cDNA Cloning, Functional Characterization, and Tissue Distribution of an Alternatively Spliced Variant of Organic Cation Transporter hOCT2 Predominantly Expressed in the Human Kidney

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Abstract. A cDNA coding a novel organic cation transporter, hOCT2-A, was isolated from human kidney. The hOCT2-A cDNA is an alternatively spliced variant of hOCT2 with an insertion of 1169 bp. The open reading frame encodes a 483-amino acid protein that has 81% amino acid identity with hOCT2. From hydropathy analysis, hOCT2-A is predicted to have nine transmembrane domains. hOCT2-A mRNA is expressed mainly in kidney and weakly in brain, liver, colon, skeletal muscle, bone marrow, spinal cord, testis, and placenta. When expressed in HEK293 cells, hOCT2-A stimulated the uptake of tetraethylammonium (TEA) in an electrogenic manner. The transport of TEA by hOCT2-A–transfected cells was saturable with the apparent Km value of 63 μM. hOCT2-A stimulated the uptake of TEA, 1-methyl-4-phenylpyridinium, and cimetidine as well as did hOCT2. The uptake of guanidine and choline by hOCT2-transfected cells also increased markedly but not that by hOCT2-A–transfected cells. The uptake of TEA mediated by hOCT2-A but not by hOCT2 was inhibited significantly by organic cations such as procainamide, N-acetylprocainamide, and levofloxacin, indicating that hOCT2-A differs from hOCT2 in its affinity for several compounds. These findings suggested that hOCT2-A contributes to the renal clearance of endogenous and exogenous organic cations.

Secretion is an important physiologic function for the maintenance of body fluid homeostasis and detoxification of drugs and xenobiotics. The proximal tubular cells play a principal role in limiting or preventing toxicity by actively secreting organic cations from the circulation into the urine (1–4). Functional studies using isolated membrane vesicles (5–7) and cultured renal epithelial cells (8,9) suggested that renal tubular secretion of cationic substances is controlled by the concerted action of two distinct classes of organic cation transporters: one driven by the transmembrane potential difference in the basolateral membranes and the other driven by the transmembrane H+ gradient in the brush border membranes.

Several organic cation transporters of the OCT gene have been identified: OCT1 (10), OCT2 (11), OCT3 (12), OCTN1 (13), and OCTN2 (14,15). ratOCT1A has been identified from rat kidney as a spliced variant of rat (r) OCT1 (16). We isolated the second member of the OCT family, ratOCT2, in rat kidney, which has 67% amino acid identity to rOCT1 (11). rOCT1 is expressed abundantly in the liver and kidney (10), whereas ratOCT2 is expressed predominantly in the kidney (11). Functional studies using Xenopus oocytes (10,11,17,18) and transfected mammalian cells (19–21) suggested that rOCT1 and ratOCT2 have multispecific (polyspecific) substrate specificity and translocate various structurally unrelated cationic compounds in an electrogenic manner. Immunohistochemical analysis showed that both rOCT1 and ratOCT2 proteins were localized in the basolateral membrane of renal tubular cells (22,23). Human (h) OCT1 and hOCT2 were isolated from the human liver and kidney, respectively (24). It is interesting that hOCT2 is expressed mainly in the kidney, whereas hOCT1 is transcribed mainly in the liver (24). A recent study by Pietig et al. (25) showed that organic cation transport across the basolateral membrane of isolated human proximal tubules, most likely mediated by hOCT2, is electrogenic.

Recently, the spliced isoforms in some genes encoding renal transporters have been identified (26). In some cases, the spliced isoforms have been functional, resulting in a variety of physiologic consequences, including changes in the polarization of isoforms, changes in pharmacologic or kinetic properties, and changes in tissue expression or intrarenal localization (26). Because the renal organic cation transporters mediate secretion of various organic cations in the basolateral or brush border membranes, we hypothesized that several spliced isoforms derived from the OCT genes would be expressed in the kidney.

We report here the identification of a novel organic cation...
transporter, hOCT2-A, isolated from a human kidney cDNA library. Functional studies indicated that hOCT2-A, along with hOCT2, is a multispecific organic cation transporter in human kidney. This is the first evidence of alternatively spliced variant of hOCT2.

