Identification and functional assessment of the novel murine organic anion transporter Oat5 (Slc22a19) expressed in kidney

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Youngblood, Geri L., and Douglas H. Sweet. Identification and functional assessment of the novel murine organic anion transporter Oat5 (Slc22a19) expressed in kidney. Am J Physiol Renal Physiol 287: F236–F244, 2004.—An uncharacterized murine cDNA clone was identified and, through sequence, phylogenetic, and functional analysis, determined to encode the newest member of the organic anion transporter family, organic anion transporter 5 (Oat5; Slc22a19). The Oat5 cDNA clone contained an insert 1,964 bp in length with a peptide 551 amino acids long. Slc22a19 was localized to mouse chromosome 19 near the genes encoding Oat1 (Slc22a6) and Oat3 (Slc22a8). Northern blot analysis revealed Oat5 is highly expressed in the kidney of adult mice and rats. No sexual dimorphism in renal or hepatic expression of Oat5 was observed. Unlike Oat1–3, Oat5 expression was not detected in the choroid plexus of either mice or rats. Murine Oat5-expressing Xenopus laevis oocytes supported increased accumulation of the mycotoxin ochratoxin A, compared with water-injected control oocytes. This uptake was significantly inhibited by probenecid and the organic anions 2,4-dichlorophenoxyacetic acid, salicylate, and estrone sulfate but not by para-aminophenol or urate. Transport of ochratoxin A by murine Oat5 was saturable, with an estimated \( K_m \) of 2.0 ± 0.45 \( \mu \)M. Oat5-mediated transport was neither \( cis \)-inhibited nor \( trans \)-stimulated by the dicarboxylic glutarate. Uptake was also completely unaffected by short-circuiting of the membrane potential. Thus the motive forces behind Oat5 function, which provide insight into its membrane localization, need to be further resolved. These data demonstrate for the first time that this newly identified gene encodes a protein that functions as an organic anion transporter. Anti-inflammatory drugs, toxins (e.g., herbicide/pesticide constituents and mycotoxins), and endogenous compounds (e.g., steroid hormones and neurotransmitter metabolites). Increased knowledge of OAT function will improve our ability to exploit these transporters as drug targets and to accurately assess their impact on drug efficacy. The recent observation that some OATs are also expressed in the brain capillary endothelium and/or choroid plexus (CP) suggests these transporters are involved in regulating the environment of the central nervous system as well (15, 26, 31, 43). Thus identification and characterization of these transporters are essential for the proper modeling of the basic physiology and toxicology of these barrier tissues.

The renal and CP epithelia use the same mechanisms and driving forces to achieve efficient vectorial transport of organic anions, despite a reversal in the polarity of OAT localization (basal vs. apical membrane). In the movement of substrates from cerebrospinal fluid to blood (CP) or from blood to urine (kidney), cellular entry is accomplished via a tertiary-active transport mechanism that is indirectly coupled to the \( Na^+ \) gradient and cellular energy. Cellular exit is driven by exchange or facilitated diffusion (2, 28, 29, 35, 47). Thus the physiological properties of transport at each membrane are distinct and simply knowing that a particular transcript is homologous to an OAT is insufficient to determine its placement within the model for organic anion transport or to assess its potential impact on the cellular flux of organic anions.

The first identified organic anion transporter, Oat1, was isolated from the kidney (24, 46). Mechanistic characterization of Oat1’s transport function, particularly the \( trans \)-stimulation of Oat1-mediated organic anion (OA) uptake by glutarate (GA), indicated that it was the postulated OA/dicarboxylate exchanger present in the basolateral membrane of renal proximal tubule cells (33, 46). Studies with an Oat1 green fluorescent protein fusion construct directly confirmed Oat1’s localization to the basolateral membrane of intact renal tubules (41). Apical targeting of the Oat1 green fluorescent protein construct in intact CP (i.e., reversal of membrane polarity with respect to kidney) was also observed (31). Subsequently, three additional OAT family members were cloned and characterized, Oat2, Oat3, and Oat4 (6, 23, 32, 36). When first analyzed, transport mediated by all three transporters did not appear to exhibit \( trans \)-stimulation, suggesting that they were facilitated diffusion carriers expressed in the apical membrane of renal proximal tubules (6, 23, 32). However, the role of Oat2 in renal OA transport is unclear, as immunolocalization studies have reported that it is apical in rat kidney, but basolateral in human

