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Interspecies comparison of the functional characteristics of plasma membrane monoamine transporter (PMAT) between human, rat and mouse

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ABSTRACT

Plasma membrane monoamine transporter (PMAT) is a newly discovered monoamine transporter belonging to the equilibrative nucleoside transporter family. Highly expressed in the brain, PMAT represents a major uptake$_2$ transporter that may play a role in monoamine clearance. Although human PMAT has been functionally characterized at the molecular level, rodent models are often used to evaluate PMAT function in *ex vivo* and *in vivo* studies. The aim of this study was to examine if there is potential species difference in the functional characteristics of PMAT between human, rat and mouse. A set of transfected cells stably expressing human PMAT (MDCK/hPMAT), rat Pmat (MDCK/rPmat) and mouse Pmat (Flp293/mPmat) were constructed. In MDCK/hPMAT, MDCK/rPmat and Flp293/mPmat cells, cellular localization analyses revealed that hPMAT, rPmat and mPmat are expressed and mainly localized to the plasma membranes of cells. The uptake of MPP^+, serotonin and dopamine by MDCK/hPMAT, MDCK/rPmat and Flp293/mPmat cells was significantly increased compared with those by the mock transfection control. In contrast, two nucleosides, uridine and adenosine, minimally interacted with PMAT/Pmat in all species. The hPMAT-, rPmat- and mPmat-mediated uptakes of MPP^+, serotonin and dopamine were saturable, with K_m values of 33.7 μM, 70.2 μM and 49.5 μM (MPP^+), 116 μM, 82.9 μM and 231 μM (serotonin), and 201 μM, 271 μM and 466 μM (dopamine), respectively, suggesting similar substrate affinities between human and rodent PMAT/Pmat. The prototypical inhibitors, decynium 22 and GBR12935, also showed similar inhibition potencies between species. In conclusion, the present study demonstrated interspecies similarities in the functional characteristics of human and rodent PMAT/Pmat, which indicate a practical utility of rat and mouse animal models for further investigating and extrapolating the *in vivo* function of PMAT in humans.

1. Introduction

The plasma membrane monoamine transporter (PMAT) belongs to the family of solute carrier (SLC) 29 transporters, which consists of four members termed human equilibrative nucleoside transporter (ENT) 1–4 (Baldwin et al., 2005). ENT1-3 exclusively interact with nucleosides and nucleobases, while human ENT4 exhibits no significant interaction with nucleosides or nucleobase analogs. We previously demonstrated that human ENT4 mainly interacts with organic cations such as monoamine neurotransmitters (Engel et al., 2004). Due to its distinct substrates specificity from other ENT members, we designated ENT4 to PMAT to be consistent with its physiological substrate profile (Engel et al., 2004).

PMAT is a plasma membrane transporter with low-affinity and high-capacity for monoamine neurotransmitters including dopamine and serotonin (Duan and Wang, 2010; Engel et al., 2004). Because PMAT is highly expressed in the brain, it is thought to play

Abbreviations: DA, dopamine; DAT, dopamine transporter; ENT, equilibrative nucleoside transporters; EPI, epinephrine; 5-HT, serotonin; MDCK, Madin-Darby canine kidney; MPP^+, 1-methyl-4-phenylpyridinium; NE, norepinephrine; NBMPR, *S*-(4-nitrobenzyl)-6-thioinosine; NET, the norepinephrine transporter; OCT, organic cation transporter; PMAT, plasma membrane monoamine transporter; SERT, serotonin transporter.

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an important role in homeostasis of brain monoamines in concert with high affinity neurotransmitter transporters such as the dopamine transporter (DAT), serotonin transporter (SERT), and norepinephrine transporter (NET) (Engel et al., 2004). PMAT is also found in the small intestine and the kidney, which are important organs for drug disposition (The Guideline Development Group, 2008; Zhou et al., 2007a). Indeed, we previously indicated that PMAT is expressed in the apical membrane of human enterocytes and may play a role in oral absorption of metformin, a positively charged antihyperglycemic drug, and possibly other cationic drugs (Zhou et al., 2007a).

Interestingly, the substrate and inhibitor specificity of PMAT largely overlaps with that of the organic cation transporters (OCTs), which belong to the SLC22 transporter family, expressed in various tissues (Engel and Wang, 2005; Koepsell, 2004; Koepsell et al., 2007, 2003). In fact, recent reports showed that biogenic amines, 1-methyl-4-phenylpyridinium (MPP^+), and metformin are transported by both PMAT and OCTs in membrane potential- and Na^+-independent manners (Engel and Wang, 2005; Engel et al., 2004; Koepsell, 2004; Koepsell et al., 2003; Sweet and Pritchard, 1999; Wright and Dantzler, 2004; Zhou et al., 2007a). Accordingly, PMAT is now regarded as a polyspecific organic cation transporter and is considered to play a role in disposition of various organic cations including biogenic amines.

The overlapping substrate specificity between PMAT and OCTs makes it difficult to distinguish the relative contribution of PMAT and OCTs to monoamine and organic cation uptake *in vivo*. As a result, the *in vivo* function of PMAT remains unclear. Experimental animal models, especially gene knock out mice, would be useful in evaluating the contribution of PMAT in monoamine uptake. In addition, rat is frequently used in pharmacokinetic studies. In this case, it is essential to consider potential species differences in PMAT function in study design, data analysis and interpretation. However, most of the functional studies carried out to date have been focused on human PMAT. Although two studies have examined the uptake properties of rat or mouse Pmat towards MPP^+, comprehensive interspecies analyses have not been performed on monoamine substrates and prototype PMAT inhibitors (Okura et al., 2011; Duan and Wang, 2013). In the present study, we focused on the most commonly used animal models rat and mouse to explore potential differences in PMAT function between human and experimental animals. Here, we established transfected cell lines stably expressing human PMAT, rat and mouse Pmats, and performed interspecies comparison for PMAT/Pmat in terms of substrate specificity, monoamine transport kinetics and inhibition potencies towards prototypical inhibitors.

