Functional Characteristics and Membrane Localization of Rat
Multispecific Organic Cation Transporters, OCT1 and OCT2,
Mediating Tubular Secretion of Cationic Drugs

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ABSTRACT

We have isolated a kidney-specific organic cation transporter, rat OCT2, which is distinct from rat OCT1 (Okuda M, Saito H, Urakami Y, Takano M and Inui K (1996) Biochem Biophys Res Commun 224:500–507). In our study, the functional characteristics and membrane localization of OCT1 and OCT2 were investigated by uptake studies using MDCK cells transfected with rat OCT1 or OCT2 cDNA (MDCK-OCT1 or MDCK-OCT2) and immunological studies. Tetraethylammonium (TEA) uptake by both MDCK-OCT1 and MDCK-OCT2 cells was markedly elevated when TEA was added to the basolateral medium, but not to the apical medium. Efflux of TEA from MDCK-OCT1 and MDCK-OCT2 cells was not changed by extracellular pH from 5.4 to 8.4, whereas TEA uptake by both transfectants was decreased by acidification of extracellular medium. Apparent $K_m$ values for TEA uptake by MDCK-OCT1 and MDCK-OCT2 cells were 38 and 45 $\mu$M, respectively. Although various hydrophilic organic cations such as 1-methyl-4-phenylpyridinium, cimetidine, quinidine, nicotine, N$^\prime$-methyl nicotine and guanidine markedly inhibited TEA uptake by both MDCK-OCT1 and MDCK-OCT2 cells, there were no significant differences in the apparent inhibition constants ($K_i$) against these organic cations between both transfectants. Furthermore, immunological studies using a polyclonal antibody against OCT1 revealed that OCT1 was expressed in the basolateral membranes but not in the brush-border membranes of the rat kidney. These results suggested that both OCT1 and OCT2 are basolateral-type organic cation transporters with broad substrate specificities, mediating tubular secretion of cationic drugs.

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ABBREVIATIONS: RT-PCR, reverse-transcription-polymerase chain reaction; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; MES, 2-(N-morpholino)ethanesulfonic acid; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Tubular secretion of various structurally unrelated organic cations including xenobiotics and endogenous compounds is critical for the maintenance of body fluid homeostasis and the defense of the body against toxic reactions. In general, the sequence of tubular secretion of organic cations is described as basolateral uptake, accumulation into the cell and subsequent extrusion from the cell into tubular fluid across brush-border membranes of renal epithelial cells (Inui et al., 1991; Pritchard and Miller, 1993). Using membrane vesicles isolated from rat (Takano et al., 1984), dog (Hoholoh and Ross, 1981) or rabbit (Hsyu and Giaconini, 1987; Dantzler et al., 1989; Montrose-Rafizadeh et al., 1989, Sokol and McKinney, 1990) kidneys and cultured epithelial cells derived from the pig kidney (Saito et al., 1992), it became clear that basolateral and brush-border transport systems for organic cations are driven by different forces; i.e., basolateral uptake is stimulated by the inside-negative membrane potential, whereas extrusive transport across brush-border membranes is stimulated by the opposite proton gradient. However, it is speculated that there are multiple transport systems for organic cations in the kidney (Miyamoto et al., 1989). Gründemann et al. (1994) isolated a rat organic cation transporter, OCT1, which is predominantly expressed in the liver and kidney. We postulated the presence of another member of the organic cation transporter family, and then screened a rat kidney cDNA library using a cDNA fragment encoding rat OCT1. A cDNA encoding an organic cation transporter distinct from OCT1 that we named rat OCT2 was isolated (Okuda et al., 1996). Rat OCT2 is homologous (67%) to rat OCT1, but its message is exclusively expressed in the kidney. However, little is known about the functional characteristics and membrane localization (basolateral or brush-border) of rat OCT1 and OCT2. In this study, we established epithelial cell lines stably expressing rat OCT1 or OCT2 and compared their transport characteristics. Our results showed that both OCT1 and OCT2 are organic cation transporters with characteristics comparable to basolateral organic cation transporters in renal tubular cells. In addition, rat OCT1 protein was shown to be expressed in the...
basolateral membranes but not in the brush-border membranes of rat kidney using a polyclonal antibody raised against rat OCT1 polypeptide.

