Functional characterization of testis-specific rodent multidrug and toxic compound extrusion 2, a class III MATE-type polyspecific H⁺/organic cation exporter

Miki Hiasa, Takuya Matsumoto, Toshinori Komatsu, Hiroshi Omote, and Yoshinori Moriyama

Department of Membrane Biochemistry, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

Submitted 3 July 2007; accepted in final form 12 August 2007

Hiasa M, Matsumoto T, Komatsu T, Omote H, Moriyama Y. Functional characterization of testis-specific rodent multidrug and toxic compound extrusion 2, a class III MATE-type polyspecific H⁺/organic cation exporter. Am J Physiol Cell Physiol 293: C1437–C1444, 2007. First published August 22, 2007; doi:10.1152/ajpcell.00280.2007.—Mammalian multidrug and toxic compound extrusion (MATE) proteins are classified into three subfamilies: classes I, II, and III. We previously showed that two of these families act as polyspecific H⁺-coupled transporters of organic cations (OCs) at final excretion steps in liver and kidney (Otsuka et al. Proc Natl Acad Sci USA 102: 17923–17928, 2005; Omote et al. Trends Pharmacol Sci 27: 587–593, 2006). Rodent MATE2 proteins are class III MATE transporters, the molecular nature, as well as transport properties, of which remain to be characterized. In the present study, we investigated the transport properties and localization of mouse MATE2 (mMATE2). On expression in human embryonic kidney (HEK)-293 cells, mMATE2 localized to the intracellular organelles and plasma membrane. mMATE2 mediated pH-dependent TEA transport with substrate specificity similar to, but distinct from, that of mMATE1, which prefers N-methylnicotinamide and guanidine as substrates. mMATE2 expressed in insect cells was solubilized and reconstituted with bacterial H⁺-ATPase into liposomes. The resultant proteoliposomes exhibited ATP-dependent uptake of TEA that was sensitive to carbonyl cyanide 3-chlorophenylhydrazone but unaffected by valinomycin in the presence of K⁺. Immunologic techniques using specific antibodies revealed that mMATE2 was specifically expressed in testicular Leydig cells. Thus mMATE2 appears to act as a polyspecific H⁺/OC exporter in Leydig cells. It is concluded that all classes of mammalian MATE proteins act as polyspecific and electroneutral transporters of organic cations.

organic cation transporter; Leydig cell; guanidine; N-methylnicotinamide

**MULITDRUG TRANSPORTERS** are responsible for multidrug resistance in prokaryotes and eukaryotes through extrusion of xenobiotics and toxic metabolites from cells. Multidrug transporters have been identified and classified into at least five major families (4, 15). These include the major facilitator superfamily, the small multidrug resistance family, the resistance nodulation cell division family, and the ATP-binding cassette family (4, 15). ATP-binding cassette transporters are membrane ATPases that directly transport drugs on hydrolysis of ATP; other families are secondary transporters, with transport activities that are coupled to an electrochemical gradient of protons or Na⁺ established across the membrane. The multidrug and toxic compound extrusion (MATE) family, the most recently classified multidrug resistance-conferring transporter protein in bacteria, mediates H⁺- or Na⁺-coupled export of cationic drugs such as norfloxacin and ethidium (1, 4, 10, 13, 15). Genome-wide analysis has revealed that the MATE family comprises 1,000 sequenced proteins from all three domains of life, Eukarya, Archaea, and Eubacteria, indicating that the MATE family is one of the common constituents of life (13). In 2005, we cloned the cDNAs encoding the human and mouse orthologs of the bacterial MATE-type transporter and named them hMATE1 (SLC47A1) and mMATE1 and hMATE2 (SLC47A2) and mMATE2, respectively, as the first MATE-type transporters from animals (14).

hMATE1 and mMATE1 share 78.1% amino acid sequence identity and are predominantly expressed in luminal membranes of renal tubular cells as well as bile canaliculi (3, 14). On expression in HEK-293 cells, these transporters localize to the plasma membrane and mediate electroneutral H⁺/organic cation (OC) exchange (3, 14). Subsequently, a rat MATE1 (rMATE1) and rabbit MATE1 (rbMATE1) with similar localization and transport properties were also identified (12, 20, 24). Hence, the substrate specificity, energetics, and localization of these transporters are very similar to those of an electroneutral H⁺/OC exchanger(s) characterized in renal brush border membrane vesicles, the molecular identity of which has been long anticipated (2, 5, 7, 21–23). MATE1 proteins are considered to be the principal transporters responsible for the elimination of various OCs at the final excretion step in liver and kidney (13, 14).