2. Materials and methods

2.1. Materials

Madin-Darby canine kidney (MDCK) cells were obtained from American Type Culture Collection (ATCC, Manassas, VA). Flp-in HEK293 (Flp293) cells were purchased from Invitrogen (Carlsbad, CA). Minimum Essential Media (MEM), Dulbecco's modified Eagle's medium (DMEM), phosphate-buffered saline (PBS), BD Falcon™ 24-well multiwell plates and 60-mm cell culture dishes were purchased from Corning (Corning, NY). Trypsin (0.25%)-EDTA (1 mM) Penicillin-streptomycin mixture was obtained from Gibco (Carlsbad, CA). Fetal bovine serum (FBS) and G418 were obtained from Invitrogen. [^{3}H]MPP^+ (80 Ci/mmol), [^{3}H]uridine (30 Ci/mmol), and [^{3}H]adenosine (30 Ci/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, MO). [^{3}H]5-HT (5-hydroxy-[1,2-^{3}H]tryptamine creatinine sulfate, 27.1 Ci/mmol) and [^{3}H]dopamine (3,4-dihydroxy-[2,5,6-^{3}H]phenylethylamine, 59.7 Ci/mmol) were from PerkinElmer Life Sciences, Inc. (Boston, MA). All other chemicals and general reagents were of analytical grade or better and were obtained from various commercial sources such as Gibco, Corning, Invitrogen or Applied Biosystems (Foster City, CA).

2.2. Cell culture

MDCK cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air using MEM supplemented with 10% FBS, 100 U/mL benzylpenicillin, 100 μg/mL streptomycin. Transfected MDCK cells were cultured in the presence of 200 μg/mL G418. Cells were routinely subcultured at 90% confluency with trypsin (0.25%)-EDTA. For the uptake experiments, cells were plated at a density of 5×10^4 cells/cm^2 in 24-well plates pretreated with 0.1% poly-L-lysine solution and allowed to grow for 3 days.

Flp293 cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air using DMEM (high glucose) supplemented with 10% FBS, 100 U/mL benzylpenicillin, 100 μg/mL streptomycin. Transfected Flp293 cells were cultured in the presence of 150 μg/mL hygromycin B. Cells were routinely subcultured at 90% confluency with trypsin (0.25%)-EDTA. For the uptake experiments, cells were plated in 24-well plates pretreated with 0.1% poly-L-lysine solution and allowed to grow for 2–3 days to reach 80–90% confluence.

2.3. Establishment of transfected cells expressing hPMAT, rPmat or mPmat

The series of full length complementary DNAs (cDNAs) of rat Pmat (rPmat) and mouse Pmat (mPmat) were isolated from respective brains by a reverse transcriptase polymerase chain reaction (RT-PCR) using primers summarized in Table 1. A MDCK cell line stably expressing yellow fluorescent protein (YFP) tagged human PMAT (hPMAT) was previously established (Engel et al., 2004). The full length rPmat cDNA was amplified from rat brain, sequenced, and subcloned into the EcoR I and Bgl II sites of the pEYFP-C1 vector. The plasmid containing rPmat were stably transfected into MDCK cells according to previously described methods (Engel et al., 2004). In brief, MDCK cells were seeded on 24-well plates at a density of 40×10^4 cells/cm^2. After 1 day culture, 2 mg plasmid DNAs of rPmat were transfected into MDCK cells using LipofectAMINE 2000 Reagent (Invitrogen). Empty pEYFP-C1 vector was also transfected into MDCK cells as a control. YFP positive cells were purified using the FACS Vantage S.E. flow cytometry sorter (BD Biosciences, San Jose, CA). Stably transfected cell lines were obtained by G418 selection and cultured in MEM containing 200 μg/mL G418. The transfected cells stably expressing hPMAT and rPamt and the mock transfection control were designated as MDCK/hPMAT, MDCK/rPmat and MDCK/mock cells, respectively.

Recently, we used the Flp-in system to generate HEK293 cell lines stably expressing mPMAT (Duan and Wang, 2013). This system uses Flp recombinase to mediate integration of the transfected gene into the flippase recognition target (FRT) site in the Flp-in host cells, allowing gene expression from a defined

Table 1
Sequences of Primers for RT-PCR Analysis.

Gene	Primer	Sequence
Human PMAT	Sense	5′-GAGAGGCTGCCATGGGCTCCGTGGGGAGC-3′
	Antisense	5′-CGGTCCTCGGAGGACTTTGCAGAACTTCAGTCC-3′
Rat Pmat	Sense	5′-ATGGGCTCCATTGGAAGCCAGCGC-3′
	Antisense	5′-TCAGGGGCCAACAAGGATGGAGTC-3′
Mouse Pmat	Sense	5′-GCCGCTAGCCGCCCGAGTGTGAACTGCCAT-3′
	Antisense	5′-GGTCTCGAGGGCTCAGGGACCGACAGGGAT-3′