THE SEQUENCING OF THE MOUSE and human genomes, combined with advances in bioinformatics, has resulted in the identification of thousands of putative genes for which no function has been determined. Comparison to well-characterized members of known gene families greatly narrows the scope of their potential role; nevertheless, direct confirmation of the predicted gene’s actual function is essential to properly integrating their encoded proteins into the various models for cellular biochemical processes. In the current work, we characterized the function of a new member of the organic anion transporter (OAT) family, a subfamily within the amphiphilic solute transporter branch (Slc22a) of the major facilitator superfamily (13, 14, 38, 44).

The OAT family plays a critical role in the renal excretion and detoxification of drugs (e.g., penicillins and nonsteroidal
Experimental Procedures

It was recently shown to be responsive to Na⁺-dependent trans-stimulation by GA arguing that it should be basolateral in renal proximal tubule (39). This conclusion was confirmed in work in Oat3 knockout mice, which demonstrated that loss of Oat3 expression correlated.

In this work, we report the identification and physiological characterization of a novel murine gene. Initial in silico analysis indicated it was a new member of the OAT family. Once functional studies confirmed the transcript encoded an OAT, the gene was designated Oat5 (Slc22a19). Inhibitor-sensitive transport of the mycotoxin, ochratoxin A (OTA), was demonstrated in Oat5-expressing Xenopus laevis oocytes. Oat5 was insensitive to inhibition by the dicarboxylates α-ketoglutarate (α-KG), GA, succinate, or malate and to trans-stimulation by GA. Oat5-mediated transport was also unaffected by membrane depolarization. Unlike Oat1–3, Oat5 expression was not detected in CP. These data indicate that murine Oat5 (mOat5) will impact the renal excretion of hormones, drugs, and xenobiotics.

Table 1. Sequence comparison of selected OAT family members to mOat5

<table>
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<tr>
<th>Name</th>
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<th>cDNA Accession Number</th>
<th>%Similarity/%Identity to mOat5 (Slc22a19)</th>
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</table>

*Designation obtained for this publication from The Mouse Genome Informatics Database using the rules and guidelines established by the International Committee on Standardized Genetic Nomenclature for Mice and implemented through the Mouse Genomic Nomenclature Committee. †Sequence was not submitted to database and was obtained from Ref. 37. OAT, organic anion transporter.

X. laevis oocyte isolation procedures and uptake assay were performed as reported previously (8, 39, 43). Briefly, adult female X. laevis were anesthetized in tricaine methanesulfonate, decapitated, and follicle-free stage V and VI oocytes were isolated from the ovaries by collagenase A treatment. After overnight recovery, oocytes were injected with 20 ng of capped cRNA. Three days postinjection, oocytes were randomly divided into experimental groups (n = 5–10 oocytes/group) and incubated for 1 h at room temperature in oocyte Ringer 2 (in mM: 82.5 NaCl, 2.5 KCl, 1 Na₂PO₄, 3 NaOH, 1 CaCl₂, 1 MgCl₂, 1 pyruvic acid, 5 HEPES, pH 7.6) containing 0.1–10 μM ³H-labeled test substrates (0.5 μCi/ml) in the absence or presence of inhibitors as indicated in the figure legends. Oocyte membrane potential difference was altered by increasing the external K⁺ concentration in the presence of 10 μM valinemycin, a condition previously demonstrated to depolarize X. laevis oocytes and to inhibit potential-sensitive transport in transporter-expressing oocytes (16, 40, 45, 46). High-K⁺-OR-2 solutions were isosmotically adjusted by lowering the Na⁺ concentration. Water-injected or un.injected oocytes were included as negative controls in every experiment. Oocyte radioactivity was measured in disintegrations per minute in a Packard Tri-Carb 2900TR liquid scintillation counter with external quench correction. All experiments were done in three to five animals to confirm results. The program for laboratory animal care at Medical University of South Carolina (MUSC) has a detailed assurance statement on file with OPRR/DHHS, and MUSC has ongoing full accreditation from AAALAC, effective November 1987. All animal use reported in this manuscript was reviewed and approved by the MUSC IACUC (MUSC protocol AR 2080, approved 10/30/2003).
Cycle parameters were as follows: denature at 94°C for 30 s; followed by 30 cycles of 94°C denature for 20 s, 60°C anneal for 20 s, and 72°C extension for 30 s; with a final extension at 72°C for 5 min. The 32P-labeled probe was generated by random prime labeling using a Prime-It II kit (Stratagene, La Jolla, CA). Blots were hybridized for 3 h with 1 × 10⁶ cpm/ml of mOat5 probe at 68°C in PerfectHyb Plus hybridization buffer (Sigma, St. Louis, MO) and washed under final conditions of high stringency (0.1× SSC/0.1% SDS). The blots were stripped in boiling 0.1% SDS and reprobed under identical conditions with murine GAPDH.