In contrast to MATE1 transporters, hMATE2 and mMATE2 exhibit only low mutual sequence identity (38.1%) and different expression patterns (14). hMATE2 is predominantly expressed in kidney, but not in other organs, including liver, whereas mMATE2 is specifically expressed in testis (14). Since hMATE2-K, a splicing variant of hMATE2, and rabbit MATE2-K, a rabbit counterpart of hMATE2-K, were shown to mediate electroneutral H⁺/OC exchange at the luminal surface of renal tubular cells (8, 24), hMATE2 seems to be a renal-specific electroneutral OC exporter (13). It is uncertain whether the physiological function of mMATE2 is similar to that of hMATE2 (14).

Phylogenet analysis has shown that mammalian MATE transporters can be classified into three subclasses: classes I, II,
FUNCTION OF CLASS III MATE TRANSPORTER

Fig. 1. Classification of mammalian multidrug and toxic compound extrusion (MATE)-type transporters. A: phylogenetic tree of mammalian MATE-type transporters clearly suggests 3 subclasses of MATE transporters: class I (red), class II (blue), and class III (green). B: multiple-sequence alignment of mammalian class III transporters. *: conserved amino acid residues. Horizontal bars indicate putative transmembrane regions. Peptide regions used in the physiological function of MATE transporters belonging to class III. Therefore, in the present study, we have characterized the transport properties and localization of mMATE2. We have shown that mMATE2 also behaves as a polyspecific H\(^+\)/OCs exchanger with substrate specificity similar to, but distinct from, that of hMATE1 and mMATE1 in testicular Leydig cells.

MATERIALS AND METHODS
cDNA. cDNA of mMATE2 (GenBank accession no. NP_001028714) was cloned by RT-PCR from mouse testis RNA (14). The sequence of mMATE2 cDNA was confirmed by comparison with the mouse genome sequence (16).

mMATE2-expressing HEK-293 cells. mMATE2 cDNA was amplified by PCR using the primers 5’-CACCGAATTCATGGAGGCGGCGAGGACA-3’ and 5’-CGTACTCGAGTTAGCCACCGTCTAGAAA-3’ and ligated into a pENTR/D-TOPO vector (Invitrogen). mMATE2 cDNA was transferred from the pENTR/D-TOPO vector to pcDNA3.1/nV5-DEST (Invitrogen). This plasmid, pcDNA3.1/nV5-DEST-mMATE2, was used to transfect HEK-293 cells by lipofection using TransIT reagent (Mirus). HEK-293 cells were grown in DMEM containing 10% fetal calf serum, penicillin, and streptomycin at 37°C under 5% CO\(_2\), as described elsewhere (14). After 24 h, 1.5 x 10\(^6\) cells per 10-cm dish were transfected with 10 \(\mu\)g of pcDNA3.1/nV5-DEST-mMATE2. After 2 days of incubation, the cells were used for immunohistochemistry and transport assay (see below). About 15% of HEK-293 cells were transfected under these conditions.

