Distinct Characteristics of Organic Cation Transporters, OCT1 and OCT2, in the Basolateral Membrane of Renal Tubules

Yumiko Urakami,[1] Masahiro Okuda,[1] Satohiro Masuda,[1] Maiko Akazawa,[1] Hideyuki Saito,[1] and Ken-ich Inui[1,2]

Received May 15, 2001; accepted July 30, 2001

Purpose. This study was performed to determine the detailed mRNA distribution of organic cation transporters, rOCT1 and rOCT2, along the rat nephron and to distinguish the substrate affinities of these transporters.

Methods. The distributions of rOCT1 and rOCT2 mRNA were determined by reverse transcriptase polymerase chain reaction analysis of microdissected nephron segments. Using MDCK cells transfected with rOCT1 or rOCT2 cDNA, the inhibitory effects of various compounds on the uptake of [^{14}C]tetraethylammonium were assessed.

Results. rOCT1 mRNA was detected primarily in the superficial and juxtamedullary proximal convoluted tubules, whereas rOCT2 mRNA was detected widely in the superficial and juxtamedullary proximal straight and convoluted tubules, medullary thick ascending limbs, distal convoluted tubule, and cortical collecting duct. The IC_{50} values for cationic drugs and endogenous cations on [^{14}C]tetraethylammonium uptake across the basolateral membranes in the transfectants indicated that rOCT1 and rOCT2 had similar inhibitor specificity for many compounds but showed moderate differences in the specificity for several compounds, such as 1-methyl-4-phenylpyridinium, dopamine, disopyramide, and chlorpheniramine.

Conclusions. rOCT1 and rOCT2 possess similar but not identical multispecificities for various compounds with distinct distributions along the nephron, indicating that the two transporters share physiologic and pharmacologic roles in the renal handling of cationic compounds.

KEY WORDS: renal tubular secretion; basolateral membranes; rOCT1; rOCT2; neurotransmitter; antiarrhythmic.

INTRODUCTION

Renal organic cation transporters play an important physiologic role in the elimination of a wide variety of positively charged molecules, including drugs, xenobiotics, exogenous toxins, and endogenous bioactive amines. Renal tubular secretion of organic cations is mediated sequentially by two distinct transport systems: a transporter stimulated by the transmembrane potential difference in the basolateral membranes, which is responsible for the first uptake step in organic cation secretion, and a secondary active transporter driven by the transmembrane H^+ gradient, i.e., an electroneutral antiporter for organic cation with luminal H^+ in the brush-border membranes mediating the second efflux step in the secretion of small organic cations (1–4).

Several homologous transporters that mediate the transport of small cationic molecules have been identified, including OCT1 (5), OCT2 (6), and OCT3 (7). Sequence alignment of these gene products has shown that rat (r) OCT2 and rOCT3 have 67% and 48% amino acid identity with rOCT1, respectively. rOCT1 is expressed abundantly in the liver and kidney (5), whereas rOCT2 is expressed predominantly in the kidney but not in the liver (6). Busch *et al.* (8) reported that the human (h) OCT2 homologue is transcribed mainly in the kidney. rOCT3 is expressed predominantly in the placenta but also has been detected in the intestine, heart, brain, lung, and very weakly in the kidney (7). Functional studies using *Xenopus* oocytes (5,6,8–11) and mammalian transfected cells (7,8,12) have suggested that these cloned transporters have multispecific substrate specificity and translocate various structurally unrelated cationic compounds in an electrogenic fashion, indicating that these transporters possess characteristics peculiar to the basolateral membrane organic cation transport system of hepatocytes or renal tubular cells. Recently, Koepsell's group and our's have demonstrated by immunohistochemic analysis that both rOCT1 and rOCT2 are localized at the basolateral membrane of renal tubular cells (13,14).

