

Short Communication

Molecular Cloning, Functional Characterization and Tissue Distribution of Rat H⁺/Organic Cation Antiporter MATE1

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Purpose. Transport characteristics and tissue distribution of the rat H⁺/organic cation antiporter MATE1 (multidrug and toxin extrusion 1) were examined.

Methods. Rat MATE1 cDNA was isolated by polymerase chain reaction (PCR) cloning. Transport characteristics of rat MATE1 were assessed by HEK293 cells transiently expressing rat MATE1. The mRNA expression of rat MATE1 was examined by Northern blot and real-time PCR analyses.

Results. The uptake of a prototypical organic cation tetraethylammonium (TEA) by MATE1-expressing cells was concentration-dependent, and showed the greatest value at pH 8.4 and the lowest at pH 6.0–6.5. Intracellular acidification induced by ammonium chloride resulted in a marked stimulation of TEA uptake. MATE1 transported not only organic cations such as cimetidine and metformin but also the zwitterionic compound cephalexin. MATE1 mRNA was expressed abundantly in the kidney and placenta, slightly in the spleen, but not expressed in the liver. Real-time PCR analysis of microdissected nephron segments showed that MATE1 was primarily expressed in the proximal convoluted and straight tubules.

Conclusions. These findings indicate that MATE1 is expressed in the renal proximal tubules and can mediate the transport of various organic cations and cephalexin using an oppositely directed H⁺ gradient.

KEY WORDS: H⁺/organic cation antiporter; kidney; MATE1; renal secretion.

INTRODUCTION

The secretion of drugs and xenobiotics is an important physiological function of the renal proximal tubules. Functional studies using isolated membrane vesicles and cultured renal epithelial cells have suggested that the renal tubular secretion of cationic substances involves concerted actions of two distinct classes of organic cation transporters: one facilitated by the transmembrane potential difference in the basolateral membrane and the other driven by the transmembrane H⁺ gradient (H⁺/organic cation antiporter) in the brush-border membrane (1,2). The membrane-potential dependent organic cation transporters (OCT1-3, SLC22A1-3) have been identified and well characterized (3–5), but the molecular nature of the H⁺/organic cation antiporter has not been elucidated for a long time.

Recently, based on in silico homology screening, human and mouse orthologues of the multidrug and toxin extrusion (MATE) family, which confers multidrug resistance to bacteria, have been identified (6). The tissue distribution, membrane localization and transport characteristics of human MATE1 suggested that this transporter is similar to transport-

ers of the renal H⁺-coupled organic cation export system (6). However, the precise substrate specificity based on direct uptake measurements and expression profiles of wide-ranging tissues for MATE1 have not been evaluated. So far, we have characterized the functional properties of H⁺/organic cation antiporters in the rat kidney (7–12). In the present study, we isolated rat MATE1 cDNA and investigated the transport characteristics and tissue and intrarenal distribution of this transporter.

MATERIALS AND METHODS

Materials

Cephalexin (Shionogi, Osaka, Japan) and cefazolin (Fujisawa Pharmaceutical Co., Osaka, Japan) were donated by the respective suppliers. [¹⁴C]Tetraethylammonium bromide (TEA) (2.035 GBq/mmol), [¹⁴C]creatinine (2.035 GBq/mmol) and [¹⁴C]procainamide (2.035 GBq/mmol) were obtained from American Radiolabeled Chemicals Inc (St. Louis, MO). [¹⁴C]Metformin (962 MBq/mmol) and [¹⁴C]guanidine hydrochloride (1.961 Gbq/mmol) were purchased from Moravek Biochemicals Inc (Brea, CA). [³H]1-Methyl-4-phenylpyridinium acetate (MPP) (2.7 TBq/mmol) and [¹⁴C]p-aminohippurate (PAH) (1.9 GBq/mmol) were from PerkinElmer Life Analytical Sciences (Boston, MA). [³H]1-Methyl-3-³H]Cimetidine (451 GBq/mmol) was obtained from

