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Gene Expression Levels and Immunolocalization of Organic Ion Transporters in the Human Kidney

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Abstract. Renal excretion of organic anions and cations is mediated by the organic ion transporter family (SLC22A). In this study, the mRNA levels of the organic ion transporters were quantified by real-time PCR in normal parts of renal tissues from seven nephrectomized patients with renal cell carcinoma, and the distributions and localization of human (h)OAT1, hOAT3, and hOCT2 proteins were investigated by immunohistochemical analyses in the human kidney. The expression level of hOAT3 mRNA was the highest among the organic ion transporter family, followed by that of hOAT1 mRNA. The hOCT2 mRNA level was the highest in the human OCT family, and the level of hOCTN2 mRNA was higher than that of hOCTN1. hOCT1 mRNA showed the lowest level of expression in organic ion transporter family. hOAT1, hOAT3, and hOCT2 proteins were detected in crude membranes from the kidney of all patients by Western blot analyses, whereas hOCT1 protein could not be detected. Immunohistochemical analyses showed that both hOAT1 and hOAT3 were localized to the basolateral membrane of the proximal tubules in the cortex, and hOCT2 was localized to the basolateral membrane of the proximal tubules in both the cortex and medullary ray. Immunohistochemical analyses of serial sections indicated that hOAT1, hOAT3, and hOCT2 were coexpressed in a portion of the proximal tubules. These results suggest that hOAT1, hOAT3, and hOCT2 play predominant roles in the transport of organic ions across the basolateral membrane of human proximal tubules.

The kidney plays an important role in the excretion of endogenous and exogenous organic anions and cations, including various drugs, toxins, and endogenous metabolites. In renal tubules, membrane transport systems mediate the tubular secretion or reabsorption, and many transporters have been identified at the molecular level (1–4). Four isoforms of the organic anion transporter (OAT) family have been characterized (3,4). Rat (r)OAT1 was cloned independently in two laboratories by expression cloning from rat kidney as renal para-aminohippurate (PAH) transporters (5,6). The human (h)OAT1 was identified, and the uptake of PAH by hOAT1-expressing oocytes was suggested to be mediated by PAH/dicarboxylate exchange (7–9). A novel liver transporter (NLT) isolated from rat liver was shown to mediate organic anion transport; this transporter was named rOAT2 (10,11). The human homolog, hOAT2, was also identified, and its nucleotide sequence was deposited in the GenBank database (accession number AF097518). Both hOAT3 and hOAT4 were isolated from a human kidney cDNA library, and mRNA of hOAT1, hOAT3, and hOAT4 were detected in the kidney (12–14). In addition to PAH, rOAT1 has been suggested to interact with various anionic agents, such as nonsteroidal antiinflammatory drugs (NSAIDs), diuretics, and antidiabetic agents (15–18), and we have suggested that OAT1 is one of the main sites for the drug interaction among the anionic compounds. PAH uptake mediated by hOAT1 was also inhibited by anionic agents, such as probenecid, the angiotensin II receptor antagonist, losartan, and the tricarboxylic acid cycle intermediate, α-ketoglutarate (8,12). hOAT3 mediated the transport of PAH, estrone sulfate, methotrexate, dehydroepiandrosterone sulfate, ochratoxin A, prostaglandin E_2, estradiol glucuronide, taurocholate, glutarate, cAMP, urate, and fluorescein (14). hOAT4 mediated the transport of estrone sulfate, dehydroepiandrosterone sulfate, and ochratoxin A, and the uptake of PAH into hOAT4-expressing oocytes was at a low rate (13).

The three isoforms of the organic cation transporter (OCT1) family were identified (2,3). rOCT1 was cloned from rat kidney by expression cloning (19), and a homologous transporter rOCT2 was isolated (20). Comparison of the functional characteristics of rOCT1 and rOCT2 suggested that rOCT1 and rOCT2 had similar substrate affinities for many compounds, but it showed moderate differences in inhibitor sensitivity for several compounds, such as 1-methyl-4-phenylpyridinium (MPP), procainamide, dopamine, and *O*-methylisoprenaline (21–23). We previously showed that rOCT1 and rOCT2 were localized to basolateral membranes of renal tubular cells (24). Although rOCT1 was concentrated in the proximal tubules in the renal cortex, rOCT2 was abundant in the proximal tubules

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in the outer stripe of the outer medulla. The two human homologs, hOCT1 and hOCT2, mediated the uptake of organic cations, such as N^1-methylnicotinamide (NMN) and n-tetraalkylammonium, including tetraethylammonium (TEA), and MPP (25,26). An additional member of the OCT family was isolated from the rat placenta as rOCT3 (27), and hOCT3, which had previously been cloned as extraneuronal monoamine transporter (EMT) (28), was also cloned from the placenta (29). By Northern blot analyses, hOCT2 and hOCT3 mRNA were detected in the kidney, but the hOCT1 mRNA was not detected (25,29). Two additional members of the organic ion transporter (SLC22A) family, OCTN1 and OCTN2, were identified (30–32). hOCTN1 was identified from the human fetal liver, and hOCTN2 was cloned as a homolog of hOCTN1 from the human kidney. hOCTN2 is expressed in the adult human kidney and has been shown to mediate the uptake of L-carnitine in a Na^+-dependent manner (31,33).