Methods

Cell culture and transfection. The parental MDCK cells (ATCC CCL-34) obtained from American Type Culture Collection (Manassas, VA) were cultured in complete medium consisting of Dulbecco's modified Eagle's medium (Life Technologies, Inc., Rockville, MD) with 10% fetal calf serum (Whittaker Bioproducts Inc., Walkersville, MD) in an atmosphere of 5% CO2, 95% air at 37°C. OCT1 cDNA was subcloned into the SalI- and NotI-cut mammalian expression vector pBK-CMV (Stratagene, La Jolla, CA) (Brewer, 1994). The open reading frame of OCT2 cDNA was amplified by PCR using a set of specific primers for the nucleotide sequence of rat OCT2 (sense strand with a PstI site, 5'-CCTGTGAGGGACCATGTCGAGTTGA-3' (bases 1–17); antisense strand with a NotI site, 5'-CCGGGCCGCACTTCCAGGGAATGAGTTGGT-3' (bases 1,761–1,785)), and then subcloned into PstI- and NotI-cut pBK-CMV. The nucleotide sequence of the subcloned cDNA insert was determined using a fluorescence DNA sequencer 373A (Applied Biosystems, Foster City, CA) and confirmed to be identical to the corresponding sequence of OCT2. MDCK cells were transfected with pBK-CMV/OCT1, pBK-CMV/OCT2 or pBK-CMV using the calcium phosphate coprecipitation technique as described previously (Terada et al., 1996). Fifteen hours after transfection, cells were rinsed with Ca2++ and Mg2+-free Dulbecco's-phosphate-buffered saline (pH 7.4) (PBS) and cultured in complete medium comprised of 137 mM NaCl, 3 mM KCl, 8 mM Na2HPO4, and 1.5 mM KH2PO4 containing 15% fetal calf serum, and 10% fetal calf serum (Whittaker Bioproducts Inc., Walkersville, MD) at a cell density of 5 × 105 cells/cm2 with complete medium, as described previously (Saito et al., 1992). In this study, MDCK cells were used between the 81st and 91st passages.

RT-PCR analysis. Total RNA was extracted from rat tissues and MDCK cells transfected by the guanidium/isothiocyanate method (Chirgwin et al., 1979) or using RNasy spin columns (Qiagen GmbH, Hilden, Germany), respectively. For RT-PCR analysis, 5 μg of total RNA from each tissue and MDCK transfectants were reverse-transcribed using Superscript II (Life Technologies, Inc.) and amplified with specific primer sets for either rat OCT1 (sense strand, 5’-CAGAAGAACGGGAAGGTGCC-3’ (bases 922–941); antisense strand with a PstI site, 5’-ACCTTCAATCCTGGACTTGG-3’ (bases 1,746–1,765)) or

Uptake study. Uptake of [14C]tetraethylammonium by the cells was measured using monolayer cultures grown in Transwell chambers. The incubation medium for uptake experiments contained 145 mM NaCl, 3 mM KCl, 1 mM CaCl2, 0.5 mM MgCl2, 5 mM d-glucose and 5 mM MES (pH 5.4 and 6.4) or 5 mM HEPES (pH 7.4 and 8.4). The pH of the medium was adjusted with NaOH or HCl. After removing the culture medium from both sides of the monolayers, the cells were washed once with 2 ml of incubation medium (pH 7.4) in each side for the 4.71-cm2 chamber (1 ml for the 1.0-cm2 chamber) and then incubated for 10 min at 37°C with 2 ml of the same medium in each side (apical 0.5 ml and basolateral 1 ml for the 1.0-cm2 chamber). This was replaced with 2 ml of incubation medium containing [14C]tetraethylammonium in either the apical or basolateral side (1 ml in the basolateral side for the 1.0-cm2 chamber) and the cells were incubated at 37°C. Unlabeled incubation medium was added to the opposite side. The medium was immediately aspirated off and the culture inserts were rapidly rinsed twice with 2 ml of ice-cold incubation medium (pH 7.4) in each side (1 ml each for the 1.0-cm2 chamber). For the efflux experiments, cell monolayers grown on the 4.71-cm2 chambers were incubated with 2 ml of incubation medium containing 50 μM [14C]tetraethylammonium for 15 min from the basolateral side. After incubation, the cells were rinsed twice with 2 ml of incubation medium (pH 7.4) in each side, and then incubated with 2 ml of unlabeled incubation medium (pH 5.4–8.4 for the basolateral side and pH 7.4 for the apical side) for 5 min. The cells were lysed with 1 N NaOH, and then the radioactivity in aliquots was determined in 5 ml of ACSII (Amersham International, Buckinghamshire, UK) by liquid scintillation counting. The amount of protein in the solubilized cell monolayers was determined by the method of Bradford (1976), using a Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Hercules, CA) with bovine γ-globulin as a standard.