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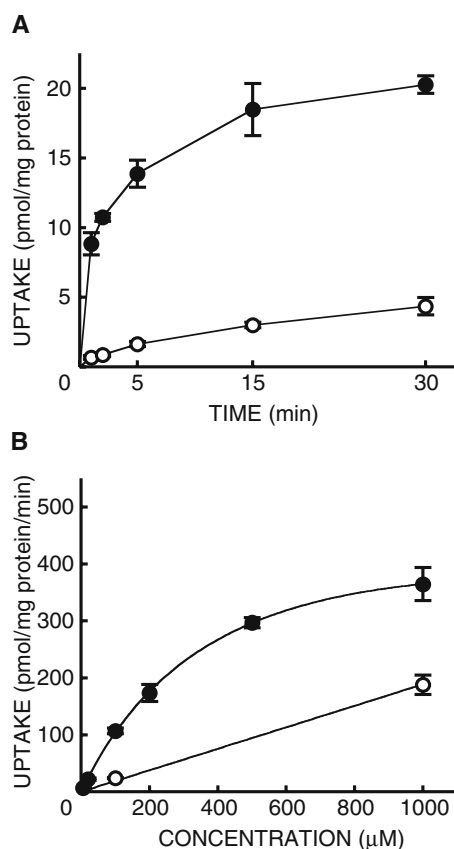


Fig. 1. Transport of [^{14}C]TEA by rat MATE1. **A** Time course of [^{14}C]TEA uptake by HEK293 cells transiently expressing rat MATE1. HEK293 cells transfected with vector alone (pcDNA3.1) (○) or MATE1 cDNA (●) were incubated with 5 μM of [^{14}C]TEA (pH 8.4) at 37°C. Each point represents the mean \pm S.E. for three monolayers. **B** Concentration-dependence of [^{14}C]TEA uptake by HEK293 cells transiently expressing rat MATE1. HEK293 cells transfected with rat MATE1 cDNA were incubated with various concentrations of [^{14}C]TEA (pH 8.4) in the absence (●) or presence of 5 mM TEA (○) for 1 min at 37°C. This figure shows representative data of three separate experiments. Each point represents the mean \pm S.E. for three monolayers.

Amersham Biosciences (Uppsala, Sweden). N^1 -Methylnicotinamide (NMN) was purchased from Sigma (St. Louis, MO). All other chemicals used were of the highest purity available.

cDNA Cloning of Rat MATE1

Rat MATE1 cDNA was isolated from Marathon-Ready rat kidney cDNA (Clontech, Palo Alto, CA) using specific primers designed based on sequence information of the NCBI reference sequence NM_001014118 and human MATE1 (6). The rat MATE1 cDNA was cloned using the following primers: forward 5'-CACATGGAGGTCTTG GAGGAGCCTGCGCCG-3' and reverse 5'-CACAGACT GAGGAGCACCTGCATTGCTGG-3'. The PCR product was subcloned into the expression vector pcDNA3.1 (+) (Invitrogen, Carlsbad, CA), and sequenced by a multicapillary DNA sequencer RISA384 system (Shimadzu, Kyoto, Japan). The nucleotide sequence for the open reading frame of rat MATE1 was identical to the NCBI reference sequence NM_001014118 except for G1608T, which substituted Tyr for

Asp at position 529 (variant 2). This transporter had the same ability to transport [^{14}C]TEA and [^{14}C]metformin as the reference type of MATE1 (variant 1). The nucleotide sequences reported here have been submitted to the DDBJ/EMBL/GenBank Data Bank with Accession No. AB248823 (variant 1) and No. AB248824 (variant 2).

Cell Culture and Transfection

HEK293 cells (American Type Culture Collection CRL-1573) were cultured as described previously (13–15). pcDNA 3.1 (+) containing cDNA encoding rat MATE1 was transfected into HEK293 cells using LipofectAMINE 2000 Reagent (Invitrogen) according to the manufacturer's instructions. At 48 h after the transfection, the cells were used for uptake experiments.