There have been several reports describing the substrate specificities of OCT1 and OCT2. On expression in *Xenopus* oocytes (5,8,15,16) and mammalian cells (8,12,17,18), the apparent K_i or K_m values for various compounds were assessed to determine the substrate specificity of OCT1 or OCT2 under various experimental conditions. To distinguish the specificity of the two transporters, comparisons should be made using kinetic values determined under identical conditions, because concentrations of inhibitors used, and the presence of membrane potential, etc, may affect both K_i and K_m values. In this study, using Madin-Darby canine kidney (MDCK) cells expressing rOCT1 or rOCT2 in the basolateral membrane (19), we examined the distinct substrate specificity of rOCT1 and rOCT2. In addition, we investigated the different distribution of rOCT1 and rOCT2 along the rat nephron segment, because the expression of rOCT2 in the kidney was regulated by testosterone (20). We report here the results of a comparative study done on the substrate specificity of rOCT1 and rOCT2 for organic cation, and the distribution of rOCT1 and rOCT2 along the rat nephron.

MATERIALS AND METHODS

Materials

[^{14}C]Tetraethylammonium bromide (88.8 Mbq/mmol) and [^{3}H]1-methyl-4-phenylpyridinium acetate (3.03 TBq/mmol) were purchased from DuPont/New England Nuclear Research Products (Boston, MA). [^{3}H]Cimetidine (814 GBq/mmol) was purchased from Amersham Int. (Buckinghamshire, UK). [^{14}C]Guanidine hydrochloride (2.04 GBq/mmol) was purchased from Moravek Biochemicals, Inc, (Brea, CA). *n*-Tetraalkylammonium compounds, histamine, serotonin, dopamine hydrochloride, L-adrenaline hydrogen tartrate, thiamine hydrochloride, guanidine hydrochloride, amiloride hydrochloride, cimetidine, (±)-chlorpheniramine maleate,

[1] Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, 606-8507, Japan.

[2] To whom correspondence should be addressed. (e-mail: inui@kuhp.kyoto-u.ac.jp)

0724-8741/01/1100-1528$19.50/0

and imipramine hydrochloride were obtained from Nacalai Tesque Inc. (Kyoto, Japan). N^1-Methylnicotinamide iodide and *N*-acetylprocainamide hydrochloride were purchased from Sigma Chemical Co (St. Louis, MO). 1-Methyl-4-phenylpyridinium was purchased from Research Biochemicals International (Natic, MA). Disopyramide was from Wako Pure Chemical Industries (Osaka, Japan). Diphenhydramine hydrochloride was obtained from Tokyo Kasei Kogyo Co (Tokyo, Japan). All other compounds used were of the highest purity available.

Reverse Transcription Polymerase Chain Reaction Detection of rOCT1 and rOCT2 mRNA in Microdissected Nephron Segments

Microdissection of nephron of rats (200 g body weight) was performed as described previously (21). Total RNA was extracted from nephron segments using an RNeasy Mini Kit (Qiagen Gmbh, Hilden, Germany). Reverse transcription (RT) was performed using the SuperScript II™ preamplification system (Gibco BRL, Life Technologies, Inc., Gaithersburg, MD). The synthesized first strand cDNA was used for subsequent polymerase chain reaction (PCR) using a set of primers for rOCT1 (sense strand, 5′-GAAAAGC-GAAGTCCTTCGTTTGCC-3′ (base 1022–1045); antisense strand, 5′-CTGGTGCATATATACAAGAGTCTG-3′ (base 1791–1814), rOCT2 (sense strand, 5′-ACCTTCAATCCTG-GACTTGG-3′ (base 1087–1106); antisense strand, 5′-ACATCAGTGCAACAGACCGT-3′ (base 1901–1920) or rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (sense strand, 5′-CGGCCTCGTCTCATAGACAA-3′ (base 10–29); antisense strand, 5′-TGGTCCAGGGGTTTCT-TACT-3′ (base 1028–1047). PCR amplification was performed under the following conditions: rOCT1, 94°C for 1 min, 60°C for 1 min, 72°C for 1 min 30 sec × 30 cycles; rOCT2, 94°C for 1 min, 59°C for 1 min, 72°C for 1 min 30 sec × 33 cycles; GAPDH, 94°C for 1 min, 59°C for 1 min, 72°C for 1 min 30 sec × 33 cycles. The nucleotide sequence of the PCR products was confirmed by the chain-termination method by using a fluorescence 373A DNA sequencer (Applied Biosystem, Foster, CA).