Polyclonal antibody against OCT1. According to our previous reports (Saito et al., 1995; Masuda et al., 1997), polyclonal antibodies were raised against a synthetic peptide corresponding to the intracellular domains of the COOH-terminal of rat OCT1 (LYQVQTVKKSSST). The peptide was synthesized with cysteine at its NH2-terminal; the purity of the peptide was shown to be 96.5% by high-performance liquid chromatography (Peptide Institute, Osaka, Japan). After obtaining premuun serum, rabbit antiserum was raised against the peptide conjugated to keyhole limpet hemocyanin. A male New Zealand White rabbit (2.5 kg) was immunized with 1 ml of conjugates (1 mg of the peptide) emulsified with Freund’s complete adjuvant. The emulsified conjugates were injected as boosters every 2 wk after the antibody was obtained. After each booster injection, blood was collected and antibody production was determined by enzyme-linked immunosorbsent assay. We also tried to raise an anti-OCT2 antibody, but we could not obtain the antibody with sufficient titer.

Affinity purification of antibody and Western blotting analysis. The antiserum used for Western blotting analysis was purified by immunoadsorption on polynylinide diffusore membrane (Immobilon-P; Millipore, Bedford, MA) strips according to the method reported by Sabolic et al. (1992) with some modifications. The synthetic antigen peptides were separated by 15% SDS-PAGE and transferred onto Immobilon-P membranes by semi-dry electrobloctitng for 30 min. A horizontal strip of membrane containing the peptide was confirmed by Western blotting, excised, washed with Tris-buffered saline containing Tween 20 (TBS-T, 20 mM Tris-HCl, 137 mM NaCl, 0.1% Tween 20, pH 7.5), and then incubated with the immune serum for 4 hr at 25°C to adsorb anti-OCT1 antibody. The strips were then washed with TBS-T buffer, and the immunoaffinity-adsorbed antibody was released by incubation in 0.1 M citrate buffer (pH 2.0) for 1 min with constant mixing, followed by neutralization to pH 7.4 with 1 M Tris-HCl buffer (pH 10.5). The affinity-purified anti-OCT1 antibody was diluted 1:10 for Western blotting.

Brush-border and basolateral membranes were purified simulaneously from the rat renal cortex and medulla as described (Inui et al., 1981; Takano et al., 1984). For Western blotting analysis, the membrane fractions were solubilized in a buffer consisting of 2% SDS, 125 mM Tris-HCl, 20% glycerol in the presence of 5% β-mercaptoethanol. The samples were separated by 10% SDS-PAGE and transferred onto Immobilon-P membranes by semi-dry electroblocitng for 30 min. The blots were incubated with purified antisemirum preabsorbed with the synthetic antigen peptide (1.0 μg/ml) or the primary purified antibody (1:10) for 2 hr at 25°C. Blots were washed three times with TBS-T, and the bound antibody was detected on x-ray film by enhanced chemiluminescence with horseradish peroxidas-conjugated anti-rabbit IgG antibody and cyclic diacylhydrazides (Amersham).
Materials. [1,14C]Tetraethylammonium bromide (185.0 MBq/mmol) was obtained from Du Pont-New England Nuclear Research Products (Boston, MA). Cimetidine, quinidine sulfate, tetraethylammonium bromide, guanidine hydrochloride, HEPES and MES were purchased from Nacalai Tesque Inc. (Kyoto, Japan), and 1-methyl-4-phenylpyridinium iodide was from Research Biochemicals International (Natick, MA). (-)-Nicotine hydrog tartrate salt and N2-methyl nicotinamide iodide were purchased from Sigma Chemical Co. (St. Louis, MO). Cephalexin (Nacalai Tesque Inc. (Kyoto, Japan), and 1-methyl-4-phenylpyridinium mide, guanidine hydrochloride, HEPES and MES were purchased from (Boston, MA). Cimetidine, quinidine sulfate, tetraethylammonium bromide was obtained from Du Pont-New England Nuclear Research Products (Boston, MA). Cephalexin was a gift from Shionogi and Co. (Osaka, Japan). All other chemicals were of the highest purity available.