Quantitative comparison and distribution of organic ion transporters should provide information for understanding the relations between the functions of renal drug excretion and the levels of transporter expression. In this study, we quantified the expression levels of organic anion and cation transporters by real-time PCR. In addition, the distribution patterns of predominant transporters were compared by immunohistochemical analyses in the human kidney.

Materials and Methods

Normal parts of renal tissues were obtained from seven surgically nephrectomized patients with renal cell carcinoma at Kyoto University Hospital (six men and one woman; age, 60.4 ± 8.4 yr [mean ± SD]). This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the Ethics Committee of Kyoto University. All patients gave their written informed consent.

Isolation of Total RNA

Total cellular RNA were isolated from specimens using a MagNA Pure LC RNA isolation Kit II (Roche Diagnostic GmbH, Mannheim, Germany) according to the manufacturer's instructions, and the concentrations of total cellular RNA were measured by spectrophotometry. Isolated total RNA were reverse-transcribed, and the reaction mixtures were used for real-time PCR.

Construction of Standard Plasmid DNA for Real-Time PCR

Primer/probe sets were designed according to parameters incorporated in the Primer Express software (PE Biosystems, Foster, CA). The specific primers, Taqman probe, and the target sequence for real-time PCR are listed in Table 1. The cDNA fragments of the target sequences were generated by RT-PCR with specific primers from human kidney mRNA. Each PCR product was ligated into the pCR-Script Cloning Vector and transformed into competent cells (Stratagene, La Jolla, CA). The exact sequences of the cloned amplicons were analyzed by the chain-termination method using a fluorescence 373A DNA sequencer (PE Biosystems). The concentrations of the purified plasmid DNA were measured by spectrophotometry, and corresponding copy numbers were calculated. Serial dilutions of respective plasmid DNA were used as standards to make calibration curves.

Real-Time PCR

Real-time PCR was performed in the ABI prism 7700 sequence detector (Applied Biosystems, Foster, CA). PCR amplification was performed in a total volume of 20 μl containing 2 μl of cDNA sample, 1 μM forward and reverse primers, 0.2 μM TaqMan probe, and 10 μl of TaqMan Universal PCR Master Mix (Applied Biosystems). The starting mRNA copy number (Cn) of the target sequence was established by determining the fractional PCR threshold cycle (Ct) number at which a fluorescence signal generated during the replication process passed above a threshold value. The initial amount of target mRNA in each sample was estimated from the experimental Ct value with a standard curve generated using known amounts of standard plasmid DNA. Glyceraldehyde-3-phosphate dehydrogenase mRNA was also measured as an internal control with glyceraldehyde-3-phosphate dehydrogenase Control Reagent (Applied Biosystems).

Antibodies

Rabbit anti-hOAT1, hOAT3, hOCT1, and hOCT2 antibodies were raised against synthetic peptides corresponding to 12 to 16 amino acids of each transporter. The amino acid sequences of the synthetic peptides are summarized in Table 2. Mouse anti-Na^+/K^+-ATPase monoclonal antibody was purchased from Upstate Biotechnology (Lake Placid, NY).

Western Blot Analyses

Crude plasma membranes were prepared from renal specimens as described previously with some modifications (34). Western blot analyses were also carried out as described previously (35). Briefly, each crude membrane fraction was solubilized in loading buffer (2% sodium dodecyl sulfate, 125 mM Tris-HCl, 20% glycerol). The samples were separated by polyacrylamide gel electrophoresis on 7.5% polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA) and transferred onto polyvinylidene difluoride membranes (Immobilon; Millipore, Bedford, MA). The blots were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS; 20 mM Tris, 137 mM NaCl, pH 7.5) containing 0.3% Tween 20 (TBS-T) for 3 h at 25°C and then incubated with anti-hOAT1, hOAT3, hOCT1, hOCT2 (1:500 dilution) or Na^+/K^+-ATPase antibody (1:10,000) for 16 h at 4°C. The bound antibody was detected on x-ray film by enhanced chemiluminescence with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG antibody and cyclic diacylhydrazides (Amersham Pharmacia Biotech, Uppsala, Sweden).

Immunohistochemical Analyses

The tissue adjacent to the specimens used for the study were fixed with 10% buffered formalin and processed for light microscopy. Light microscopic study of the tissues revealed that histology of the glomeruli and interstitium adjacent to the samples used for this study was almost normal with a minimal abnormality of glomeruli and some tubules (data not shown).