Fig. 1. Peptide sequence alignment of murine organic anion transporters (OATs) and evolutionary analysis of the OAT gene family. A: predicted amino acid sequence of the single large open reading frame in the mOat5 (Slc22a19) cDNA. Peptide sequence alignment of the known murine OATs is displayed with positions of identity between all 4 transporters highlighted. B: unrooted phylogenetic tree representing the relationships between mouse (m), rat (r), and human (h) members of the OAT family. The bootstrap values are given at the branch points and represent the number of times (out of 1,000) each original grouping occurred in the subsequent trees.
**RESULTS**

**Identification of a novel mouse gene and its relationship to the OAT family.** A novel murine gene sequence, located on mouse chromosome 19 near Oat1 (Slc22a6) and Oat3 (Slc22a10), was identified via BLAST search of the mouse genome using the sequence of hOat4 (SLC22A11) as a query. DNA and peptide sequence comparisons of this putative murine gene with the members of the OAT family of transporters suggested that it was a new member of this gene family (Fig. 1 and Table 1). Evolutionary (phylogenetic) analysis (Fig. 1B) indicated that this gene is the murine ortholog of an uncharacterized rat sequence in the database referred to as rat Oat5 (GenBank Accession Number AB051836). However, it is most likely not the ortholog of the gene for human OAT5 (SLC22A10). mOat5 is equally homologous to human UST3 (SLC22A9) and human OAT5 (SLC22A10), which are both expressed in the liver and encode proteins of unknown functions (37). Because the murine and rat genes appear orthologous to each other, but not to SLC22A9 or SLC22A10, a unique gene designation, Slc22a19, was applied for and obtained from The Mouse Genome Informatics Database (http://www.informatics.jax.org/mgihome/nomen/) using the rules and guidelines established by the International Committee on Standardized Genetic Nomenclature for Mice and implemented through the Mouse Genomic Nomenclature Committee. Therefore, we refer to this novel murine gene and its rat ortholog as Oat5 (Slc22a19).

Topological analysis employing four different modeling programs yielded variable profiles, as previously observed with rOat1 (46), predicting 10–12 potential α-helical transmembrane segments. All of the predicted models agree that there is a large extracellular loop from approximately amino acid residues 33 to 149, and within this domain are several potential modification sites including possible NH2-linked glycosylation sites at Asn-39, Asn-56, Asn-62, and Asn-102; four cysteine residues (at positions 49, 78, 99, and 122) that are conserved throughout the OAT family; and three serine residues (positions 46, 60, and 68) and a threonine (Thr-109) that are potential phosphorylation sites (3). In addition, throughout the remainder of the peptide sequence, there are 11 more serine residues, 3 more threonine residues, and a tyrosine residue that could potentially be sites for phosphorylation (3).