Results

When cultured on a solid matrix, MDCK cells derived from the canine kidney develop epithelial features such as asymmetric localization of membrane proteins and vectorial transport of various solutes (Handler et al., 1980). We introduced rat OCT1- and OCT2-cDNAs into MDCK cells by calcium phosphate coprecipitation as described in "Methods." Seven and eight G418-resistant clones from the cells transfected with OCT1- and OCT2-cDNA, respectively, were selected for further evaluation of [14C]tetraethylammonium uptake activity. When tetraethylammonium was added to the medium on the basolateral side, its accumulation into the cells transfected with OCT1- and OCT2-cDNAs was markedly elevated compared to that of control cells transfected with pBK-CMV (MDCK-pBK cells). In addition, the basolateral uptake of tetraethylammonium by all clones was markedly higher than the apical uptake (14- to 87-fold and 10- to 98-fold higher for the basolateral uptake than the apical uptake by the cells transfected with OCT1- and OCT2-cDNAs, respectively), suggesting that both rat OCT1 and OCT2 transporters are expressed in the basolateral membranes of these transfectants. Among these clones, single cells retaining growth rate and morphological features of MDCK cells were selected and named MDCK-OCT1 and MDCK-OCT2, respectively.

Figure 1 shows RT-PCR detection of mRNAs encoding rat OCT1 and OCT2 in rat organs and the transfectants. OCT1 mRNA was expressed in the rat liver, kidney and MDCK-OCT1 cells, although OCT2 mRNA was expressed only in the kidney and MDCK-OCT2 cells. Tissue distributions of mRNAs encoding rat OCT1 and OCT2 were comparable to those reported by Gründemann et al. (1994) and Okuda et al. (1996), respectively.

The time courses of tetraethylammonium accumulation by MDCK-OCT1 and MDCK-OCT2 cells were measured. When added to the basolateral medium, tetraethylammonium accumulation by MDCK-OCT1 and MDCK-OCT2 cells was markedly elevated compared to MDCK-pBK cells (fig. 2). However, when tetraethylammonium was added to the apical medium, its accumulation by MDCK-OCT1 and MDCK-OCT2 cells was only slightly elevated or did not change, respectively, compared to the control MDCK-pBK cells. These results suggest that both OCT1 and OCT2 are functionally expressed in the basolateral membranes but not in the apical membranes of these transfectants. In this experiment, radioactivity in the medium on the opposite side (transcellular transport) was minimal or absent for MDCK-OCT1 or MDCK-OCT2 cells, respectively, suggesting that the transport of tetraethylammonium across the apical membranes is negligible (data not shown).

To examine the pH dependence of tetraethylammonium transport by OCT1 and OCT2, monolayers of MDCK-OCT1 and MDCK-OCT2 cells were exposed for 15 min to medium at various pHs containing tetraethylammonium on the basolateral side. As shown in figure 3, tetraethylammonium uptake via the basolateral membranes was decreased in accordance with decreases in pH from 8.4 to 5.4. Furthermore, the effects of pH on the efflux of tetraethylammonium from MDCK-OCT1 and MDCK-OCT2 cells were examined (fig. 4). Cells were incubated for 15 min with [14C]tetraethylammonium on the basolateral side, and then the media on both sides were replaced with fresh incubation media (without [14C]tetraethylammonium). Radioactivity remaining in the cells after 5 min incubation was determined. As shown in figure 4, tetraethylammonium efflux from the cells was not affected by the external pH (5.4–8.4), suggesting that extrusion of tetraethylammonium from MDCK-OCT1 and MDCK-OCT2 cells is not pH dependent.