For immunofluorescence histochemistry, specimens were fixed with 4% paraformaldehyde in phosphate-buffered saline at 4°C for 30 min. Fixed tissues were embedded in O.C.T. compound (Sakura Finetechnical, Tokyo, Japan) and rapidly frozen in liquid nitrogen. Sections (4-μm-thick) were cut and covered with 5% bovine serum albumin for 1 h. The covered sections were incubated with anti-hOAT1, hOAT3, or hOCT2 serum (1:100 dilution) for 1 h and then incubated with Cy3-labeled donkey anti-rabbit IgG (CALTAG Lab-

Table 1. Primer sets and probes of organic ion transporters for real-time PCR

	Sequence	Position	Accession Number[a]
hOAT1			
forward primer	gcgccttttttgccttct	1030–1048	AB009698
reverse primer	ttcccgcttcccattgatc	1161–1143	
Taqman probe	catctactcctggttcttcattgagtcggc	1050–1079	
hOAT2			
forward primer	ccatccaggacgtggagaga	1663–1682	AF097518
reverse primer	cccacttagttctggacctgctt	1747–1725	
Taqman probe	tgccccaaccagtcttcaggaggaag	1688–1713	
hOAT3			
forward primer	caccatcctctccttaagctacct	1109–1130	AF097491
reverse primer	actgtctccacggtctgcaagt	1229–1208	
Taqman probe	catcttggctctcacctttgtgccctt	1179–1205	
hOAT4			
forward primer	caagcacttcaggagctcagaaa	923–945	AB026198
reverse primer	gctggacatcagcacctctatg	1009–988	
Taqman probe	tggccaggataaatggccacaagga	948–972	
hOCT1			
forward primer	tcttccatcgtcactgagttcaac	447–470	U77086
reverse primer	agaagcccgcattcaaacag	531–512	
Taqman probe	ctgactcctggaagctggacctctttca	481–508	
hOCT2			
forward primer	gatggcagcaagaccaaaagta	1849–1870	X98333
reverse primer	actccactggctgtagacctaggt	1957–1934	
Taqman probe	aaatccctgcactcatcacaaagcccatac	1872–1901	
hOCT3			
forward primer	ccctgtggtctctgacccatta	352–433	AF078749
reverse primer	cattcttgatggagctgtcatgag	493–470	
Taqman probe	aaagagagacaagagaagcccccaacctga	438–467	
hOCTN1			
forward primer	cagacaggtttggcaggaaga	637–657	AB007448
reverse primer	gcccacgatgacaaataacaca	758–737	
Taqman probe	tacagactggcttcagcttcctgcagattt	682–711	
hOCTN2			
forward primer	ctgtgtctgacttgctcctggat	2012–2034	AF057164
reverse primer	ttgtctgtaggtagccccagtgt	2112–2090	
Taqman probe	acccacactcagaggctacatatggcccta	2039–2068	

[a] From GenBank database.

oratory, San Francisco, CA), 1 μg/ml 4',6-diamidino-2-phenylindole (DAPI; Roche Diagnostic GmbH), and 5 units/ml Allexa 488-phalloidin (Molecular Probe, Eugen, OR) for 1 h. These sections were examined with BX-50-FLA fluorescence microscope (Olympus, Tokyo, Japan) at $\times$100 magnification. Images were captured with a DP-50 CCD camera (Olympus) using Studio Lite software (Olympus). As controls, specific rabbit antibodies were replaced with preimmune rabbit antibodies, and positive staining could not be detected

Table 2. Target sequences of anti-hOAT1, hOAT3, hOCT1, and hOCT2 antibodies

Antibody	Sequence	Position	Accession Number[a]
hOAT1	NH_2-KYMVPLQASASEKNGL-COOH	535–550	AB009698
hOAT3	NH_2-SLGLRAKKPKQEPEVEK-COOH	535–551	AF097491
hOCT1	NH_2-NTIYLKVQTSEPSGT-COOH	540–554	U77086
hOCT2	NH_2-KMIYLQVQKLDIPLN-COOH	541–555	X98333

[a] From GenBank database.

(data not shown). To confirm the specificity of the antibodies, each antibody was absorbed with an excess amount of peptide used as immunogen and processed similarly. Specimens were also examined with a Zeiss LSM 410 confocal laser microscope (Carl Zeiss, Oberkochen, Germany) for more detailed analysis (×400).

Results

Patient Profile

The average age of patients was 60.4 ± 8.4 yr in a relatively short range from 47 to 65. The creatinine levels of all patients studied were normal before the surgery. None of the patients had any diseases that affected the kidney other than renal cell carcinoma.

Quantification of hOAT1, hOAT2, hOAT3, hOAT4, hOCT1, hOCT2, hOCT3, hOCTN1, and hOCTN2 mRNA Expression in the Human Kidney Cortex

Figure 1 shows the levels of renal organic ion transporter mRNA. The hOAT1, hOAT3, hOCT1, and hOCT2 mRNA levels were 9.82 ± 4.61, 26.94 ± 10.02, 0.02 ± 0.00, and 5.30 ± 2.28 amol(10^{-18}mol)/μg total RNA (mean ± SEM), respectively. The level of hOAT3 mRNA was the highest in this family, and the level of hOCT2 mRNA was the highest in the hOCT family. hOAT2, hOAT4, hOCT1, hOCT3, hOCTN1, and hOCTN2 mRNA were also detected, whereas hOCT1 mRNA showed the lowest level of expression among the organic ion transporter family. The level of hOCTN2 mRNA was higher than that of hOCTN1. When total RNA, which had not been reverse-transcribed, was used for real-time PCR, we could not detect any amplification (data not shown). Therefore, contamination by genomic DNA was excluded.