**Materials and Methods**

**cDNA Cloning of Human Organic Cation Transporters**

The full-length hOCT2 and hOCT2-A cDNA were isolated from a human kidney Rapid-Screen cDNA library panel (OriGene Technologies, Rockville, MD), according to the manufacturer’s instructions, that was screened by PCR using the following hOCT2-specific primers derived from the published sequence (24): forward 5'-TGTT-CACATTGAGGATAC-3' (bases 18 to 38) and reverse 5'-AA-CATCCACAGTATAGGTGGGG-3' (bases 734 to 755); forward 5'-CATTTCCCAAACCTATACGTTG-3' (bases 729 to 750) and reverse 5'-AGTTTCCTCTTCAGTTCAGG-3' (bases 1104 to 1125). The isolated hOCT2 and hOCT2-A cDNA clones were sequenced on both strands by the chain-termination method using a fluorescence 373A DNA sequencer (Applied Biosystem, Foster, CA). Multiple sequence alignments were produced using GENETYX-MAC Version 10 (Software Development Co., LTD, Tokyo, Japan).

**Northern Blot and Reverse Transcription–Coupled PCR Analyses**

Multiple tissue Northern blots (Clontech, Palo Alto, CA) were hybridized with high stringency (50% formamide, 5× SSPE [1× SSPE; 0.15 M NaCl, 10 mM NaH2PO4, 1 mM ethylenediaminetetraacetic acid (pH 7.4)], 5× Denhardt’s solution, 0.2% sodium dodecyl sulfate [SDS], and 10 μg/ml salmon sperm DNA at 42°C) with cDNA encoding either hOCT2-A or human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) labeled with [α-32P]dCTP. The cDNA probes corresponded to the nucleotide positions 1497 to 2626 (hOCT2-A) or human GAPDH labeled with [3H]cimetidine, and [14C]guanidine was measured with monolayer cultures grown on poly-d-lysin-coated 24-well plates. The composition of the incubation medium was as follows (in mM): 145 NaCl, 3 KCl, 1 CaCl2, 0.5 MgCl2, 5 d-glucose, and 5 HEPES (pH 6.4, 7.4, 8.4) or MES (2-[N-Morpholino]ethanesulfonic acid; pH 5.4). The composition of K+ high incubation medium was as follows (in mM): 3 NaCl, 145 KCl, 1 CaCl2, 0.5 MgCl2, 5 d-glucose, and 5 HEPES (pH 7.4). The cells were preincubated with 0.2 ml of incubation medium for 10 min at 37°C. The medium was then removed, and 0.2 ml of incubation medium containing [14C]TEA (5 μM) was added. The medium was aspirated at the end of the incubation period, and the monolayers were rapidly washed twice with 1 ml of ice-cold incubation medium. The cells were solubilized in 0.5 ml of 0.5N NaOH, and then the radioactivity in aliquots was determined by liquid scintillation counting. For the efflux experiment, the transfected cells were incubated for 15 min at 37°C with incubation medium containing [14C]TEA (5 μM, pH 7.4). The cells were washed and then incubated for the specified periods with incubation medium at pH 6.4 or 7.4. The [14C]TEA remaining in the cells was measured. The protein content of the solubilized cells was determined by the method of Bradford (27), using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA) with bovine γ-globulin as a standard.

**Statistical Analyses**

Data were analyzed statistically using nonpaired t test or the one-way ANOVA followed by Scheffe’s test.