Expression of mMATE2 in insect cells. Recombinant baculoviruses containing mMATE2 cDNA were constructed using the Bac-to-Bac baculovirus expression system (Invitrogen) according to the manufacturer’s protocol. mMATE2 cDNA was transferred from the pENTR/D-TOPO vector to a destination vector (pDEST10-mMATE2). DH10Bac cells carrying bacmid DNA were transformed with pDEST10-mMATE2. Recombinant bacmid was isolated from DH10Bac cells and used for transfection of Sf9 cells to generate recombinant baculoviruses. Sf9 cells (5 x 10\(^6\) cells per 10-cm dish) were grown in complete TMN-FH insect culture medium (GIBCO) supplemented with 10% fetal calf serum, 0.25 \(\mu\)g/ml amphotericin B (Fungizone), and 100 \(\mu\)g/ml penicillin-streptomycin at 27°C. Sf9 cells were infected with recombinant baculoviruses at a multiplicity of infection of 2 and cultured for 72 h. Then the cells were harvested for membrane preparation. mMATE1 was also expressed in Sf9 cells as described above.

Solubilization and reconstitution of mMATE2. Sf9 cells (1–2 x 10\(^7\)) were suspended in a buffer containing 20 mM Tris·HCl (pH 8.0), 0.1 M potassium acetate, 10% glycerol, 0.5 mM dithiothreitol, 1 \(\mu\)g/ml pepstatin A, and 1 \(\mu\)g/ml leupeptin and disrupted by sonication with a tip sonifier (model UD200, Tomy). Debris was removed by centrifugation of cell lysates at 700 g for 10 min, and the resultant supernatant was centrifuged again at 160,000 g for 1 h. The pellet was suspended in buffer containing 20 mM MOPS·Tris (pH 7.0), 10% glycerol, 1 \(\mu\)g/ml pepstatin A, and 1 \(\mu\)g/ml leupeptin to give a protein concentration of ~1.5 mg/ml. Then octylglucoside was added to the mixture to give a final concentration of 2%. The mixture was vigorously vortexed and centrifuged at 260,000 g for 30 min, and the supernatant (solubilized mMATE2 fraction) was carefully collected. Coreconstitution of solubilized mMATE2 fraction with bacterial F-type ATPase into liposomes was carried out by the freeze-thaw method, as previously described (6, 11). Briefly, 300 \(\mu\)g of solubilized mMATE2 fraction were mixed with 60 \(\mu\)g of F-type ATPase and 0.5 mg of asolectin liposomes. The mixture was frozen at ~80°C, thawed rapidly, and diluted 60-fold with a buffer containing 20 mM MOPS·NaOH (pH 7.0), 0.5 mM dithiothreitol, 0.1 M potassium acetate, and 5 mM magnesium acetate. Reconstituted proteoliposomes were pelleted by centrifugation at 160,000 g for 1 h at 4°C and suspended in 0.4 ml of buffer containing 20 mM MOPS·NaOH (pH 7.0), 100 mM KCl, and 5 mM magnesium acetate. Bacterial F-type ATPase was prepared as described previously (11).

Transport assay. For HEK-293 cells expressing mMATE2, pH-dependent TEA uptake was measured by previously published procedures (3, 14, 19). Briefly, the cells were suspended in a buffer containing 25 mM tricine-NaOH (pH 8.0), 125 mM NaCl, 4.8 mM...
KCl, 5.6 mM d-glucose, 1.2 mM CaCl2, 1.2 mM KH2PO4, and 1.2 mM MgSO4. The assay was initiated by addition of 50 µM radiolabeled TEA (5 kBq/assay; PerkinElmer Life Science) at 37°C. At the times indicated, aliquots (200 µl) were taken and filtered through 0.45-µm HA membrane filters (Millipore). The filter was washed once with 5 ml of ice-cold assay medium, and the radioactivity remaining on the filter was counted. Reconstituted proteoliposomes (5 µg of protein) were suspended in a buffer containing 20 mM MOPS-NaOH (pH 7.0), 5 mM magnesium acetate, and 100 mM KCl. ATP, at a final concentration of 4 mM, was added to the assay medium, and the mixture was incubated further for 5 min. The assay was initiated by addition of 50 µM radiolabeled TEA (5 kBq/assay; PerkinElmer Life Science). At the times indicated, aliquots (130 µl) were taken, applied to a Sephadex G-50 (fine) spin column poured in the cylinder of a 1-ml disposable syringe, and immediately centrifuged at 180 g for 1 min for separation of the proteoliposomes from the assay medium (6, 11). The radioactivity and protein concentration in the eluate were measured.