Uptake Experiments in HEK293 Cells

The cellular uptake of various radiolabeled compounds was measured by monolayers grown on poly-D-lysine-coated 24-well plates as described previously (13–15). Typically, the cells were preincubated with 0.2 ml of incubation medium (pH 7.4) for 10 min at 37°C. The medium was then removed, and 0.2 ml of incubation medium (pH 8.4) containing a radiolabeled compound such as [^{14}C]TEA was added. After an appropriate period of incubation, the medium was aspirated, and the monolayers were washed twice with 1 ml of ice-cold incubation medium. The cells were solubilized in 0.5 ml of 0.5 N NaOH, and then the radioactivity in aliquots was determined by liquid scintillation counting. Furthermore, the cellular uptake of cephalosporin antibiotics was measured as described previously (15). For cellular uptake of NMN, HEK293 cells expressing rat MATE1 were incubated with NMN for 1 h, washed two times, and scraped with 0.5 ml of incubation medium (pH 7.4). The accumulation of NMN was determined according to the method of Musfeld *et al.* (16). The conditions for high-performance liquid chromatography (HPLC) were as follows: column, Zorbax ODS column 4.6 mm inside diameter \times 250 mm (Du Pont, Wilmington, DE, USA); mobile phase, 5 mM sodium heptanesulfonate containing 0.5% triethylamine (pH 3.2):acetonitrile = 78:22; flow rate, 1.0 ml/min; excitation and emission wavelengths, 366 and 418 nm, respectively; temperature, 40°C.

Northern Blot Analysis and Real-time PCR for Various Tissues and Microdissected Nephron Segments

The preparation of total RNA and Northern blot analysis under the high-stringency conditions were performed as described previously (17). Total RNA from various rat tissues was purchased from BioChain (Hayward, CA), and reverse transcribed (18). Isolation of total RNA from microdissected nephron segments and reverse transcription (RT) were previously reported (19,20). Using these RT products, real-time PCR was carried out (18). The primer-probe set used for rat MATE1 was as follows: forward primer, 5'-GGG CATCGCTGCTAACCTT-3' (bp 567–585); reverse primer, 5'-CCCCAAGATGTAGCTGATGGA-3' (bp 654–634); fluorescence probe, 5'-(6-Fam) TCAACGCCCTGGCCAA