Next, we examined the concentration dependence of tetraethylammonium uptake by MDCK-OCT1 and MDCK-OCT2 cells (fig. 5). The transfectants were exposed to various concentrations of tetraethylammonium (0.01–1 mM) for 5 min from the basolateral side, then radioactivity in the cells was determined. Tetraethylammonium uptake across the basolateral membranes of MDCK-OCT1 and MDCK-OCT2 cells was markedly elevated compared to MDCK-pBK cells.
MDCK-OCT1 and MDCK-OCT2 cells were 38 and 45 m 


g 


of MDCK-OCT1 and MDCK-OCT2 cells. Monolayers of MDCK-OCT1 (A) and MDCK-OCT2 (B) in the 4.71-cm² chambers were incubated for 15 min at 37°C with 2 ml of 50 µM [14C]tetraethylammonium in medium at various pHs added to the basolateral side. After incubation, the radioactivity of solubilized cells was counted. Each point represents the mean ± S.E. of three monolayers.

![Fig. 3. Effects of pH on [14C]tetraethylammonium accumulation by MDCK-OCT1 (A) and MDCK-OCT2 (B) cells from the basolateral side. Monolayers of MDCK-OCT1 or MDCK-OCT2 in the 4.71-cm² chambers were incubated for 15 min at 37°C with 2 ml of 50 µM [14C]tetraethylammonium in medium at various pHs added to the basolateral side. After incubation, the radioactivity of solubilized cells was counted. Each point represents the mean ± S.E. of three monolayers.](image)

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was saturated at high concentrations. Kinetic parameters were calculated using the Michaelis-Menten equation, and apparent $K_m$ values for tetraethylammonium uptake by MDCK-OCT1 and MDCK-OCT2 cells were 38 and 45 µM, respectively. Eadie-Hofstee plots (insets of fig. 5) for these experiments were linear, suggesting that a single transport system for each transfectant was involved in tetraethylammonium uptake.

To examine the characteristics of substrate recognition, the effects of various compounds on tetraethylammonium uptake by MDCK-OCT1 and MDCK-OCT2 cells were examined. Accumulation of tetraethylammonium by both MDCK-OCT1 and MDCK-OCT2 cells for 15 min was measured in the presence of various concentrations of organic cations. As shown in figure 6, all organic cations inhibited tetraethylammonium uptake by MDCK-OCT1 and MDCK-OCT2 cells. In contrast, the inhibitory effect of cephalaxin, a zwitterionic compound that is secreted from renal tubules, on tetraethylammonium uptake by MDCK-OCT1 or MDCK-OCT2 cells was minimal, suggesting that both OCT1 and OCT2 recog-

![Fig. 4. Effects of pH on [14C]tetraethylammonium efflux from monolayers of MDCK-OCT1 and MDCK-OCT2 cells. Monolayers of MDCK-OCT1 (●) or MDCK-OCT2 (▲) in the 4.71-cm² chambers were incubated for 15 min at 37°C with 50 µM [14C]tetraethylammonium added to the basolateral side (2 ml, pH 7.4). Cell monolayers were washed, and then incubated for 5 min with unlabeled incubation medium at various pHs. After incubation, the radioactivity of solubilized cells was counted. Each point represents the mean ± S.E. of three monolayers.](image)

Fig. 4. Effects of pH on [14C]tetraethylammonium efflux from monolayers of MDCK-OCT1 and MDCK-OCT2 cells. Monolayers of MDCK-OCT1 (●) or MDCK-OCT2 (▲) in the 4.71-cm² chambers were incubated for 15 min at 37°C with 50 µM [14C]tetraethylammonium added to the basolateral side (2 ml, pH 7.4). Cell monolayers were washed, and then incubated for 5 min with unlabeled incubation medium at various pHs. After incubation, the radioactivity of solubilized cells was counted. Each point represents the mean ± S.E. of three monolayers.

nize a wide variety of cationic charged molecules. Inhibitory potencies of organic cations were in the order of 1-methyl-4-phenylpyridinium > cimetidine ≈ quinidine > tetraethylammonium ≈ nicotine > N1-methylnicotinamide ≈ guanidine. Inhibition constants ($K_i$) of these compounds against tetraethylammonium uptake by MDCK-OCT1 and MDCK-OCT2 cells were calculated and are summarized in table 1. The $K_i$ values for each compound were comparable between MDCK-OCT1 and MDCK-OCT2 cells.