**Distribution of Oat5 mRNA expression.** A strong signal corresponding to a transcript ~2.0 kbp in size was detected exclusively in the mouse kidney by Northern blot analysis using mOat5 as a probe (Fig. 2). Hybridization of the mOat5 probe to a blot prepared with RNA from adult rat tissues also revealed kidney-specific expression (Fig. 2). No Oat5 mRNA expression was detected in the brain, heart, lung, liver, spleen, stomach, small intestine, skeletal muscle, thymus, testis, unimpregnated uterus, or placenta of either mouse or rat. The blot was stripped and reprobed with murine GAPDH to confirm the integrity of RNA transfer (Fig. 2). Species and sex differences in the expression of OATs, particularly in the kidney and liver, have been observed (5, 20, 43). To eliminate the possibility that sexually dimorphic expression in the kidney and liver would not have been observed in the samples used in the preparation of the commercial Northern blots, RT-PCR analysis of Oat5 gene expression in the kidney and liver from adult male and female mice and rats was conducted (Fig. 3). Although no conclusions can be drawn as to differences in the levels of expression, Oat5 is clearly expressed in the kidney of males and females of both species. No Oat5 expression was detected in the liver of males or females of either species; thus no sex or species differences in hepatic Oat5 expression were observed (Fig. 3). The expression of Oat1–3 in the CP of mice and rats was recently reported (43); therefore, expression of Oat5 mRNA in the CP of adult mice and rats was also investigated by RT-PCR (Fig. 3). No signal was detected in the CP of either species. Integrity of reactions was shown by amplification of β-actin.

![Fig. 2. Northern blot analysis of Oat5 expression in adult mouse and rat tissues. Oat5 mRNA expression was kidney specific (Ki) in both wild-type mice and rats. No Oat5 signal was observed in the brain (Br), heart (He), lung (Lu), liver (Li), spleen (Sp), stomach (St), small intestine (SI), skeletal muscle (SM), thymus (Th), testis (Te), unimpregnated uterus (Ut), or placenta (Pl) of either mouse or rat. The blots were stripped and reprobed with murine GAPDH to confirm RNA integrity.](http://ajprenal.physiology.org.org/)
Functional Characterization of mOat5

Organic anion transport. No function had been demonstrated for this putative transport protein. Therefore, we demonstrated the uptake of the organic anion OTA in X. laevis oocytes expressing murine Oat5 (Fig. 4). Oat5-expressing oocytes exhibited substantial OTA uptake (~8-fold increase) over that measured in water-injected control oocytes (Fig. 4). This OTA accumulation was significantly inhibited (70–80%) by the model organic anion transport inhibitor Prob. Kinetic analysis revealed that OTA transport by mOat5 was a saturable process (Fig. 5A). Uptake in water- and mOat5 cRNA-injected oocytes was measured in medium containing 0.1–30 μM OTA (n = 3 animals). The diffusion-corrected data were analyzed by double reciprocal analysis and an estimated value for OTA of $K_m = 2.0 \pm 0.45 \mu M$ was obtained (Fig. 5B).

Inhibition profile. Subsequent analysis demonstrated that mOat5-mediated transport was significantly inhibited by the organic anions 2,4-D (reduced uptake by 80%), salicylate (73% inhibition), and estrone sulfate (virtually eliminated uptake) (Fig. 6A). Transport was unaffected by urate or PAH (Fig. 6A). When examined directly as a substrate, accumulation of the model organic anion PAH was not observed in mOat5-expressing oocytes (Fig. 6B). Oocytes expressing rat Oat1 showed marked Prob-sensitive PAH uptake under the same conditions (Fig. 6B). Water-injected control oocytes had negligible background PAH uptake.

The renal basolateral OA/dicarboxylate exchangers Oat1 and Oat3 use -KG as a counterion for exchange and, experimentally, GA can substitute for -KG (28, 30, 39, 46). Therefore, the effect of externally applied GA on mOat5 function was examined. As illustrated in Fig. 7, GA failed to cis-inhibit OTA uptake in mOat5-expressing oocytes, yet it produced significant cis-inhibition of rOat1-mediated OTA uptake. The additional dicarboxylate citric acid cycle intermediates succinate and malate did not cis-inhibit either transporter (Fig. 7). α-KG was also without effect on mOat5 OTA uptake (data not shown).