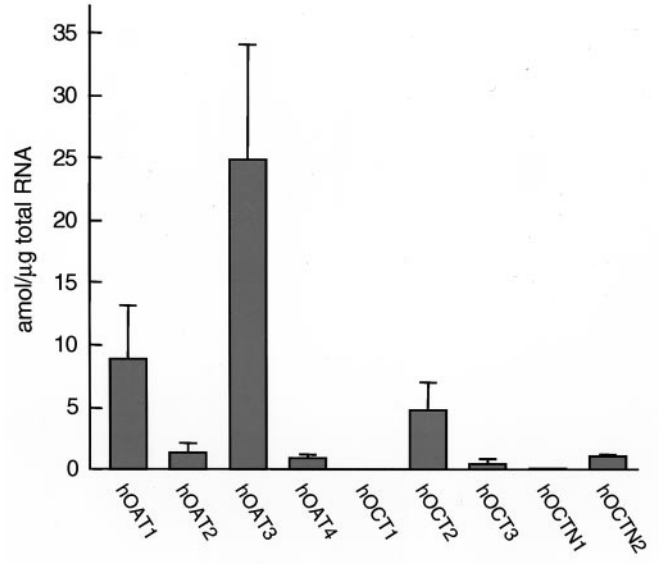

Figure 1. mRNA expression of hOAT, hOCT, and hOCTN transporters in the human kidney cortex. Total cellular RNA was extracted from the human kidney cortex, and extracted RNA was reverse transcribed. The mRNA levels of hOAT, hOCT, and hOCTN transporters were determined by real-time PCR using an ABI prism 7700 sequence detector. Each column represents the mean ± SEM of seven patients.

Western Blot Analyses

Western blot analyses with anti-hOAT1, hOAT3, hOCT1, or hOCT2 antibody were carried out using crude plasma membrane fractions from the human kidney to clarify whether these transporter proteins were expressed in the kidney. Immunoreactive proteins at 84, 80, and 93 kD, corresponding with hOAT1, hOAT3, and hOCT2, respectively, were detected in the specimens from all patients, whereas hOCT1 protein was not detected (Figure 2, A through D). The protein band of Na^+/K^+-ATPase, which is expressed in the basolateral mem-

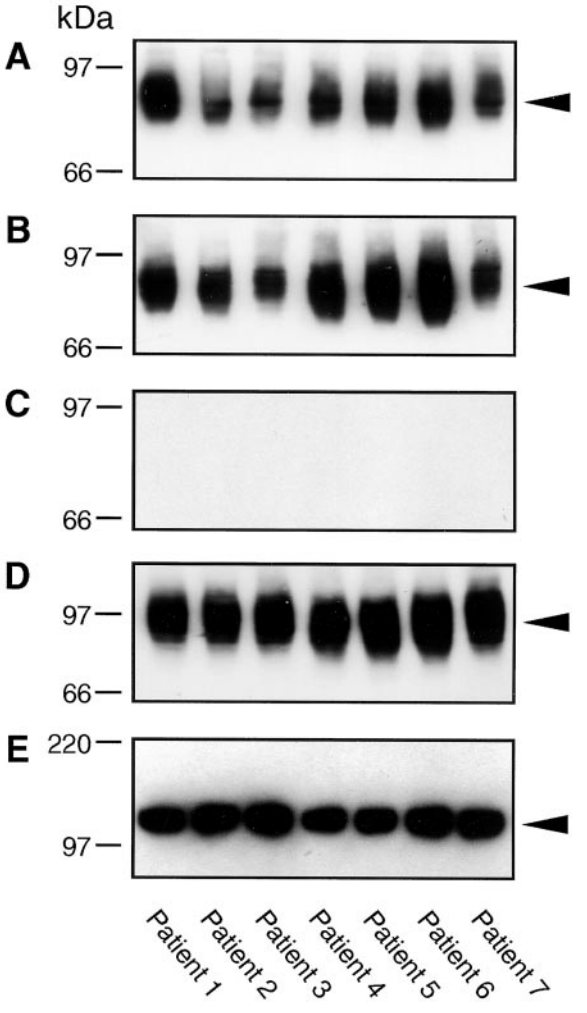

Figure 2. Western blot analyses of crude plasma membrane fraction from human kidney cortex for hOAT1 (A), hOAT3 (B), hOCT1 (C), hOCT2 (D), and Na^+/K^+-ATPase (E). Crude membranes (30 μg) were separated by sodium dodecyl sulfate-polyacrylamide get electrophoresis (7.5%) and blotted onto polyvinylidene difluoride membranes. The antisera for hOAT1, hOAT3, hOCT1, and hOCT2 (1:500 dilution) and the mouse monoclonal anti–Na^+/K^+-ATPase antibody (1:10,000) were used as primary antibodies. Horseradish peroxidase-conjugated anti-rabbit and anti-mouse IgG antibodies were used for detection of bound antibodies. The arrowheads indicate the positions of each transporter.

brane of renal tubules, was also detected (Figure 2E). All positive bands disappeared when the antiserum was preabsorbed with the corresponding antigen peptide, demonstrating the specificity of the antisera (data not shown).