Furthermore, we examined membrane localization of rat OCT1 using a polyclonal antibody against rat OCT1. Figure 7 shows the membrane localization of OCT1 in the brush-border and basolateral membranes of rat kidney cortex and medulla. When blots were incubated with affinity-purified antibody against rat OCT1 polypeptide, an immunoreactive protein with an apparent molecular mass of ~66 kDa was detected at high levels in the basolateral membranes of the rat kidney cortex and a weak band was observed in those of the kidney medulla, but no immunoreactive band was detected in the brush-border membranes (fig. 7A). These immunoreactive bands were completely abolished when the antibody was preabsorbed with OCT1 antigen peptide (1.0 µg/ml) (fig. 7B).

![Fig. 5. Concentration dependence of [14C]tetraethylammonium accumulation by MDCK-OCT1 (A) and MDCK-OCT2 (B) cells from the basolateral side. [14C]Tetraethylammonium accumulation (●) by cell monolayers in 4.71-cm² chambers was measured at various concentrations (0.01–1 mM) for 15 min at 37°C after addition to the basolateral side (2 ml, pH 7.4). Unlabeled incubation medium was added to the apical side (2 ml, pH 7.4). After incubation, the radioactivity of solubilized cells was determined. Insets represent Eadie-Hofstee plots (●) for each experiment after correction of the nonsaturable component. Each point represents the mean ± S.E. of three monolayers.](image)

Fig. 5. Concentration dependence of [14C]tetraethylammonium accumulation by MDCK-OCT1 (A) and MDCK-OCT2 (B) cells from the basolateral side. [14C]Tetraethylammonium accumulation (●) by cell monolayers in 4.71-cm² chambers was measured at various concentrations (0.01–1 mM) for 15 min at 37°C after addition to the basolateral side (2 ml, pH 7.4). Unlabeled incubation medium was added to the apical side (2 ml, pH 7.4). After incubation, the radioactivity of solubilized cells was determined. Insets represent Eadie-Hofstee plots (●) for each experiment after correction of the nonsaturable component. Each point represents the mean ± S.E. of three monolayers.

**Discussion**

In this study, we constructed MDCK transfectants expressing rat OCT1 or OCT2 to clarify the functional characteristics of these transporters. Our results indicated that rat OCT1 is a basolateral organic cation transporter in the rat kidney, and that rat OCT2 is also a basolateral-type organic cation transporter, mediating tubular secretion of various organic cations.