**Antibodies.** Site-specific rabbit polyclonal antibodies against mMATE2 were produced by repeated injections of glutathione S-transferase-fusion polypeptides comprising amino acid residues M1–A46 of mMATE2, a region specific to mMATE2 (Fig. 1B): MEPAEDSLGATQIPPELVRVPRGRSLRILLGLRGALSPDVRREAAA. Preabsorbed antiserum was prepared on incubation of antiserum (50 µl) with antigenic peptide (2 mg) in an ice bath for 10 h. Antiserum against mMATE1 was prepared as described previously (3). Monoclonal antibodies against Rab5 and early endosome antibody (EEA)-1 for early endosomes were obtained from Transduction Laboratories. Alexa Fluor 568-labeled anti-peptide (2 mg) in an ice bath for 10 h. Antiserum against mMATE2 was preabsorbed with 58, 45, and 26% sequence identity to hMATE1, a member of class 1; hMATE2, a member of class 2; and Vibrio parahaemolyticus NorM Na+/multidrug antiporter, a prototype of the MATE family (1, 10), respectively (Fig. 1). Similar to other mammalian MATE transporters, mMATE2 hypothetically has 12 transmembrane helices.

As the first step of the study, we expressed mMATE2 in HEK-293 cells with the intention of characterizing the transport properties of mMATE2. We generated a specific antiserum against mMATE2 (Fig. 1B). The antiserum recognized mMATE2 as a ~60-kDa protein, but not mMATE1 (Fig. 2A). The slightly lower mobility of mMATE1 and mMATE2 is due to the histidine tag fused to the MATE proteins expressed in S9 cells. When mMATE2 was expressed in HEK-293 cells, the protein migrated on the SDS-polyacrylamide gels with a relative mobility corresponding to 52 kDa and localized not only to the plasma membrane, but also to intracellular organelles (Fig. 2, B and C). The immunoreactivity disappeared when the antiserum was preabsorbed (Fig. 2, B and C). Double labeling with Rab5 and EEA-1, markers for early endosomes, indicated that intracellular mMATE2 and these markers roughly colocalized, suggesting that mMATE2 was present in early endosomes and plasma membrane (Fig. 2D).

Both hMATE1 and mMATE2 are predominantly found in the plasma membrane on expression in HEK-293 cells; thus the pH-dependent translocation of OCs across the plasma membrane could be easily measured (3, 14). However, here, the presence of mMATE2 in the plasma membrane and the intracellular organelles of HEK-293 cells made interpretation of the kinetic properties of mMATE2 difficult. Thus we carefully assessed mMATE2-mediated OC transport using two independent procedures: 1) pH-dependent uptake of TEA by mMATE2-expressing HEK-293 cells and 2) pH-dependent uptake of TEA by proteoliposomes containing mMATE2.

mMATE2-expressing cells exhibited time-dependent transport activity of TEA (Fig. 3A), a typical substrate for H+-coupled OC exporter (2, 4, 5, 7, 13, 21–23). The transport activity of mMATE2 was saturable with respect to substrate concentration, with and Vmax for TEA of 710 µM and 400 pmol·min⁻¹·mg protein⁻¹, respectively (Fig. 3B). The transport was weakly pH dependent (Fig. 3C); transport activity was lower at pH 6.0–6.5, increased slightly with increasing extracellular pH, and was maximal at pH ~8.5. The addition of 10 µM cyanide 3-chlorophenylhydrazine (CCCP), which dissipates the proton electrochemical gradient across the membranes, inhibited the uptake, whereas 1 µM valinomycin in the presence of 65 mM KCl, which causes membrane depolarization, did not have much effect (Fig. 3D). Neither Na⁺ nor Cl⁻ was required for the transport activity (Fig. 3E). The pharmacology of the cis inhibition of TEA transport by mMATE2 was similar to, but distinct from, that of hMATE1 (14) and mMATE1 (3) (Table 1). It is strongly inhibited by cimetidine, methylphénylpyridinium, and rhodamine 123 (Table 1), but not by organic anions such as p-aminohippurate and uric acid (data not shown). N-methyl nicotinamide (NNN) and guanidine inhibited mMATE2-mediated TEA uptake (Table 1), whereas