Immunohistochemical Analyses

To compare the distributions of organic ion transporter proteins, which had been detected by Western blot analyses, we performed immunohistochemical examination of these transporters. By immunofluorescence microscopy for hOAT1, positive staining was seen in the proximal tubules but not in the glomeruli of the kidney cortex (Figure 3A). Fluorescein-phalloidin strongly labeled F-actin in the brush border region of proximal tubules (Figure 3B), and comparison of F-actin and hOAT1 staining revealed that hOAT1 was concentrated in the proximal tubules. Tubules that did not have brush borders, including distal tubules or collecting ducts, were negative for hOAT1. The preabsorption of anti-hOAT1 antisera with excess immunogen abolished these positive stainings, confirming the specificity of the primary antibody (Figure 3C). At a higher resolution, the labeling with anti-hOAT1 antibody was localized to the basolateral membrane (Figure 3E).

Similar to hOAT1, positive staining for hOAT3 was also detected in the proximal tubules in comparison with the simultaneous F-actin staining (Figure 4). No staining was seen in glomeruli, distal tubules, or collecting ducts. Some proximal tubules in the cortex region were negative for hOAT3 staining. These positive stainings disappeared by the preabsorption of anti-hOAT3 antisera with excess antigen peptide, confirming the specificity of the primary antibody (Figure 4C). At a higher resolution, staining with anti-hOAT3 antibody was also seen in the basolateral membrane (Figure 4E).

A cluster of hOCT2 staining was observed in the tubules but not in glomeruli, distal tubules, or collecting ducts (Figure 5). The preabsorption of anti-hOCT2 antisera with excess antigen

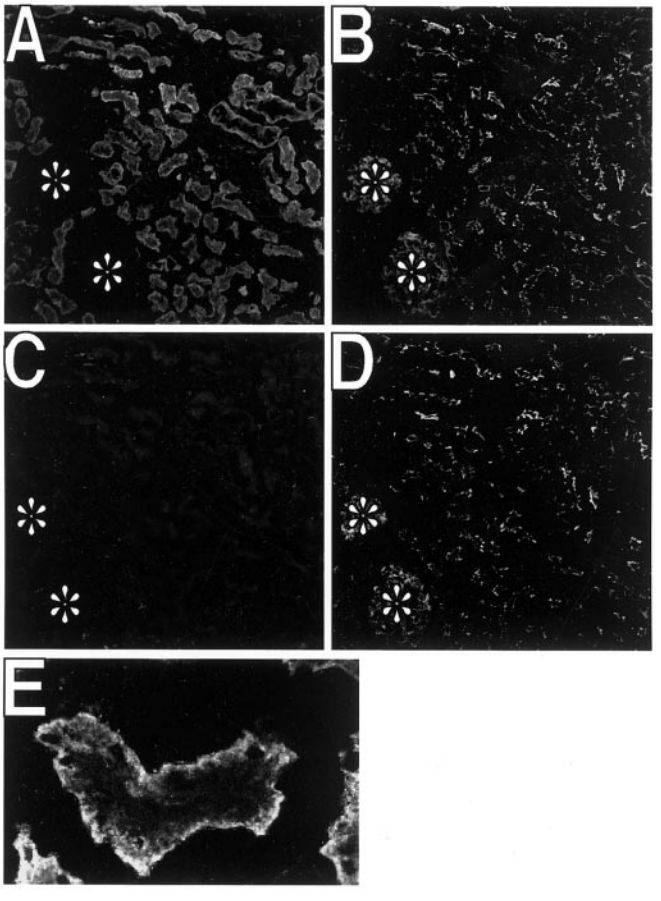

Figure 3. Immunofluorescence localization of hOAT1 in the human kidney. hOAT1 (A) and F-actin (B) in the same section. The brush border of the proximal tubules clearly showed F-actin staining. hOAT1 was concentrated in the proximal tubular cells in the cortex. hOAT1 antisera in the presence of hOAT1 antigen peptide (C) and F-actin (D) in adjacent section of panel A. No positive staining for hOAT1 was observed. (E) A confocal image of the proximal tubules in the cortex hOAT1 was localized along the basolateral membranes. *glomeruli. Magnifications: ×100 in A through D; ×400 in E.