Stimulation of transport function by the proton gradient is the most critical feature of the H⁺/organic cation antiporter localized in the brush-border membranes, but not of the basolateral organic cation transporter. In our previous studies, tetraethylammonium uptake by renal brush-border membrane vesicles was shown to be stimulated by the proton...
In this study, we examined the effects of pH on the basolateral transport of organic cations. The basolateral uptake of tetraethylammonium across basolateral membranes, rather than that on brush-border membranes, is markedly stimulated by lowering apical pH (Saito et al., 1984), and extrusive transport of tetraethylammonium from kidney epithelial cells across apical membranes was markedly stimulated by lowering apical pH (Saito et al., 1992). However, little is known about the effects of pH on the basolateral transport of organic cations. Previously, we examined the effects of pH on tetraethylammonium uptake via basolateral membranes of LLC-PK₁ cells (Inui and Saito, 1992). The basolateral uptake of tetraethylammonium was decreased by lowering basolateral or intracellular pH, suggesting that the basolateral organic cation transporter is regulated by environmental pH, but not by a pH-gradient across the basolateral membranes of LLC-PK₁ cells. Recently, Kim and Dantzler (1997) also reported that tetraethylammonium uptake across basolateral membranes of snake renal tubules is inhibited by acidification of the basolateral medium. In our study, tetraethylammonium uptake via basolateral membranes of MDCK-OCT1 and MDCK-OCT2 cells was inhibited by lowering the pH of the medium (fig. 3). However, efflux of tetraethylammonium from these transfectants was not affected by the pH of the basolateral medium. These results suggest that transport functions of neither OCT1 nor OCT2 are stimulated by the proton gradient, but rather they are regulated by environmental pH. Furthermore, in studies using *Xenopus* laevis oocytes, decreasing transmembrane electrical potential by displacement of sodium ions with potassium ions resulted in decreased accumulation of tetraethylammonium in oocytes injected with either OCT1 RNA (Gründemann et al., 1994) or OCT2 RNA (uptake of 64.4 ± 7.4 pmol/oocyte/hr for low potassium buffer with 101 mM Na⁺ and 1 mM K⁺ decreased to uptake of 44.5 ± 1.0 pmol/oocyte/hr for high potassium buffer with 2 mM Na⁺ and 100 mM K⁺) (M. Okuda, Y. Urakami, H. Saito and K.-I. Inni, unpublished observation). These results suggest that the driving force for the tetraethylammonium transport by OCT1 and OCT2 is not the pH gradient but the transmembrane electrical potential. We speculate that OCTs themselves and/or certain environmental factor(s) might be regulated by pH, although the precise mechanisms remain to be determined.

In general, substrate specificities of the brush-border and the basolateral organic cation transporters are similar, but not identical. David et al. (1995) reported that cationic molecules with low hydrophobicity and low basicity interact with basolateral organic cation transport systems more strongly than that in the brush-border membranes. In this study, we analyzed inhibitory potencies of several organic cations that are known to be secreted into the urine across renal tubular epithelial cells. The order of inhibitory potencies of various organic cations on tetraethylammonium uptake by OCT1 and OCT2 was comparable to that on N¹-methyl nicotinamide uptake across basolateral membranes, rather than that on 1-methyl-4-phenylpyridinium uptake across brush-border membranes of rat renal tubules (David et al., 1995). These

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### TABLE 1

Inhibition constants for organic cations to compete with 
[¹⁴C]tetraethylammonium accumulation by MDCK-OCT1 and MDCK-OCT2 cells

<table>
<thead>
<tr>
<th>Compounds</th>
<th>OCT1 (µM)</th>
<th>OCT2 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Methyl-4-phenylpyridinium</td>
<td>0.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>5.7</td>
<td>9.4</td>
</tr>
<tr>
<td>Quinidine</td>
<td>14.6</td>
<td>19.1</td>
</tr>
<tr>
<td>Tetraethylammonium</td>
<td>46.6</td>
<td>52.2</td>
</tr>
<tr>
<td>Nicotine</td>
<td>64.3</td>
<td>50.5</td>
</tr>
<tr>
<td>N¹-Methyl nicotinamide</td>
<td>669.2</td>
<td>403.4</td>
</tr>
<tr>
<td>Guanidine</td>
<td>724.2</td>
<td>713.6</td>
</tr>
</tbody>
</table>

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**Fig. 6.** Effects of various compounds on [¹⁴C]tetraethylammonium accumulation by MDCK-OCT1 (A) and MDCK-OCT2 (B) cells. Monolayers of MDCK-OCT1 or MDCK-OCT2 in 1.0-cm² chambers were incubated for 15 min at 37°C with 50 µM [¹⁴C]tetraethylammonium in the presence of 1-methyl-4-phenylpyridinium (●), cimetidine (○), quinidine (▲), tetraethylammonium (□), nicotine (●), N¹-methyl nicotinamide (△), guanidine (◇) and cephalxin (▼) added to the basolateral side (1 ml, pH 7.4). After incubation, the radioactivity of solubilized cells was counted. Each point represents the mean ± S.E. of three monolayers.