**RESULTS**

mMATE2 acts as a polyspecific OC transporter. The cDNA for mMATE2 encodes a 573-amino acid protein with 44–95% sequence identity to proteins of the MATE3 subclass, but with 58, 45, and 26% sequence identity to hMATE1, a member of class 1; hMATE2, a member of class 2; and Vibrio parahaemolyticus NorM Na⁺/multidrug antiporter, a prototype of the MATE family (1, 10), respectively (Fig. 1). Similar to other mammalian MATE transporters, mMATE2 hypothetically has 12 transmembrane helices.
these compounds did not significantly affect TEA transport by hMATE1 and mMATE1 (3, 14). Testosterone, procainamide, and choline moderately inhibited TEA uptake (Table 1). Although this transport assay did not consider any contributions from intracellular mMATE2, these results are consistent with the idea that mMATE2 is an H⁺-coupled OC exporter.

**Reconstitution of mMATE2 with bacterial F-type ATPase.** To obtain direct evidence for H⁺ coupling to mMATE2-mediated TEA uptake, mMATE2 expressed in insect Sf9 cells was solubilized with octylglucoside and incorporated with purified bacterial F-type ATPase into liposomes. This assay system contains no latent transporter activity, such as that

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**Fig. 2.** Expression of mouse MATE2 (mMATE2) in HEK-293 cells. **A:** immunologic specificity of anti-mMATE2 antibodies. Membrane fractions (10 μg of protein each) prepared from mMATE1- and mMATE2-expressing Sf9 cells were solubilized with SDS sample buffer and subjected to electrophoresis. After proteins were transferred to a nitrocellulose membrane, blots were incubated with antibodies to mMATE1 or mMATE2 (left). Blots with mMATE2 incubated with antiserum preabsorbed with antigenic peptide are shown at right. Immunologic reactivity was visualized by enhanced chemiluminescence. Positions of molecular weight markers are indicated. **B:** HEK-293 cells transfected with pcDNA3.1 (mock control) or pcDNA3.1/nV5-DEST-mMATE2 (mMATE2) were prepared, and membrane fraction (50 μg of protein) was analyzed by Western blotting with antiserum against mMATE2 or antiserum preabsorbed antiserum preabsorbed antibodies (preabsorbed). **C:** HEK-293 cells from the same experiment were immunostained with antiserum against mMATE2 or antiserum preabsorbed with antigenic peptide. Scale bar, 5 μm. Sections were also observed under Nomarski microscope, and merged images are shown. **D:** pcDNA3.1/nV5-DEST-mMATE2-transfected HEK-293 cells were doubly immunostained with antibodies against mMATE2 (a) and Rab5 (b) or mMATE2 (d) and EEA1 (e) and then observed. Superimposed (merged) images (c and f) are also shown. Scale bars, 5 μm.
present in HEK-293 cells, and, thus, provides more conclusive and quantitative results on transport activity. Since F-type ATPase is an ATP-dependent proton pump, the proteoliposomes can be acidified on the addition of ATP (6, 11), and H+/TEA exchange should be observed. As shown in Fig. 4A, mMATE2 in Sf9 cells was successfully solubilized and reconstituted into liposomes. SDS-PAGE of the proteoliposomes after reconstitution revealed that relative mobility of mMATE2 immunoreactive protein bands was much slower and corresponded to \( \sim 110 \) and \( >300 \) kDa (Fig. 4A, arrowheads), suggesting formation of complexes composed of two or more molecules. On addition of ATP, the proteoliposomes took up TEA with a \( \sim 50\)-fold increase in its specific activity over that in HEK-293 cells (Fig. 4B). The total TEA uptake activity decreased by \( \sim 70\% \) in the absence of ATP. Proteoliposomes lacking mMATE2 exhibited a background level of TEA uptake (Fig. 4B). The ATP-dependent uptake was inhibited (by \( \sim 75\% \)) by CCCP, but not by valinomycin in the presence of K+ (Fig. 4C). These results indicate that mMATE2 took up TEA at the expense of transmembrane pH gradient, but not the membrane potential established by the proton-translocating ATPase. TEA uptake in the absence of ATP seems to be due to downhill transport through mMATE2. Similar downhill transport of TEA was observed in the proteoliposomes containing purified hMATE1 (unpublished observations).