Fig. 3. Examination of potential sexual dimorphism of Oat5 expression in adult mice and rats. Total RNA was isolated from the kidney (K) and liver (L) of male (M) and female (F) wild-type mice and rats and from choroid plexus (CP; from mixed sexes). Gene expression was examined by RT-PCR using Oat5-specific primers that amplify a full-length product (~1.6 kbp). Oat5 was clearly expressed in the kidneys of both males and females in each species. No Oat5 expression was detected in the livers of males or females of either species. No PCR reaction product was obtained for Oat5 in CP indicating lack of expression in this tissue in either sex of both species. A control amplification of β-actin (372 bp) was performed for each sample. ● Indicates the 1.6-kbp band and - indicates the 344-bp band of a 1-kbp ladder. The experiment was repeated with 2 independent groups of animals with the same results.

Fig. 4. Murine Oat5-mediated transport of ochratoxin A (OTA) in Xenopus laevis oocytes. Three days after injection with Oat5 cRNA or water, oocytes were randomly sorted into test groups and 1-h uptake was determined. Inhibitor-sensitive [±1 mM probenecid (Prob)] uptake of 0.5 μM [3H]OTA was observed in Oat5-expressing oocytes demonstrating mOat5 to be an organic anion transporter. Data are mean values ± SE from a representative animal.

Fig. 5. Kinetic analysis of mOat5-mediated OTA uptake. Water-injected and Oat5 cRNA-injected oocytes were exposed to increasing concentrations (0.1–30 μM) of OTA for 60 min. A: saturation analysis. Total OTA uptake was corrected for diffusion by subtracting the value for OTA uptake in water-injected (control) oocytes at each concentration. B: double reciprocal plot of the diffusion-corrected data with linear regression analysis performed. $K_m$ was estimated to be 2.0 ± 0.45 μM (n = 3 animals). Data are mean values ± SE from a representative animal.
Trans-Stimulation. Increasing the outwardly directed gradient of the dicarboxylate counterion (α-KG) accelerates uptake mediated by the basolateral OA/dicarboxylate exchangers Oat1 and Oat3 (39, 46). Therefore, if mOat5 functions as a basolateral OA/dicarboxylate exchanger, increasing the intracellular concentration of α-KG (or glutarate) should induce trans-stimulation of OTA uptake in mOat5-expressing oocytes. For this determination, GA is the preferred counterion because, as opposed to α-KG, it is not extensively metabolized (30). In contrast to the significant trans-stimulation produced in rOat1-expressing oocytes, incubating mOat5-expressing oocytes in 2.5 mM glutarate for 90 min before exposure to OTA (i.e., preloading) did not stimulate uptake, compared with nonpreloaded oocytes (Fig. 8). GA preloading had no effect on water-injected (control) oocytes. Thus the energetics of mOat5-mediated organic anion transport do not correspond with those established for the OA/dicarboxylate exchangers Oat1 and Oat3, which mediate the basolateral entry of OA into renal proximal tubule cells.

Potential dependence. The well-characterized luminal organic anion facilitative-diffusion carrier(s) in renal proximal tubule uses the membrane potential as a driving force (25, 47).

Fig. 7. Effect of external dicarboxylates on OTA uptake. Three days postinjection, oocytes injected with water (control), mOat5 cRNA, or rat Oat1 cRNA were incubated for 1 h in OR-2 containing 0.5 μM [3H]OTA with and without various dicarboxylates (25 μM). As previously shown for the basolateral OA/dicarboxylate exchanger rOat1, external glutarate (GA) significantly inhibited rOat1-mediated uptake. In marked contrast, mOat5-mediated transport of OTA was completely unaffected by external GA, suggesting that it does not function as a renal basolateral OA/dicarboxylate exchanger. The dicarboxylates succinate and malate were without effect on either transporter. Data are means ± SE values from a representative animal. ***P ≤ 0.0001 compared with corresponding no inhibitor group.

