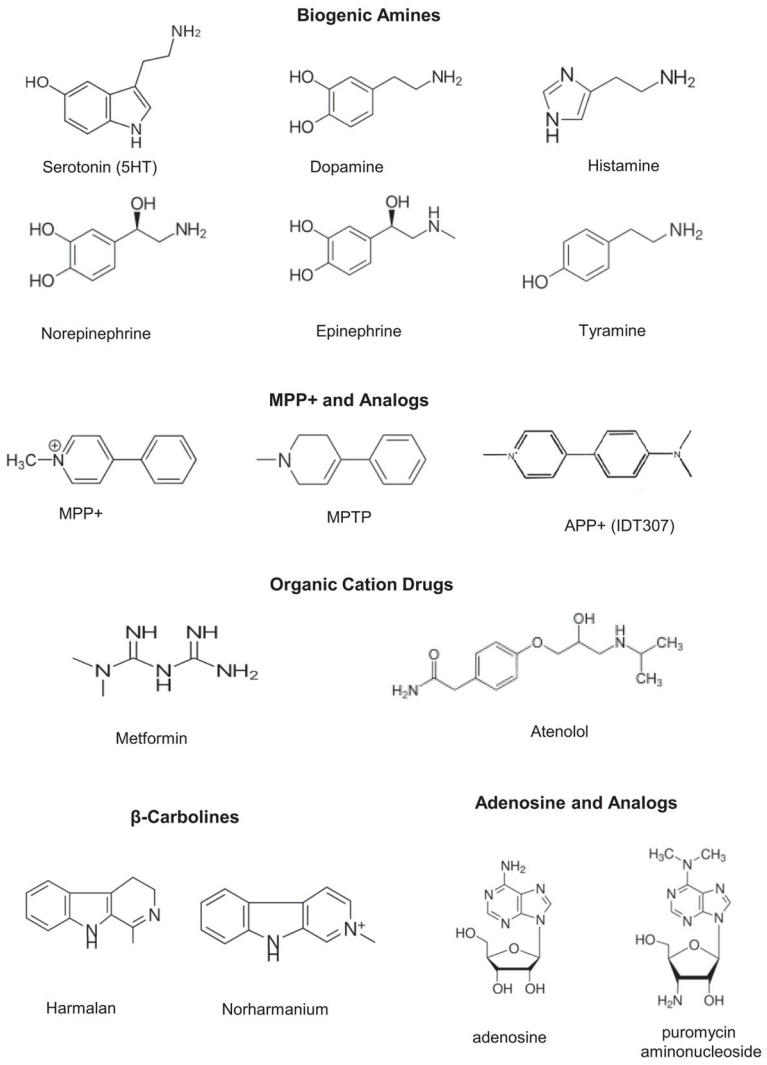

Figure 2.
Structures of selected substrates of PMAT.