**Materials**

[ethyl-1-14C] Tetraethylammonium bromide (55 mCi/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). [3H]1-methyl-4-phenylpyridinium (MPP), [3H]cimetidine, and [14C]guanidine was measured with monolayer cultures grown on poly-d-lysin-coated 24-well plates. The composition of the incubation medium was as follows (in mM): 145 NaCl, 3 KCl, 1 CaCl2, 0.5 MgCl2, 5 d-glucose, and 5 HEPES (pH 6.4, 7.4, 8.4) or MES (2-[N-Morpholino]ethanesulfonic acid; pH 5.4). The composition of K+ high incubation medium was as follows (in mM): 3 NaCl, 145 KCl, 1 CaCl2, 0.5 MgCl2, 5 d-glucose, and 5 HEPES (pH 7.4). The cells were preincubated with 0.2 ml of incubation medium for 10 min at 37°C. The medium was then removed, and 0.2 ml of incubation medium containing [14C]TEA (5 μM) was added. The medium was aspirated at the end of the incubation period, and the monolayers were rapidly washed twice with 1 ml of ice-cold incubation medium. The cells were solubilized in 0.5 ml of 0.5N NaOH, and then the radioactivity in aliquots was determined by liquid scintillation counting. For the efflux experiment, the transfected cells were incubated for 15 min at 37°C with incubation medium containing [14C]TEA (5 μM, pH 7.4). The cells were washed and then incubated for the specified periods with incubation medium at pH 6.4 or 7.4. The [14C]TEA remaining in the cells was measured. The protein content of the solubilized cells was determined by the method of Bradford (27), using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA) with bovine γ-globulin as a standard.

**Cell Culture and Transfection**

HEK 293 cells (American Type Culture Collection CRL-1573), a transformed cell line derived from human embryonic kidney, were cultured in complete medium consisting of Dulbecco’s modified Eagle’s medium (Sigma Chemical Co., St. Louis, MO) with 10% fetal calf serum (Whittaker Bioproducts Inc., Walkersville, MD) in an atmosphere of 5% CO2, 95% air at 37°C. pCMV6-XL4 plasmid vector DNA containing hOCT2 or hOCT2-A cDNA was purified using the CONCERT High Purity Plasmid Purification Midiprep System (Life Technologies, Inc., Rockville, MD). The day before the transfection, HEK 293 cells were seeded onto poly-d-lysine-coated 24-well plates at a density of 2.0 × 105 cells/well. The cells were transfected with 0.8 μg of total plasmid DNA per well using LipofectAMINE 2000 (Life Technologies) according to the manufacturer’s instructions. At 48 h after transfection, the cells were used for uptake experiments.
ylnicotinamide iodide, N-acetylprocainamide hydrochloride, and amantadine were purchased from Sigma Chemical Co. 1-Methyl-4-phenylpyridinium iodide was purchased from Research Biochemicals International (Natick, MA). All other compounds used were of the highest purity available.

Results

**cDNA Cloning of the hOCT2 Alternatively Spliced Variant**

Using primers derived from the published cDNA sequence of hOCT2 (24), we obtained two cDNA clones of approximately 2.4 kb and 3.7 kb from a human kidney cDNA library. Sequence analysis demonstrated that the sequence of the 2.4-kb cDNA was identical to the published hOCT2 cDNA sequence (data not shown). The 3.7-kb cDNA clone designated as hOCT2-A was distinct from hOCT2. Figure 1 shows the nucleotide sequence of hOCT2-A cDNA in comparison with that of hOCT2. The hOCT2-A cDNA consists of 3694 bp with an insertion of 1169 bp. The hOCT2 gene (SLC22A2) consists of 11 exons (28). hOCT2-A uses exon 1 to exon 11, and the intron between exon 7 and exon 8 as the new exon (Figure 1B).

On the basis of the Kozak consensus sequence (29), the insertion results in a stop codon at position 1628 and the open reading frame of the cloned hOCT2-A cDNA consist of 1449 nucleotides, coding for a 483-amino acid protein. Figure 2A shows the deduced amino acid sequence of hOCT2-A and its alignment with hOCT2. hOCT2 and hOCT2-A showed an amino acid identity of 81%. hOCT2-A also showed amino acid identity of 62% to rOCT2, 58% to hOCT1, 56% to rOCT1, 42% to hOCT3, and 40% to rOCT3. A Kyte-Doolittle (30) hydropathy analysis suggested that hOCT2-A has nine putative membrane-spanning domains (Figure 2B), thus indicating four potential N-linked glycosylation sites in the extracellular loop. There is one potential cAMP-dependent kinase phosphorylation site at position 345 and two potential protein kinase C phosphorylation sites at positions 286 and 327 (29).