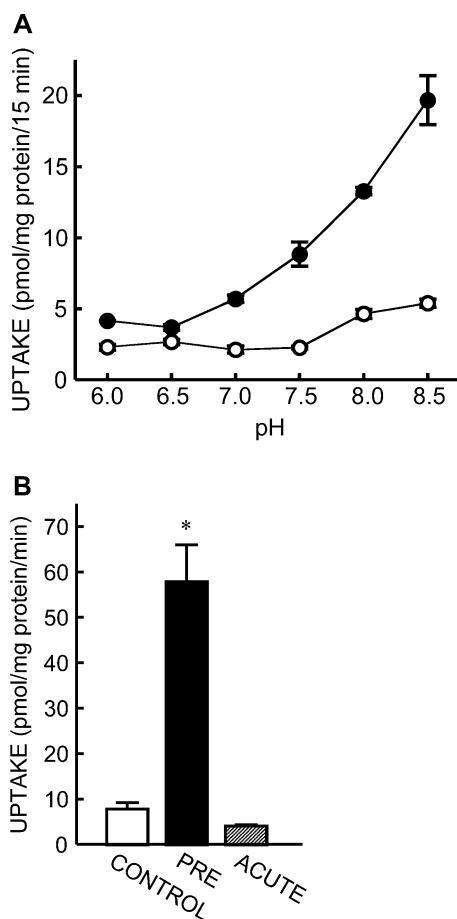


Fig. 2. Effect of pH on the transport of [^{14}C]TEA by rat MATE1. **A** Effect of extracellular pH on [^{14}C]TEA uptake by HEK293 cells transiently expressing rat MATE1. HEK293 cells transfected with vector alone (pcDNA3.1) (○) or rat MATE1 cDNA (●) were incubated with 5 μM of [^{14}C]TEA (pH 8.4) for 15 min at 37°C. **B** Effect of intracellular pH on [^{14}C]TEA uptake by HEK293 cells transiently expressing rat MATE1. HEK293 cells transfected with MATE1 cDNA were preincubated with incubation medium (pH 7.4) in the absence (CONTROL and ACUTE) or presence (PRE) of 30 mM ammonium chloride for 20 min (21). Then, the preincubation medium was removed, and the cells were incubated with 5 μM of [^{14}C]TEA (pH 8.4) in the absence (CONTROL and PRE) or presence (ACUTE) of 30 mM ammonium chloride for 1 min at 37°C. Each point represents the mean \pm S.E. for three monolayers. * $P < 0.05$, significantly different from CONTROL.

CTATCTGTTT (Tamra)(phosphate)-3' (bp 587–612). The primer-probe sets used for rat OCT1 and OCT2 were pre-developed TaqMan Assay Reagents (Applied Biosystems, Foster, CA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was also measured as an internal control with GAPDH Control Reagent (Applied Biosystems).

Statistical Analyses

Data were analyzed statistically using a non-paired t test (Fig. 3B) or a one-way analysis of variance followed by Sheffé's test (Fig. 2B). P values of less than 0.05 were considered significant. In all figures, when error bars are not shown, they are smaller than the symbol.

RESULTS

We cloned rat MATE1 cDNA from rat kidney by PCR cloning. Rat MATE1 showed 79% amino acid identity with human MATE1, 91% with mouse MATE1, 48% with human MATE2 and 57% with mouse MATE2 (6). When rat MATE1 was expressed in HEK293 cells, a time- and concentration-dependent uptake of [^{14}C]TEA was observed (Fig. 1). The uptake by MATE1 exhibited saturable kinetics, following the Michaelis–Menten equation. The apparent K_m value of the uptake was calculated at $570 \pm 64 \mu\text{M}$.

Figure 2 shows the effects of changes in extra- and intracellular pH on [^{14}C]TEA uptake by MATE1. When the extracellular pH was changed from 6.0 to 8.4, the uptake was greatest at pH 8.4 and lowest at pH 6.0–6.5. Intracellular pH