DISCUSSION

The transport of many small organic anions, including drugs, hormones, neurotransmitter metabolites, and xenobiotics, is handled by the OAT family, which is part of the amphiphilic solute transporter (Slc22a) branch of the major facilitator superfamily. Every OAT identified thus far is expressed in the kidney where their function is a major determinant of toxicity and the therapeutic action of drugs. OATs are also found in other barrier epithelia, such as placenta, retinal pigmented epithelium, and CP. Expression and function in the CP allow the OATs to actively regulate the composition of the cerebrospinal fluid. Thus a detailed understanding of OAT function at the molecular level will aid the development of more efficac-
Uptake under these trans-stimulation conditions was also determined in the presence of Prob (GA preload + 1 mM Prob). Water-injected control oocytes were included in all treatments. Unlike the OA/dicarboxylate exchanger rOat1, mOat5-mediated uptake was not trans-stimulated by GA. Data are means ± SE values from a representative animal. + + P ≤ 0.01 compared with no preload control. + + + P ≤ 0.001 compared with no preload control. *P ≤ 0.05 compared with corresponding no preload group.

After identification of the novel murine transcript, the first issue to be addressed was establishing the correct phylogenetic relationship with other known transporter genes and properly designating it to prevent confusion in the literature. The Ensembl database predicted a rat homolog (ENSRNOG0000021214) for murine Slc22a19 on rat chromosome 1 near rOat1 (Slc22a6) and rOat3 (Slc22a8). A BLAST search of the GenBank database with the murine Slc22a19 sequence identified an unpublished rat sequence (GenBank accession number AB051836) referred to as rOat5. A pairwise BLAST of the cDNA sequence for the Ensembl predicted rat gene with the rOat5 cDNA revealed the two sequences are 100% identical. Therefore, we concluded that the novel Ensembl-predicted rat gene is the gene coding for the rOat5 cDNA and rOat5 is therefore the rat ortholog of murine Slc22a19.

We refer to Slc22a19 as mouse Oat5 and suggest that rat Oat5 should be referred to with the gene designation Slc22a19. Potentially lending support to this conclusion, recent literature (17, 21) refers to a rat kidney cDNA as rOat5 and reports that it is 1) exclusively expressed in the kidney, 2) 55% identical to the amino acid sequence of hOAT5, and 3) transports OTA; all characteristics we report here for mOat5 (Slc22a19). Unfortunately, no means were published for confirming that the cDNA described is the same rOat5 in the GenBank database, which we identify here as the ortholog to mOat5. As indicated in Table 1 and Fig. 1B, the two human genes, hUST3 (SLC22A9) and hOAT5 (SLC22A10), have the greatest sequence identity (54.6 and 54.9% at the amino acid level, respectively) and the closest evolutionary relationship to mOat5. To date, neither human gene has been functionally characterized and it remains to be conclusively shown that they mediate the transport of organic anions. Furthermore, in contrast to rat and mouse Oat5, both human genes were found to be exclusively expressed in the liver by Northern blot analysis (37). It therefore seems unlikely that either of these genes represents the human ortholog for Slc22a19.

There are, however, several examples of tissue, species, and sex differences in the expression patterns of OATs. In the rat, Oat2 is more highly expressed in the male vs. female liver, whereas in the mouse hepatic expression of Oat2 is observed exclusively in females (19, 20, 32, 36). Oat3 is not expressed in the liver of either sex in mice; however, it is highly expressed in the liver of male, but not female, rats (19, 23, 43). Because it could not be determined whether the hepatic samples on the Northern blots used to investigate Oat5 expression (Fig. 2) were male, female, or mixed in origin, we examined renal and hepatic Oat5 expression in male and female mice and rats (Fig. 3). No detectable Oat5 expression was found in the liver from either sex or species, thus, unlike Oat2 and Oat3, there is no species difference or sexual dimorphism in the hepatic expression of Oat5. The transporters Oat1–3 are all expressed in the CP of both mice and rats and it has been proposed that they play a role in the clearance of organic anions from cerebrospinal fluid (38, 43). In marked contrast, Oat5 is the first OAT to be identified in these species that is not expressed in the CP and, therefore, it clearly does not play a direct role in the regulation of the central nervous system environment (Fig. 3). Finally, there are currently three human OATs without any apparent rat or murine orthologs, hOAT4.
(SLC22A11), hUST3 (SLC22A9), and hOAT5 (SLC22A10), and all three exhibit tissue expression patterns distinct from Oat5 (Slc22a19). Human OAT4 is expressed in the placenta as well as the kidney, whereas hUST3 and hOAT5 are exclusively expressed in the liver (6, 37). Thus the expression pattern of Oat5 (Slc22a19) is unique among the OAT family members identified thus far, as it was found exclusively in the kidney in both male and female mice and rats. This difference may provide a biochemical basis for making the renal organic anion transport process unique from that found in the brain.