**Fig. 7.** Western blotting analysis of rat brush-border and basolateral membranes purified from either rat kidney cortex or medulla with antiserum for rat OCT1. Aliquots of 50 µg of each membrane preparation were separated by SDS-PAGE (10%) and blotted onto PVDF membranes. A, Affinity-purified antiserum for rat OCT1 (1:10 dilution) was used as the primary antibody. B, Affinity-purified anti-OCT1 antiserum (1:10) preabsorbed with the rat OCT1 peptide (1.0 µg/ml) was used as a control. Horseradish peroxidase-conjugated anti-rabbit IgG antibody was used for detection of bound antibodies, and strips of the blots were visualized by chemiluminescence on x-ray film. The arrows indicate the positions of OCT1. BBM, brush-border membranes; BLM, basolateral membranes.
results support the interpretation that both OCT1 and OCT2 are basolateral-type organic cation transporters, mediating accumulation of organic cations in the kidney.

Recently, Gründemann et al. (1997) reported cDNA cloning of the porcine organic cation transporter, OCT2p, from the renal epithelial cell line LLC-PK1. They investigated the transport characteristics of OCT2p using several organic compounds as inhibitors of tetraethylammonium uptake. By comparing the order of inhibitory potencies of several compounds on cellular accumulation of tetraethylammonium between OCT2p-transfected cells and LLC-PK1 cells from the apical side, they speculated that OCT2p is an apical organic cation transporter expressed in LLC-PK1 cells. In contrast, we found that rat OCT2 is a basolateral-type organic cation transporter based on the following evidence. First, the driving force for tetraethylammonium transport by rat OCT2 was similar to that for the basolateral organic cation transporter. Tetraethylammonium efflux from MDCK-OCT2 cells was not proton gradient dependent, and tetraethylammonium uptake by OCT2 RNA-injected oocytes was inhibited by decreasing the transmembrane electrical potential. These results were similar to those for rat OCT1, expression of which was shown immunologically to be dominant in the basolateral membranes rather than brush-border membranes (fig. 7). Second, when rat OCT1 and OCT2 cDNAs were introduced in MDCK cells, both OCT1 and OCT2 were functionally expressed in the basolateral membranes. Third, the specificities of substrate affinity for rat OCT1 and OCT2 were comparable to those observed for the basolateral membranes of rat renal tubules (David et al., 1995). Although Gründemann et al. (1997) deduced that OCT2p is the porcine homologue of rat OCT2, we speculated that OCT2p is another member of the OCT family. It is also possible that there are species-related differences between rat OCT1 and porcine OCT2p.

Recently, Gorboulev et al. (1997) reported the membrane localization of the human isofrom of OCT2 (hOCT2) in the brush-border membranes of distal tubules by immunological staining. They used anti-rat OCT1 antibody, raised against a partial rat OCT1 amino acid sequence, to detect hOCT2 protein in the kidney. As the corresponding amino acid sequence of hOCT2 is not identical to that of rat OCT1, their results may merely indicate the presence of immunoreactive material in the brush-border membranes of human distal tubules. As reported previously (Okuda et al., 1996), the level of mRNA encoding OCT2 was greater in the medulla than in the cortex, although the level of expression of OCT1 mRNA was greater in the cortex than in the medulla (Gründemann et al., 1994). However, the transport characteristics of OCT1 and OCT2 are similar. This raises the question of why these two similar transporters are expressed in the kidney. One possibility is that there are differences in the regulation of transport activity between rat OCT1 and OCT2. However, we propose another possibility as follows. Removal of toxic or unnecessary cationic compounds in the body is critical for defense against their harmful effects. Recently, it has become clear that mutations in the transporter genes result in serious diseases such as malabsorption of glucose in the intestine (Turk et al., 1991) and defects in biliary excretion of bilirubin in the liver (Paulusma et al., 1996). OCT1 and OCT2 may compensate for each other under conditions of transporter dysfunction.

In conclusion, we studied the transport characteristics and membrane localization of the rat organic cation transporters OCT1 and OCT2. Our results indicated that both rat OCT1 and OCT2 are basolateral-type organic cation transporters with broad substrate specificities, and contribute to the extrusion of cationic drugs from blood into the urine.

References