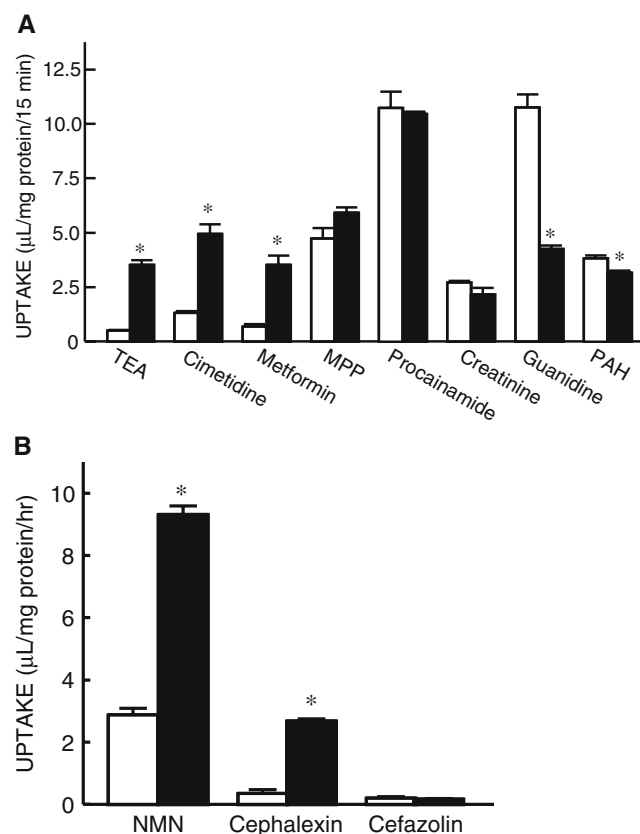


Fig. 3. Uptake of various compounds by rat MATE1. **A** Uptake of various radiolabeled compounds by HEK293 cells transiently expressing rat MATE1. HEK293 cells transfected with vector alone (pcDNA3.1) (open column) or MATE1 cDNA (closed column) were incubated with [^{14}C]TEA (5 μM), [^3H]cimetidine (23 nM), [^3H]MPP (3.8 nM), [^{14}C]metformin (10 μM), [^{14}C]creatinine (5 μM), [^{14}C]guanidine (5 μM), [^{14}C]procainamide (5 μM) or [^{14}C]PAH (5 μM) for 15 min at 37°C. After the incubation, the radioactivity of solubilized cells was determined. **B** Uptake of NMN, cephalixin and cefazolin by HEK293 cells transiently expressing rat MATE1. HEK293 cells transfected with vector alone (pcDNA3.1) (open column) or MATE1 cDNA (closed column) were incubated with each compound (1 mM) for 1 h at 37°C. After the incubation, amounts of each compound extracted from cells were determined by HPLC. Uptake was expressed as clearance, which was obtained by dividing the net uptake value by the concentration of each substrate in the medium. Each column represents the mean \pm S.E. for three monolayers.

was manipulated by treating the cells with ammonium chloride according to a previous report (21). When ammonium chloride is added to a preincubation medium and then removed (pretreatment), the intracellular pH falls. However, the exposure of cells to ammonium chloride (acute treatment) causes a rapid alkalization of the intracellular pH. As shown in Fig. 2B, intracellular acidification through pretreatment resulted in a marked stimulation of [14 C]TEA uptake, whereas the uptake was reduced by acute treatment.

We then examined the substrate specificity of MATE1. As shown in Fig. 3, MATE1 mediated the transport of several organic cations with different chemical structures such as [14 C]TEA, [3 H]cimetidine, [14 C]metformin, and NMN. In addition, MATE1 transported the zwitterionic cephalosporin cephalixin, which was demonstrated to be a substrate for the rat H⁺/organic cation antiporter in the membrane vesicle studies (8). In contrast, MATE1 did not transport cationic compounds such as [3 H]MPP, [14 C]procainamide, [14 C]creatinine and [14 C]guanidine, the anionic compound [14 C]PAH, or the anionic cephalosporin cefazolin.