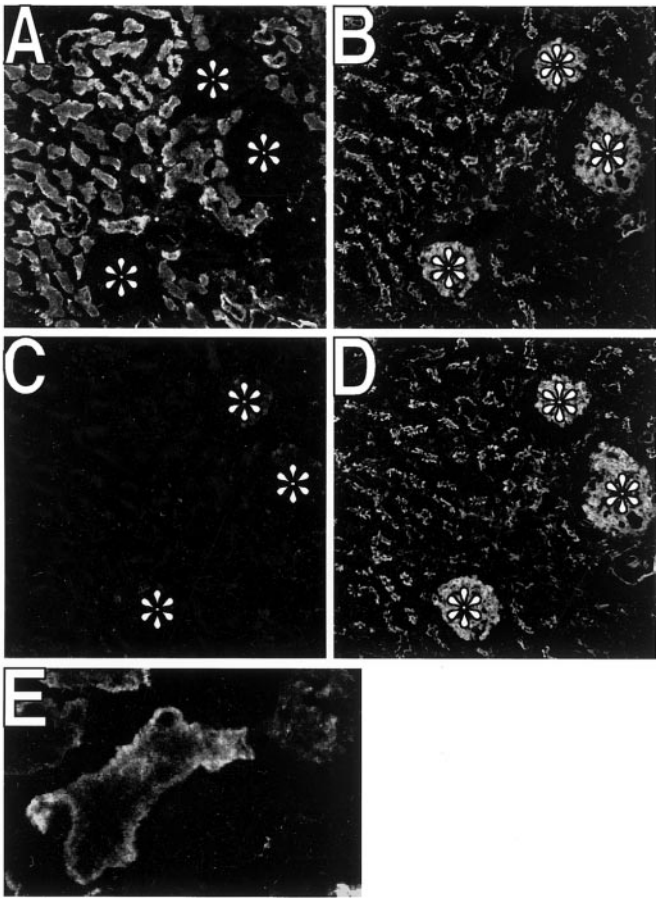

Figure 4. Immunofluorescence localization of hOAT3 in the human kidney. hOAT3 (A) and F-actin (B) in the same section. The brush border membrane of the proximal tubules clearly showed F-actin staining. hOAT3 was concentrated in the proximal tubular cells in the cortex. hOAT3 antisera in the presence of hOAT3 antigen peptide (C) and F-actin (D) in the adjacent section of panel A. No positive staining for hOAT3 was observed. (E) A confocal image of the proximal tubules in the cortex. hOAT3 was localized along the basolateral membranes. *glomeruli. Magnifications: ×100 in A through D; ×400 in E.

peptide abolished the positive staining, confirming the specificity of the anti-hOCT2 antibody (Figure 5C). At a higher resolution, hOCT2 signals were restricted to the basolateral membrane of proximal tubules (Figure 5E).

To compare the localization of hOAT1, hOAT3, and hOCT2, immunohistochemical analyses of these transporters were performed using serial sections. As shown in Figure 6, positive signals for hOAT1, hOAT3, and hOCT2 were observed in the same proximal tubules. However, in some proximal tubules of the kidney cortex, positive signals for hOAT1 and hOCT2 were clearly detected, but there were no signals for hOAT3. In some proximal tubules of the medullary ray, only positive signals for hOCT2 were detected.

Discussion

Organic ion transporters are expressed in the human kidney and have been suggested to play important roles for tubular secretion and reabsorption. This study was performed to quantify the expression levels and compare the distributions of the organic ion transporters in the human kidney. The profiles and

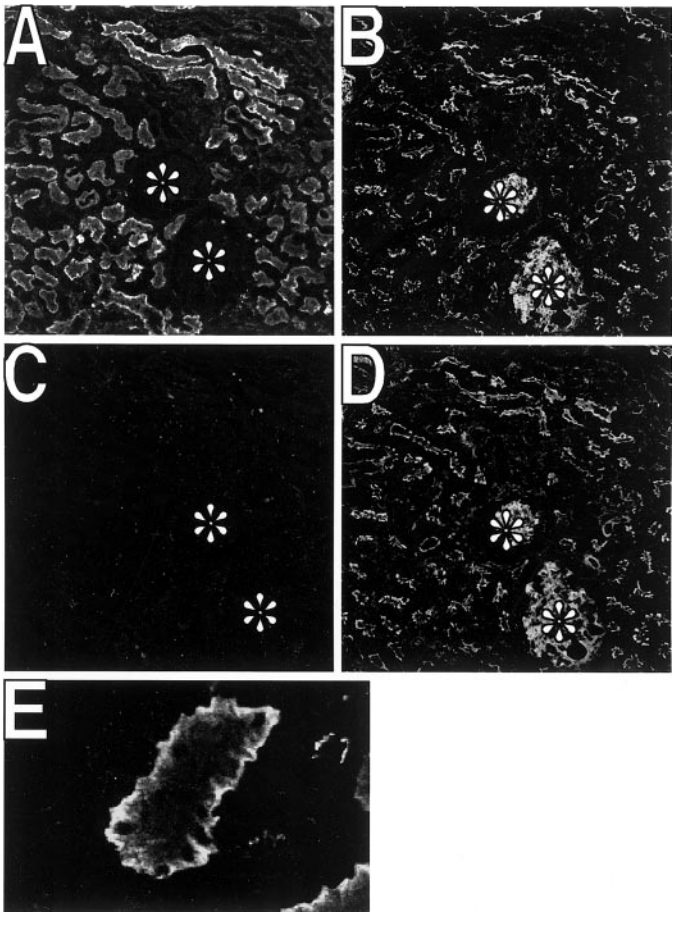

Figure 5. Immunofluorescence localization of hOCT2 in the human kidney. hOCT2 (A) and F-actin (B) in the same section. The brush border membrane of the proximal tubules clearly showed F-actin staining. hOCT2 was concentrated in the proximal tubular cells in the cortex and in medullary ray. hOCT2 antisera in the presence of hOCT2 antigen peptide (C) and F-actin (D) in the adjacent section of panel A. No positive staining for hOCT2 was observed. (E) A confocal image of the proximal tubules in the cortex. hOCT2 was localized along the basolateral membranes. *glomeruli. Magnifications: ×100 in A through D; ×400 in E.