Functional evidence that Oat5 (Slc22a19) is an organic anion transporter was obtained using the X. laevis oocyte expression assay system (Figs. 4–6). The mechanisms and driving forces governing the renal uptake and efflux of small (300–500 Da) OA via the classic organic anion transport system are well defined (38, 44). Therefore, physiological characterization of the transport properties of Oat5 should allow us to predict where Oat5 may be positioned (i.e., basolateral or apical membrane) in the model describing the flux of OA through renal proximal tubule cells. In the current model, the basolateral OA/dicarboxylate exchangers, Oat1 and Oat3, are characterized by cis-inhibition and trans-stimulation by the dicarboxylates α-KG and GA (39, 46). Luminal efflux of organic anions is mediated by either facilitated diffusion or anion exchange that is insensitive to α-KG or GA (4, 25). Therefore, the influence of the dicarboxylate GA on mOat5 function may be diagnostic of mOat5’s membrane localization.

The inability of GA to cis-inhibit OTA uptake in mOat5-expressing oocytes suggests that mOat5 does not function as a basolateral OA/dicarboxylate exchanger in renal proximal tubule (Fig. 7). Furthermore, if mOat5 did function as a basolateral OA/dicarboxylate exchanger, increasing the intracellular (trans) concentration of GA should induce stimulation of OA uptake, as previously demonstrated for Oat1 and Oat3 (39, 46). Increasing the intracellular GA concentration in mOat5-expressing oocytes produced no effect on Oat5-mediated uptake, but it produced a significant effect on Oat1-mediated transport (Fig. 8). Thus the failure of GA to produce cis-inhibition or trans-stimulation of mOat5-mediated OTA uptake indicates that mOat5 does not function as a basolateral OA/dicarboxylate exchanger. Alternatively, mOat5 may exhibit a counterion specificity that is unique with respect to these well-characterized exchangers.

Oat5-mediated OTA accumulation was also unaffected by urate (Fig. 6a), distinguishing it functionally from the identified apical anion exchange mechanism and the apical urate transporter, URAT1 (4, 11). Similar to the apical organic anion transporter OAT4, but unlike the basolateral exchangers Oat1 and Oat3, Oat5 is not inhibited by, and does not transport, PAH (Fig. 6) (6, 39, 46). In addition, the cDNA referred to in the literature as rOat5 was immunolocalized to the apical membrane (17, 21). Thus, if this rat gene is indeed the same gene we identify in this report as the rat ortholog to murine Oat5 (Slc22a19), this would suggest an apical localization for mOat5. However, Oat5 function was insensitive to membrane depolarization, confirming the major driving force for Oat5-mediated uptake is not the membrane potential and indicating Oat5 is not an apical facilitative-diffusion carrier (Fig. 9). Clearly, more work is needed to conclusively define the energetics of mOat5 function and determine its membrane targeting in the polarized renal epithelium.

In summary, we identified a new member of the OAT family in the mouse, mOat5 (Slc22a19), and obtained data confirming its function as an organic anion transporter. Expression of Oat5 is unique among the OATs in that it is solely found in the kidney. This may provide a molecular basis for differentiating renal organic anion transport from that in other barrier epithelia. Current data on expression and energetics leave mOat5’s membrane localization undetermined, and further experiments are required to conclusively elucidate the driving forces governing Oat5 activity. Discovery of the substrate specificity of Oat5 increases our understanding of the organic anion secretory pathway and will aid our ability to accurately model the biochemical mechanisms contributing to the elimination of drugs, hormones, and toxic substances.

GRANTS

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