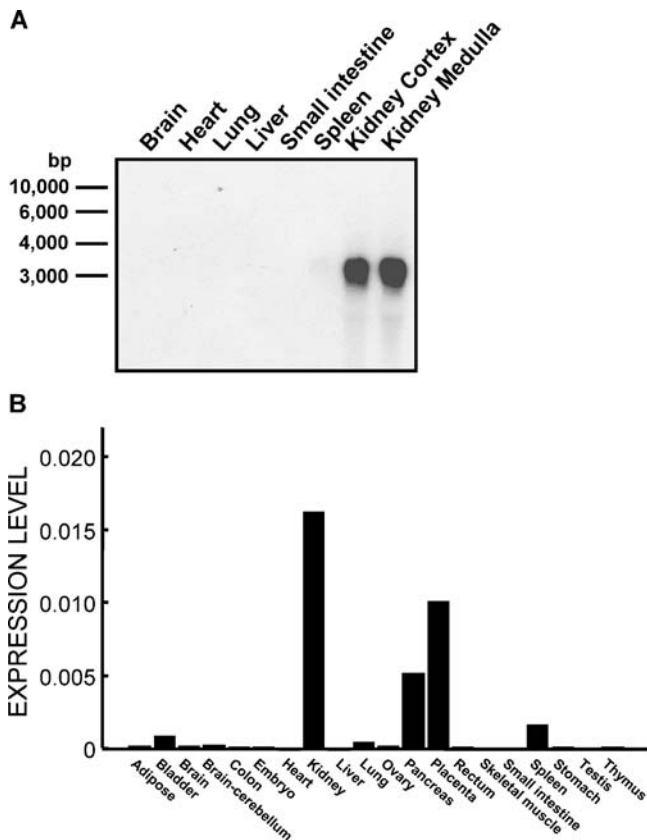


Fig. 4. Tissue distribution of rat MATE1. **A** Northern blot analysis of MATE1 mRNA in rat tissues. Total RNA (30 μ g) from the indicated tissues was electrophoresed, blotted, and hybridized with a specific probe for rat MATE1 under high stringency conditions. **B** Real-time PCR for MATE1 mRNA in rat tissues. RNA from various rat tissues was reverse-transcribed, and rat MATE1 and GAPDH mRNA levels were determined by real-time PCR using an ABI PRISM 7700 sequence detector. The mRNA expression level of rat MATE1 is represented as a ratio of that of GAPDH in each tissue. Each column represents the mean for two separate experiments.

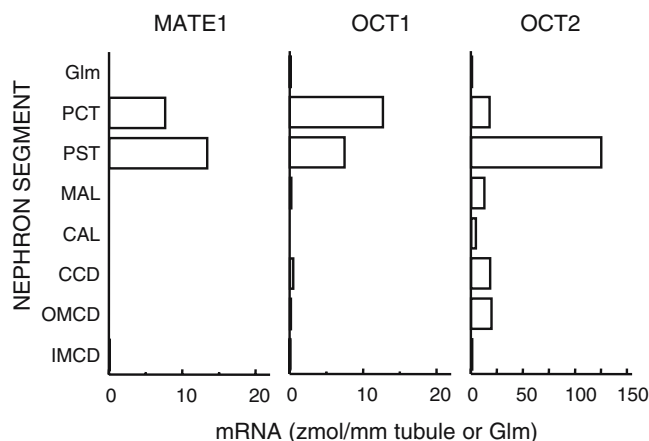


Fig. 5. Distribution of rat MATE1, OCT1 and OCT2 mRNAs along the microdissected renal nephron segments. Each PCR was performed using part of RT reaction derived from 10 glomeruli and 5 mm of renal tubules. The mRNA expression levels of rat MATE1, OCT1 and OCT2 were determined by real-time PCR using an ABI PRISM 7700 sequence detector. Each column represents the mean for two separate experiments. *Glm* glomerulus, *PCT* proximal convoluted tubule, *PST* proximal straight tubule, *MAL* medullary thick ascending limb, *CAL* cortical thick ascending limb, *CCD* cortical collecting duct, *OMCD* outer medullary collecting duct, *IMCD* inner medullary collecting duct.

Finally, tissue distribution of rat MATE1 mRNA was examined. High-stringency Northern blot analysis revealed that a transcript (about 3.5 KB) of rat MATE1 was strongly expressed in the kidney cortex and medulla, and faintly in the spleen (Fig. 4A). No positive signal was detected in other tissues, including the brain, small intestine and liver (Fig. 4A). The results of real-time PCR analyses using RNA from various rat tissues were consistent with the results of the Northern blot analysis. MATE1 mRNA was highly expressed in the kidney and placenta, and slightly expressed in the pancreas, spleen, bladder and lung (Fig. 4B).

To determine the distribution of rat MATE1 mRNA along the nephron segments, real-time PCR analysis using microdissected nephron segments was performed, as comparing with those of rat OCT1 and OCT2 mRNA. As shown in Fig. 5, rat MATE1 mRNA was primarily expressed in the proximal convoluted tubule (PCT) and proximal straight tubule (PST). The expression of rat OCT1 mRNA was also found in the PCT and PST. Rat OCT2 mRNA was highly expressed in PST with about 10 fold higher expression level as compared with that of rat MATE1 or OCT1 mRNA, and significant expression was also detected in other segments such as PCT, outer medullary collecting duct (OMCD) and cortical collecting duct (CCD). Distribution of rat OCT1 and OCT2 mRNA was corresponded to a previous report (20).