![Figure 1](image1.png)

![Figure 2](image2.png)

**Figure 1.** The nucleotide and amino acid sequences of hOCT2-A (A) and a diagram of the hOCT2 gene (SLC22A2) showing a possible mechanism for alternative splicing giving rise to hOCT2-A (B). (A) The insertion of 1169 bp is lined under the sequence. (B) Lines and boxes show the introns and exons, respectively, of SLC22A2. The novel sequence region of hOCT2-A cDNA (top) that is not hOCT2 cDNA (bottom) is shown by a solid box.

**Figure 2.** Comparison of the deduced amino acid sequences (A) and hydropathy plots (B) of hOCT2-A and hOCT2. (A) Residues conserved between the two transporters are indicated by dots. Putative transmembrane-spanning domains are lined over the sequence with numbers (M1 to M9), and potential N-linked glycosylation sites are indicated by asterisks. Potential protein kinase C phosphorylation site (●), and protein kinase A phosphorylation site (●) are indicated. (B) Kyte and Doolittle hydropathy plots with a window of 13 amino acid residues. Numbers between plots indicate putative membrane-spanning regions.
Northern blot analysis of poly(A)$^+$ RNA from several human tissues using a specific probe for hOCT2-A cDNA revealed that the hOCT2-A mRNA transcript was predominantly expressed in the human kidney (Figure 3A). Two bands of 4.2 kb and 2.7 kb were detected under high-stringency conditions. No hybridization signal was detected in mRNA from any other tissues. Furthermore, the expression of hOCT2 and hOCT2-A mRNA in human tissues was investigated by reverse transcriptase–PCR amplification. As shown in Figure 3B, the PCR product with an expected size of 512 bp for hOCT2-A was found in kidney and weak signal for hOCT2-A was detected in brain, liver, colon, skeletal muscle, bone marrow, spinal cord, testis, and placenta. However, the specific PCR product for hOCT2 was found in kidney and weak signal for hOCT2 was detected in brain, lung, spinal cord, cerebellum, testis, uterus, and placenta.

**Figure 3.** Northern blot analysis (A) and detection by PCR amplification (B) of hOCT2-A mRNA in human tissues. (A) Commercially available hybridization-ready poly(A)$^+$ RNA blots were hybridized with the hOCT2-A (a) or glyceraldehyde 3-phosphate dehydrogenase (GAPDH; b) cDNA as a probe at high stringency. Tissues were as follows: 1, brain; 2, heart; 3, skeletal muscle; 4, colon (no mucosa); 5, thymus; 6, spleen; 7, kidney; 8, liver; 9, small intestine; 10, placenta; 11, lung; 12, peripheral blood leukocyte. (B) One microgram of total RNA from human tissues was reverse-transcribed and amplified using a set of primers specific for the hOCT2 (a), hOCT2-A (c), or GAPDH (e) cDNA as described in the Materials and Methods section. The PCR products were separated by electrophoresis through 1.5% agarose gels, blotted to a nylon membrane, and hybridized with specific cDNA probes for hOCT2 (b) or hOCT2-A (d). Lanes were as follows: 1, hOCT2/pCMV6-XL4; 2, hOCT2-A/pCMV6-XL4; 3, brain; 4, lung; 5, heart; 6, liver; 7, stomach; 8, pancreas; 9, small intestine; 10, colon; 11, kidney; 12, skeletal muscle; 13, bone marrow; 14, spinal cord; 15, cerebellum; 16, thymus; 17, spleen; 18, thyroid; 19, adrenal gland; 20, mammary gland; 21, testis; 22, prostate; 23, uterus; 24, placenta.