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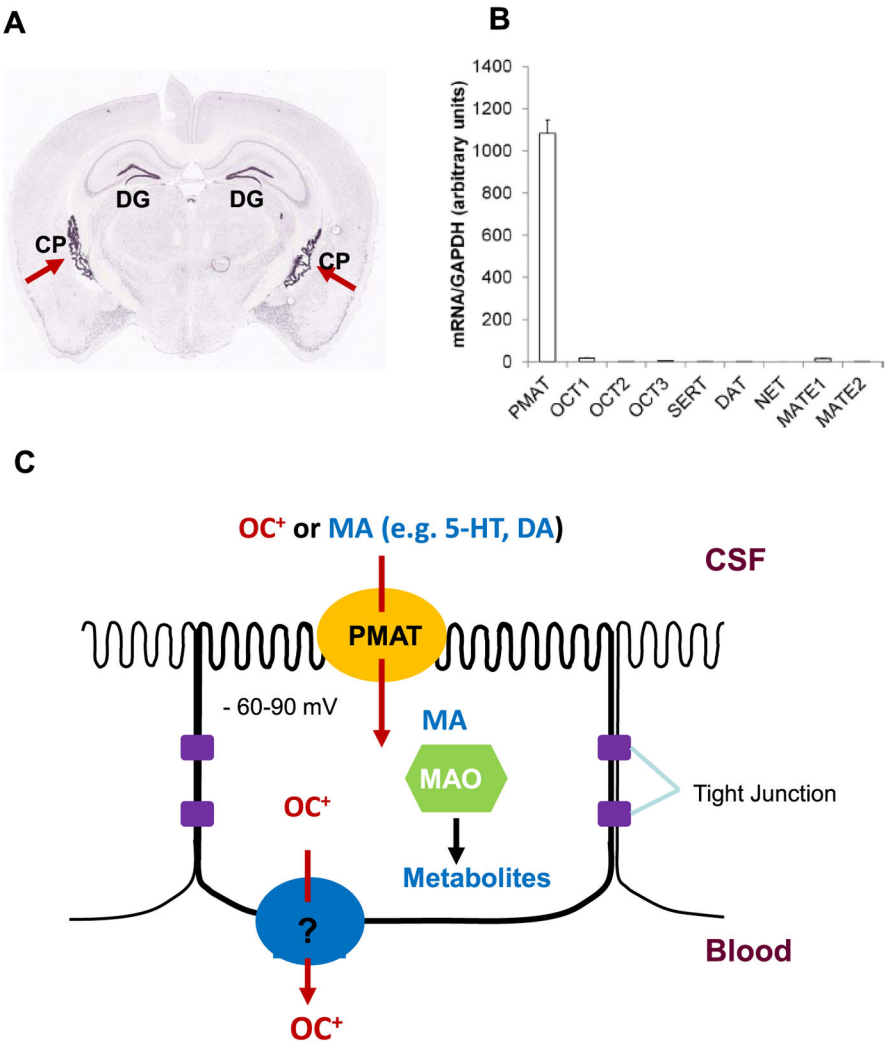

Figure 3.

(**a**) Distribution of Pmat mRNA in a coronal cryosection of mouse brain analyzed by *in situ* hybridization. Image was from the Allen Mouse Brain Atlas (http://mouse.brain-map.org/). Red arrows indicate choroid plexus (CP) in lateral ventricles. DG denotes dentate gyrus of the hippocampal formation. (**b**) Expression of PMAT and functionally related monoamine and organic cation transporters in human choroid plexus quantified by real-time PCR (data taken from Ref. 15). A similar expression pattern was observed in mouse choroid plexus.[15] (**c**) A cellular model for monoamine and organic cation transport at the blood–CSF barrier. MA, monoamine; OC^+, organic cation; MAO, monoamine oxidase; 5-HT, serotonin; DA, dopamine.

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Table 1

Members of the human SLC29 family

	SLC29A1	SLC29A2	SLC29A3	SLC29A4
Gene locus	6p21.1	11q13	10q22.1	7p22.1
Encoded protein (a.a. length)	hENT1 (456 a.a.)	hENT2 (456 a.a)	hENT3 (475 a.a.)	PMAT or ENT4 (530 a.a)
Subcellular location	plasma membrane	plasma membrane	intracellular (lysosomes, mitochondria)	plasma membrane
Transport mode	facilitated carrier electroneutral	facilitated carrier electroneutral	not determined pH-sensitive	electrogenic pH-sensitive
Substrate	nucleosides and related analogs	nucleosides and related analogs	nucleosides and related analogs	monoamines, organic cations (e.g., MPP^+, metformin), adenosine

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Table 2

Ki or IC_{50} values (μM) of selected inhibitors of human PMAT and OCT1-3

	hPMAT	hOCT1	hOCT2	hOCT3
Decynium 22 (D22)	0.10 ± 0.03[a]	0.98 ± 0.31[b]	1.13 ± 0.19[b]	0.09 ± 0.01[b]
Corticosterone	450.5 ± 76.5[a]	21.7 ± 2.44[b]	34.2 ± 6.47[b]	0.29 ± 0.04[b]
Lopinavir	1.4 ± 0.2[c]	174 ± 40.1[c]	N.I.[c]	N.I.[c]

Data are compiled from references 9,[a]22,[b] and 27[c] N.I. denotes no significant inhibition at highest tested inhibitor concentrations.