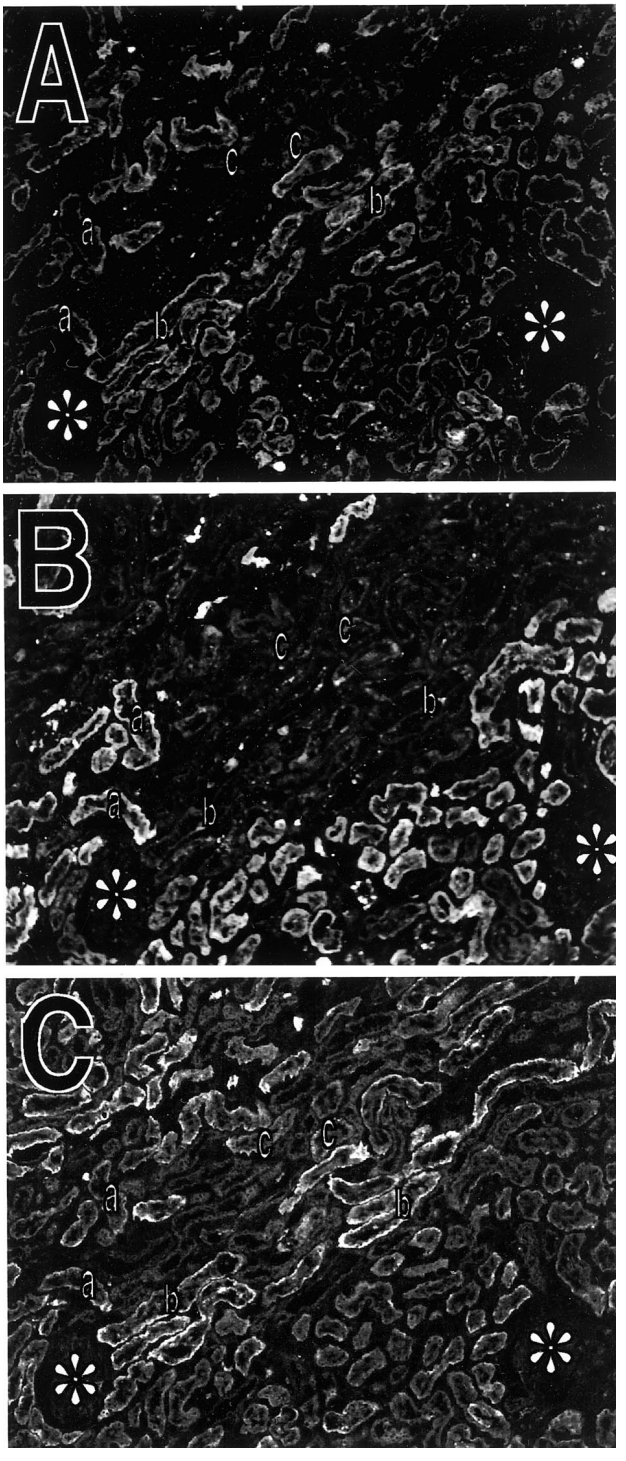

Figure 6. Staining of serial sections of normal human kidney with hOAT1 (A), hOAT3 (B), and hOCT2 (C). a, proximal tubules positive for hOAT1, hOAT3, and hOCT2; b, proximal segments positive for hOAT1 and hOCT2 and negative for hOAT3. c, proximal tubules positive for hOCT2 in the medullary ray. *glomeruli. Magnification, ×100.

histologies demonstrated that patients involved in this study had normal renal function and histology.

hOAT1 was cloned from the human kidney as PAH transporter and appeared to mediate transport of various anionic compounds (3,4). It is widely accepted that OAT1 is a key component of the renal tubular secretory pathway of organic anions. The level of hOAT1 mRNA was 9.82 ± 4.61 amol/μg total RNA, and the amount of hOAT1 mRNA expression was the second highest among the organic ion transporters (Figure 1), suggesting that hOAT1 mRNA is highly expressed in the human kidney. Hosoyamada *et al.* (8) showed that hOAT1 was expressed in the basolateral membrane of proximal tubules by immunohistochemical analyses. Consistent with their findings, strong staining for hOAT1 was observed in the basolateral membrane of tubular cells in this study (Figure 3). Therefore, the hOAT1 should serve as one of the major routes of organic anion transport across the basolateral membrane. In the rat kidney, rOAT1 was located in the S2 segment of proximal tubules, but there was no staining for rOAT1 in the S1 segment of proximal convoluted tubules and the S3 segment of the proximal straight tubules (36). In contrast, as shown in Figure 6, hOAT1 was distributed more widely than hOAT3, implying that hOAT1 expression is not restricted to the S2 segment. Although further studies are required to define hOAT1 distribution along the proximal tubules, it could be possible that the tubular distribution of OAT1 is different between humans and rats.