**Functional Expression of hOCT2-A in HEK293 Cells**

The transport function of hOCT2-A was investigated in HEK293 cells transfected with hOCT2-A cDNA by measuring the uptake of $[^{14}C]$TEA and comparing it with that in hOCT2 transfecants (Figure 4). As shown in Figure 4A, the accumulation of $[^{14}C]$TEA increased time-dependently in hOCT2- or hOCT2-A–transfected cells, whereas the accumulation in cells transfected with expression vector (pCMV6-XL4) alone exhibited a negligible increase with time. In hOCT2- or hOCT2-A–transfected cells, the accumulation of TEA was linear for up to 2 min and then reached a steady state. To investigate the influence of membrane potential on hOCT2-A transport activity, we measured the accumulation of TEA in the transfected cells under altered conditions of K$^+$ permeability (Figure 4B). With this approach, increasing the concentration of K$^+$ in the uptake buffer depolarized the cell membrane potential (31). The amount of TEA accumulated in hOCT2-A–transfected cells decreased in the presence of high K$^+$ medium as well as in hOCT2-transfected cells. Furthermore, the accumulation of TEA in hOCT2- or hOCT2-A–transfected cells decreased in the presence of Ba$^{2+}$ (10 mM), K$^+$ channel blocker (Figure 4B). These results suggest that the transport activity of hOCT2-A is not sodium dependent but potential sensitive as well as that of hOCT2. Next, we examined the effect of pH on hOCT2-A transport activity (Figure 4C). The accumulation of TEA in hOCT2 or hOCT2-A–transfected cells decreased in accordance with decreases in the medium pH from 8.4 to 5.4. Furthermore, to investigate the influence of the external pH on efflux via hOCT2-A, we measured TEA remaining of hOCT2- or hOCT2-A–transfected cells under the altered medium pH (Figure 5). TEA efflux from hOCT2-A–transfected cells was inhibited by quinine (1 mM), and then TEA efflux via hOCT2-A was not affected by the external pH 6.4 to 7.4.

**Concentration Dependence of $[^{14}C]$TEA Accumulation in HEK293 Cells Transfected with hOCT2-A cDNA**

We examined the concentration dependence of $[^{14}C]$TEA accumulation in hOCT2-A–transfected HEK293 cells (Figure 6). The uptake of TEA by the cells was saturated at high concentrations. With the use of a nonlinear least squares regression analysis, kinetic parameters were calculated according to the Michaelis-Menten equation from three separate experiments on three monolayers. The apparent Michaelis–Menten constant (Km) values for hOCT2 and hOCT2-A were 431 ± 87 and 63 ± 1 μM (mean ± SEM), respectively. Maximal uptake rate (Vmax) values for hOCT2 and hOCT2-A were 3770 ± 634 and 314 ± 17 pmol/mg protein/min (mean ± SEM), respectively.

**Uptake of Various Cationic Compounds in HEK293 Cells Transfected with hOCT2 or hOCT2-A cDNA**

To characterize the substrate specificity of hOCT2-A, we measured the uptake of various cationic compounds in hOCT2-A–transfected cells, comparing the results with those for hOCT2-transfected cells. As shown in Figure 7,
hOC T2 and hOCT2-A stimulated the uptake of TEA, MPP, and cimetidine. The uptake of guanidine by hOCT2-transfected cells increased markedly but not that by hOCT2-A-transfected cells. However, the uptake of levofloxacin and 3’-azido 3’-deoxythymidine was not stimulated by either hOCT2 or hOCT2-A.

Figure 4. Time course of (A) and effect of membrane potential (B) or extracellular pH (C) on [14C]tetraethylammonium (TEA) accumulation in HEK293 cells transfected with hOCT2 or hOCT2-A cDNA. (A) The cells transfected with hOCT2 (●), hOCT2-A (▲), or pCMV6-XL4 vector (□) were incubated for the specified periods at 37°C with incubation medium containing [14C]TEA (5 μM, pH 7.4). Each point represents the mean ± SEM of 12 monolayers of four separate experiments. (B) The cells transfected with hOCT2 (●), hOCT2-A (▲), or pCMV6-XL4 vector (□) were incubated for 1 min at 37°C with incubation medium at the indicated ion concentrations containing [14C]TEA (5 μM, pH 7.4). Each column represents the mean ± SEM of three monolayers. (C) The cells transfected with hOCT2 (○) or hOCT2-A (●) were incubated for 1 min at 37°C with incubation medium containing [14C]TEA (5 μM) at various pH. Each point represents the mean ± SEM of three monolayers.

Figure 5. Effect of pH on [14C]TEA efflux from HEK293 cells transfected with hOCT2 (A) or hOCT2-A (B) cDNA. The cells transfected with hOCT2 cDNA or hOCT2-A cDNA were incubated for 15 min at 37°C with incubation medium containing [14C]TEA (5 μM, pH 7.4). The cells were washed and then incubated for the specified periods at 37°C with incubation medium at pH 7.4 (●), pH 6.4 (▲) or pH 7.4 containing with 1 mM quinine (○). [14C]TEA remaining in the cells was measured. Data represent percentages of the values at time 0. The values at time 0 were 42.6 ± 1.4 pmol/mg protein/15 min (A; hOCT2) and 40.2 ± 1.8 pmol/mg protein/15 min (B; hOCT2-A). Each point represents the mean ± SEM of three monolayers.