Expression of mMATE2 in Leydig cells. Our previous Northern analysis indicated that the mMATE2 gene is predominantly expressed in testis and below the detection limit in other organs such as kidney, liver, and brain (14). Consistent with this
Table 1. Cis inhibition of TEA transport in mMATE2

<table>
<thead>
<tr>
<th>Compounds</th>
<th>mMATE2</th>
<th>mMATE1</th>
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<tbody>
<tr>
<td>Control</td>
<td>100.0±1.8</td>
<td>100.0±4.7</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>48.2±3.5†</td>
<td>29.3±5.2†</td>
</tr>
<tr>
<td>0.1 mM</td>
<td>36.0±1.2†</td>
<td>3.1±5.1†</td>
</tr>
<tr>
<td>Quinidine</td>
<td>50.3±5.4†</td>
<td>65.7±8.2†</td>
</tr>
<tr>
<td>0.05 mM</td>
<td>50.2±5.4†</td>
<td>19.7±3.7†</td>
</tr>
<tr>
<td>0.1 mM</td>
<td>26.6±7.3†</td>
<td>96.0±8.5</td>
</tr>
<tr>
<td>TEA (5 mM)</td>
<td>3.9±0.1†</td>
<td>0.0±7.6†</td>
</tr>
<tr>
<td>MPP (0.1 mM)</td>
<td>33.1±9.5*</td>
<td>115.3±8.9</td>
</tr>
<tr>
<td>Nicotine (5 mM)</td>
<td>3.3±2.4*</td>
<td>62.0±8.8*</td>
</tr>
<tr>
<td>NMN (1 mM)</td>
<td>13.3±2.2†</td>
<td>36.7±3.1†</td>
</tr>
<tr>
<td>Choline (5 mM)</td>
<td>17.3±0.5*</td>
<td>10.0±6.7†</td>
</tr>
<tr>
<td>Corticosterone (0.1 mM)</td>
<td>71.6±4.8*</td>
<td>99.5±5.0</td>
</tr>
<tr>
<td>Testosterone</td>
<td>40.7±6.8*</td>
<td>59.5±8.4*</td>
</tr>
<tr>
<td>0.001 mM</td>
<td>20.8±0.3†</td>
<td>31.6±3.7†</td>
</tr>
<tr>
<td>0.1 mM</td>
<td>13.9±0.6†</td>
<td>0.5±6.2†</td>
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Values are means ± SD (n = 3–9). Uptake of 50 μM radiolabeled TEA by mouse multidrug and toxic compound extrusion 2 (MATE2)-expressing HEK-293 cells at pH 8.0 was determined after 20 min in the presence of the listed compounds. MPP, methylphenylpyridinium; NMN, N-methyl nicotinamide. After the background value, which was obtained from the mock control, was subtracted, values were expressed as percentages of radiolabeled TEA uptake under control conditions (without addition of test substance). Cis inhibition of TEA transport by mMATE1 (from Ref. 3) is shown for comparison. *P < 0.05; †P < 0.001 vs. control.

DISCUSSION

In the present study, we investigated function and localization of mMATE2. We showed for the first time that mMATE2, a mammalian class III MATE transporter, acts as an electroneutral polyspecific H+/OC antiporter.