hOAT3 was isolated from human kidney cDNA library, and the strong band of hOAT3 mRNA was detected in the kidney (14). In this study, the level of hOAT3 was the highest among the organic ion transporter family. Similar to hOAT1, strong staining for hOAT3 was observed in the basolateral membrane of proximal tubules, which is consistent with the findings of Cha *et al.* (14) that hOAT3 protein was shown to be localized to the basolateral membrane of renal proximal tubules. Thus, hOAT3 is one of the predominant transporters in the basolateral membranes. Although substrate specificity of hOAT3 overlaps with that of hOAT1, the affinities for the anionic compounds are probably different between these transporters. For example, the Km values of hOAT1 and hOAT3 for PAH transport were 9.3 μM and 87.2 μM, respectively (8,14). In contrast to the fact that OAT1 plays as the PAH/dicarboxylate exchanger at the basolateral membrane, OAT3 did not mediate the exchange of estrone sulfate for PAH (37). A couple of polyspecific transporters with different substrate affinities and transport mechanisms are coexpressed in the basolateral membrane, mediating the efficient renal uptake of the diverse toxic agents from the blood circulation.

Recent studies demonstrated that rat, rabbit, and mouse OCT1 mRNA were detected in the kidney at relatively high levels (19,38,39). In addition, rOCT1 protein was clearly detected in the basaolateral membrane of the proximal tubules in the rat kidney cortex (24). Therefore, rOCT1 may be one of the basolateral type organic cation transporters. However, Gorboulev *et al.* (25) reported that hOCT1 mRNA could not be detected by Northern blot analyses, but the hOCT1 cDNA fragment was amplified by PCR with specific primers. In our study, we found that expression of hOCT1 mRNA was extremely low and that hOCT1 protein could not be detected by Western blot analyses. These findings suggests that hOCT1 may not play important roles for renal uptake of organic cations.

hOCT2 was isolated from the human kidney cDNA library, and its mRNA was predominantly expressed in the kidney (25). In this study, the level of hOCT2 mRNA was the highest among the human OCT family, and it was suggested that hOCT2 may be the major organic cation transporter in the kidney. In our previous study (24), rOCT2 was localized to the basolateral membrane of the proximal tubules, and hOCT2 immunostaining was also detected in the basolateral membrane, as shown in Figure 5. On the other hand, Gorboulev *et al.* (25) reported that hOCT2 was located at the luminal membrane in the distal tubules in the human kidney. However, in their recent study (40), they stated that crossreactivity of their antibody with closely related transporter subtypes or a splice variant in the distal tubules needed to be excluded. In addition, they used sections in which the proximal tubules were collapsed in the first study (40). Therefore, they could not detect the hOCT2 protein localized to the basolateral membrane of proximal tubules. The present results suggest that hOCT2 plays more important roles in the transport of organic cations across the basolateral membrane than other members of hOCT family in the proximal tubules.

In the rat kidney, rOCT1 was localized to the basolateral membrane of the proximal tubular cells in the cortex, and rOCT2 was expressed along the basolateral membrane of the proximal tubular cells in the outer stripe of the outer medulla (24,40). It is suggested that rOCT1 mediates the transport of organic cations across the basolateral membrane of the cortical proximal tubules and that rOCT2 mediates the transport in the medulla. However, in the human kidney, hOCT1 is expressed scarcely and hOCT2 could be distributed in proximal tubules more widely compared with rOCT2. Therefore, renal expression and the distribution pattern of OCT family are different between humans and rats.

hOCTN1 was identified as organic cation transporter and expressed in the kidney (30). hOCTN1 mediates the bidirectional and pH-dependent transport of organic cations and has been suggested to function as a proton/organic cation antiporter at the apical membrane of renal tubules (41). However, in this study, the expression of hOCTN1 mRNA was extremely low in human kidney. Therefore, it seems that hOCTN1 may not play an important role as the proton/organic cation antiporter, and other transporters remain to be identified at the apical membrane of the renal tubules.

In this study, we quantified the expression levels of organic ion transporters and examined the renal distributions of hOAT1, hOAT3, hOCT1, and hOCT2 proteins. The findings provide new information regarding the human renal organic ion transporters as follows: (*1*) in the renal cortex, the levels of hOAT1, hOAT3, and hOCT2 mRNA are much higher than those of other organic ion transporters, and hOCT1 mRNA is scarcely expressed; (*2*) renal expression and distribution of hOCT are different from those in the rats, and hOCT2 should

be more important for the transport of organic cations than other members of the hOCT family in the proximal tubules; and (*3*) hOAT1, hOAT3, and hOCT2 appear to be coexpressed at the basolateral membrane of the same cortical proximal tubules. These results suggest that hOAT1, hOAT3, and hOCT2 play predominant roles in organic ion transport across basolateral membrane of proximal tubules.

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