Figure 6. Concentration dependence of [14C]TEA accumulation in HEK293 cells transfected with hOCT2 (A) and hOCT2-A (B) cDNA. The cells transfected with hOCT2 or hOCT2-A cDNA were incubated for 1 min at 37°C (pH 7.4) with various concentrations of [14C]TEA in the absence (●) or presence (○) of 5 mM unlabeled 1-methyl-4-phenylpyridinium (MPP). Each point represents the mean ± SEM of three monolayers. Kinetic parameters for the saturable transport of TEA were calculated using the Michaelis-Menten equation from three separate experiments on three monolayers.

hOCT2 and hOCT2-A stimulated the uptake of TEA, MPP, and cimetidine. The uptake of guanidine by hOCT2-transfected cells increased markedly but not that by hOCT2-A-transfected cells. However, the uptake of levofloxacin and 3’-azido 3’-deoxythymidine was not stimulated by either hOCT2 or hOCT2-A.
Effect of Various Cationic Compounds on the Uptake of \([^{14}\text{C}]\)TEA in HEK293 Cells Transfected with hOCT2 or hOCT2-A cDNA

Next, we examined the accumulation of \([^{14}\text{C}]\)TEA in hOCT2- and hOCT2-A-transfected cells under conditions of cis-inhibition. MPP, nicotine, quinidine, grepafloxacin, imipramine, and amantadine inhibited markedly the hOCT2- and hOCT2-A-mediated uptake of \([^{14}\text{C}]\)TEA (Figure 8). Cimetidine, TEA, choline, thiamine, and N\(^1\)-methylnicotinamide inhibited markedly the hOCT2-A-mediated uptake of \([^{14}\text{C}]\)TEA but had relatively weak though significant inhibitory effects on the hOCT2-mediated uptake of \([^{14}\text{C}]\)TEA. Furthermore, procainamide, N-acetyl procainamide, and levofloxacin inhibited markedly the hOCT2-A-mediated uptake of \([^{14}\text{C}]\)TEA but not the hOCT2-mediated uptake at these concentrations. In contrast, hOCT2 showed higher affinity for noradrenaline and guanidine than hOCT2-A. The inhibitory effects of serotonin, histamine, and dopamine were comparable between hOCT2 and hOCT2-A.

Discussion

We have identified and characterized cDNA encoding a novel organic cation transporter, hOCT2-A, an alternatively spliced variant of hOCT2 with an insertion. The deduced amino acid sequence of hOCT2-A has 81\% identity with hOCT2, another organic cation transporter in the kidney.

DNA sequence alignment of hOCT2 and hOCT2-A demonstrated that the hOCT2-A cDNA had an insertion of 1169 bp. The insertion would lead to a stop codon at position 1628 and
result in a shorter protein than hOCT2 (Figure 1A). On the basis of a hydropathy analysis using the Kyte-Doolittle algorithm, hOCT2-A is predicted to have nine transmembrane domains. However, the OCT family are predicted to have 12 transmembrane domains (32). As a result of the insertion, hOCT2-A lacks the tenth to twelfth transmembrane domains of hOCT2. Nevertheless, hOCT2-A has activity to transport TEA, a typical organic cation substrate (Figure 4A). This result suggested that the last three transmembrane domains were not essential for the TEA transport function. It was also reported that rOCT1A had the activity to transport TEA, despite lacking the first two transmembrane domains of rOCT1 that are well conserved in the OCT family. These results suggested also that the sites essential for the transport of TEA by hOCT2 were in the third to ninth transmembrane domains.