We showed that mMATE2 mediates pH-dependent TEA antiport by the standard filtration technique with mMATE2-expressing HEK-293 cells. The transport properties, such as kinetics, pH dependence, independence of Na⁺, and cis inhibition profile, are similar to those of mMATE1 and hMATE1. Localization of mMATE2 in early endosomes is probably an artifact due to overexpression; thus there is latent TEA uptake ability when HEK-293 cells were used for transport assay. To
Together, these results provide convincing evidence for the transport of protons across the proteoliposomal membrane. Accordingly, the addition of ATP led to the formation of an electrochemical gradient of protons in the presence of K⁺/H⁺ exchange by mMATE2-expressing HEK-293 cells. Bacterial F-type ATPase is one of the most well-characterized electrogenic proton pumps. The ATP-dependent uptake of TEA is sensitive to CCCP but unaffected by valinomycin in the presence of K⁺. The molecular mechanism of testosterone secretion from the cells is poorly characterized. It is of interest to note that mMATE2 seems to recognize NMN and guanidine as substrates, in addition to TEA, choline, procainamide, cimetidine, and quinidine (Table 1). It is well established that an electroneutral H⁺/OC exchanger(s) located in renal brush border membrane transports NMN and guanidine (5, 7, 9, 18, 21–23). However, hMATE1 and mMATE1 exhibit only weak affinity to these substances (3, 14). We noticed that a small amount of mMATE2 is present in renal tubular cells (unpublished observation). Taken together, these observations suggest that mMATE2 may be responsible for excretion of NMN and guanidine and may play a role in excretion of OCs in combination with mMATE1 in renal tubular cells. Further studies on the localization of mMATE2 in renal tubular cells and its transport ability for guanidine and NMN are needed to clarify these issues.

Because of its site of expression, mMATE2 is significant compared with other characterized mammalian MATE transporters. As shown here, mMATE2 is specifically expressed in testicular Leydig cells. The rat counterpart (rMATE2) was also present in testicular Leydig cells, as revealed by immunohistochemical analysis with anti-mMATE2 antiserum (unpublished observations). Although the physiological function of mMATE2 in Leydig cells is unknown, a fascinating hypothesis is that mMATE2 could act as a testosterone exporter and, hence, is at least partially responsible for secretion of this substance from Leydig cells. Testosterone is a hydrophobic, cationic sex hormone produced in Leydig cells. The molecular mechanism of testosterone secretion from the cells is poorly characterized.

It is of interest to note that mMATE2 seems to recognize NMN and guanidine as substrates, in addition to TEA, choline, procainamide, cimetidine, and quinidine (Table 1). It is well established that an electroneutral H⁺/OC exchanger(s) located in renal brush border membrane transports NMN and guanidine (5, 7, 9, 18, 21–23). However, hMATE1 and mMATE1 exhibit only weak affinity to these substances (3, 14). We noticed that a small amount of mMATE2 is present in renal tubular cells (unpublished observation). Taken together, these observations suggest that mMATE2 may be responsible for excretion of NMN and guanidine and may play a role in excretion of OCs in combination with mMATE1 in renal tubular cells. Further studies on the localization of mMATE2 in renal tubular cells and its transport ability for guanidine and NMN are needed to clarify these issues.

The present results support the idea that members of all MATE subclasses of mammalian origin mediate similar polytopic H⁺/OC exchange. Therefore, we can summarize the overall features of mammalian MATE transporters as follows. Class I is ubiquitously present throughout the body but is predominantly found in kidney and liver. Classes II and III have more specific expression patterns than class I and are expressed primarily in kidney and testes, respectively. The functions of these classes may be coordinated and may play a role in the elimination of metabolic OCs and xenobiotics at the final step of excretion in kidney. The physiological role(s) of class III does not seem to involve excretion of OCs, because rodent MATE2 is predominantly expressed in Leydig cells. Whether all mammals, especially humans, possess a counterpart to mMATE2 or in class III is unknown and awaits further study.
ACKNOWLEDGMENTS

We thank Dr. M. Otsuka, S. Arioka, and K. Shimizu for help in the initial stage of the study.

GRANTS

M. Hiasa and T. Matsumoto were supported by Research Fellowships from the Japan Society for the Promotion of Science for Young Scientists. This work was supported in part by Japanese Ministry of Education, Science, Sport, and Culture Grant-in-Aid for Research 16017264 and the Smoking Research Foundation (to Y. Moriyama).

REFERENCES