DISCUSSION

Using renal brush-border membrane vesicles, the transport of TEA (7,22,23), aminocephalosporins (8), cimetidine (9,24,25), NMN (26) and procainamide (27) was examined, and demonstrated to be actively driven by an outwardly directed H⁺ gradient via the H⁺/organic cation antiport

system. By using a renal epithelial cell line LLC-PK₁, the apical transport of TEA (28,29) and procainamide (30) was demonstrated to be mediated by the H⁺/organic cation antiport system. In the present study, we found that most of these organic compounds were transported by rat MATE1. However, procainamide was not transported, although rat OCT1 and OCT2 can transport procainamide (unpublished data). As the transport of procainamide via the H⁺/organic cation antiport system was demonstrated in rabbit renal brush-border membrane vesicles (27) and porcine LLC-PK₁ cells (30), a species difference may be responsible for this discrepancy. Using human renal brush-border membrane vesicles, Chun *et al.* (31) found that transport of guanidine was stimulated by H⁺ gradient, and that the mechanism involved was distinct from that for the transport of TEA and NMN. Although species differences should be taken into consideration, the present findings that guanidine was not transported by rat MATE1 may support the membrane vesicle study (31). Taken together, these findings suggest that other MATE isoforms reported in humans (6) may be involved in the transport of procainamide and guanidine in the renal brush-border membrane. Further studies are needed to clarify the molecular mechanisms responsible for the diversity of organic cation transport in the renal brush-border membrane.

Otsuka *et al.* (6) reported that [¹⁴C]TEA transport by human MATE1 was inhibited by MPP, but not by NMN. In the present study, rat MATE1 was able to transport NMN, but not MPP. These findings suggest that human and rat MATE1 have different properties of substrate recognition for these compounds. Experimental conditions such as inhibition studies (6) and our uptake studies may also explain the different findings.

Rat MATE1 mRNA is highly expressed in the kidney, especially in the proximal tubules, and placenta, but not in the liver. In contrast, human MATE1 is preferentially expressed in the kidney and liver, but not in the placenta (6), indicating a clear species difference in the distribution of MATE1 mRNA between human and rat. Previous studies using vesicles prepared from rat sinusoidal membranes (32) and from human term placenta (33) revealed the presence of a H⁺/organic cation antiport system in these tissues. Detailed functional characterization suggested that the system in the liver and placenta differs from the renal system (32,33), and MATE1 mRNA expression in rat and human tissues supports these assumptions. Other molecules than MATE1 may be responsible for the H⁺/organic cation antiport in the rat liver and human placenta.

The transport of organic cations such as TEA and cimetidine in the renal brush-border membrane was mediated by H⁺/organic cation antiport system energized by a transmembrane H⁺ gradient (1,2). Since the luminal pH is more acidic than the intracellular pH in the proximal tubules (34), due to an Na⁺/H⁺ antiporter and/or ATP-driven H⁺-pump, it is reasonable to assume that the inward H⁺ gradient (luminal pH < intracellular pH) can drive the secretion of organic cations *in vivo*. Human MATE1 exhibited pH-dependent transport of TEA in cellular uptake and efflux studies (6), but these analyses are not enough to prove the H⁺/TEA antiport mechanism. It is possible that pH-dependent transport of TEA may be due to the modulation of

transport activity caused by changes in extracellular pH. In the present study, we found that intracellular acidification by ammonium chloride pretreatment resulted in the marked stimulation of [¹⁴C]TEA uptake by MATE1, suggesting that outwardly directed H⁺ gradient serves as a driving force for MATE1 (Fig. 2B).

In conclusion, rat MATE1 is abundantly expressed in the renal proximal tubules, and can accept various compounds including organic cations and zwitterionic cephalosporin as substrates. Changes in extra- and intracellular pH suggested that an oppositely directed H⁺ gradient works as a driving force for MATE1. Although further studies are needed to elucidate the physiological and pharmacokinetic roles of this transporter, the present findings can provide important information about the renal tubular secretion of organic cations.

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