Despite that the amino acid sequences of hOCT2 and hOCT2-A are highly conserved, hOCT2-A seemed to differ from hOCT2 in substrate affinity. As shown in Figure 6, hOCT2-A has less transport activity for guanidine than hOCT2. However, hOCT2-A had a higher affinity for cationic compounds, TEA, procainamide, N-acetyl procainamide, levofloxacin, thiamine, and N1'-methylnicotinium than hOCT2 (Figure 8). These results suggest that hOCT2-A has the narrow spectrum of substrates compared with hOCT2, although it shows high affinity for cationic compounds. Gorboulev et al. (33) reported that selectivity of rOCT1 was changed by mutation of aspartate 475 (Asp475). It is interesting that the affinity of rOCT1 for specific cations was increased after the mutation of Asp475 to glutamate in the middle of the proposed eleventh transmembrane domain. Asp475 is conserved in the OCT family, OCT1, OCT2, and OCT3. Therefore, differences in substrate affinity between hOCT2-A and hOCT2 might be due to the truncation of the last three transmembrane domains including Asp475 as a result of alternate splicing.

As shown in Figure 3, both the hOCT2 and hOCT2-A mRNA are expressed mainly in the kidney. Furthermore, hOCT2-A mRNA was detected in brain, liver, colon, skeletal muscle, bone marrow, spinal cord, testis, and placenta, whereas hOCT2 mRNA was detected predominantly in the kidney. The tissue distribution of hOCT2-A mRNA therefore seems to be different from that of hOCT2. The similarities of events were observed in the sodium bicarbonate cotransport protein (NBC) (34). kNBC is predominantly expressed in the proximal tubular cells in kidney. In contrast, pNBC, a spliced variant of NBC, is highly expressed in pancreas and at a lower level in several other organs. It is suggested that the alternative splicing in a single SLC22A2 gene may contribute to the profile of tissue expression of the spliced isoform and thereby result in a variety of physiologic consequence in the handling of organic cations.

In previous studies, the efflux of TEA via rOCT1 and rOCT2 was not changed by the extracellular pH, whereas the uptake of TEA via rOCT1 and rOCT2 was decreased by acidification (18,21). Similar results were observed in this study; the uptake of TEA via hOCT2 and hOCT2-A was decreased by lowering the extracellular pH, whereas the efflux from these transfectants was not affected by the pH (Figures 4C and 5). It is suggested that the transport function of hOCT2 and hOCT2-A is regulated by environmental pH and not stimulated by the proton gradient.

Recently, Pietig et al. (25) reported the mechanisms and properties of organic cation transport and its regulation across the basolateral membrane of isolated tubules from the human kidney in a functional study. They showed that transport of organic cations across the basolateral membrane of human proximal tubules is electrogenic. Moreover, they demonstrated that mRNA for the cation transporters hOCT1, hOCT2, hOCTN1, and hOCTN2 were detected in these tubules. They suggested that hOCT2 most likely mediated the transport of organic cations across the basolateral membrane. Immunohistochromy showed that hOCT2 protein was localized in the basolateral membrane of the proximal tubules in the human kidney (35). In the present study, we indicated that hOCT2-A mRNA was expressed in the human kidney and that the transport of TEA via hOCT2-A was electrogenic (Figures 3 and 4B). Our functional data suggested that hOCT2-A also might play a role in the transport of organic cations across the basolateral membrane in the human kidney. However, this raises the question of why two such transporters are expressed in the human kidney. One possibility is that there are differences in distribution along the nephron between hOCT2-A and hOCT2. It was reported that the mRNA encoding spliced variant of plasma membrane Ca2+ pump was detected differently in rat nephron segments (36). Another possibility is that there are differences in the regulation of renal expression between hOCT2-A and hOCT2. Additional study is required to determine the expression level of the RNA or protein of hOCT2-A and hOCT2 in the kidney, the distributions and membrane localization of hOCT2-A and hOCT2 along the nephron, or whether the alternative splicing represents a regulatory mechanism.

In conclusion, we isolated cDNA encoding a new organic cation transporter, hOCT2-A, from the human kidney. hOCT2-A is an alternatively spliced variant of hOCT2. The predominant expression of the hOCT2-A mRNA in the kidney and its functional properties suggested that hOCT2-A contributes to the renal clearance of endogenous and exogenous organic cations from the blood.

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The nucleotide sequence reported in this article has been submitted to the GenBank/EMBL Data Bank with accession number AB075951.

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