Uptake by MDCK Transfectants

The uptake experiments using MDCK cells stably transfected with cDNA encoding for either rOCT1 or rOCT2, MDCK-rOCT1 or MDCK-rOCT2, were performed as described (19). The uptake of ligands by the cells was measured using cell monolayers grown on microporous membrane filters (3.0–μm pores, 1.0 cm^2 growth area) in Transwell cell chambers (Costar, Cambrige, MA). The amount of protein in the solubilized cells was determined by the method of Bradford (22), using Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA) with bovine γ-globulin as a standard. For *cis*-inhibition study, the uptake of [^{14}C]tetraethylammonium was achieved by adding various concentrations of unlabeled inhibitors to the incubation medium. The IC_{50} values were calculated from the inhibition plots based on the equation, $V = V_0 / [1 + (I / IC_{50})^n]$ by nonlinear least square regression analysis with Kaleidagraph Version 3.5 (Synergy Software). V and V_0 are the uptake of [^{14}C]tetraethyammonium in the presence and absence of inhibitor, respectively, I is the concentration of inhibitor, and n is the Hill coefficient. The apparent K_i values were calculated from the IC_{50}, $K_i = IC_{50} / (1 + K_m/ S)$. K_m is the Michaelis–Menten constant of tetraethyammonuim uptake by MDCK cells expressing rat OCT1 or rat OCT2 (19), and S is the concentration of [^{14}C]tetraethyammonium. For *trans*-stimulation study, the cells were preincubated with either incubation medium (control) or incubation medium plus the indicated concentration of unlabeled compounds on both sides at 37°C for 1 h. The cells were rinsed twice with 1 mL of ice-cold incubation medium on each side before the uptake experiments.

Statistical Analysis

Data were analyzed statistically using non-paired Student's *t* test or the one-way analysis of variance followed by Scheffé's test.

RESULTS

To determine the detailed distributions of rOCT1 and rOCT2 mRNA in the rat nephron, RT-PCR analysis was performed to detect the mRNA expression. As shown in Figure 1, the PCR product with the expected size of 793 bp for rOCT1 mRNA was detected in proximal convoluted tubule from superficial and juxtamedullary nephron segments. In contrast, the PCR product (834 bp) for rOCT2 mRNA was detected in the superficial and juxtamedullary proximal straight and convoluted tubules, medullary thick ascending limb, distal convoluted tubule, and cortical collecting duct. These results clearly demonstrated that the distributions of rOCT1 and rOCT2 mRNA along the nephron were different.

To compare the substrate specificity of rOCT1 and rOCT2, the inhibitory effects of several groups of cationic compounds on the uptake of [^{14}C]tetraethylammonium were examined to assess the IC_{50} values. First, the interactions of various *n*-tetraalkylammonium compounds with rOCT1 and rOCT2 were evaluated (Fig. 2). These inhibitors at various concentrations were added simultaneously to the incubation medium containing [^{14}C]tetraethylammonium on the basolateral side, and the inhibitory effects on uptake were compared. *n*-Tetraalkylammonium compounds inhibited [^{14}C]tetraethylammonium uptake in a dose-dependent manner with the following inhibitory potencies: tetrapentylammonium > tetrabutylammonium > tetrapropylammonium > tetraethylammonium > tetramethylammonium (Fig. 2, Table I). The order of inhibition appeared to be correlated with the length of the alkyl side chain. rOCT1 had a higher affinity for tetrapropylammonium and tetrametylammonium than rOCT2 by a factor of 0.59 and 0.68, respectively (Table I).

Next, the specificity of rOCT1 and rOCT2 for other cationic drugs was determined. As shown in Figure 3, imipramine had the strongest inhibitory effect on [^{14}C]tetraethylammonium uptake by both rOCT1 and rOCT2 transfectants followed by amiloride > *N*-acetylprocainamide, chlorpheniramine, diphenhydramine > disopyramide, procainamide. rOCT2 showed moderately higher affinities for procainamide and disopyramide than rOCT1 by a factor of 1.4 and 2.2, respectively (Table I). In contrast, rOCT1 had higher inhibitor specificity for chlorpheniramine and diphenhydramine than rOCT2 by a factor of 0.55 and 0.74, respectively (Table I).

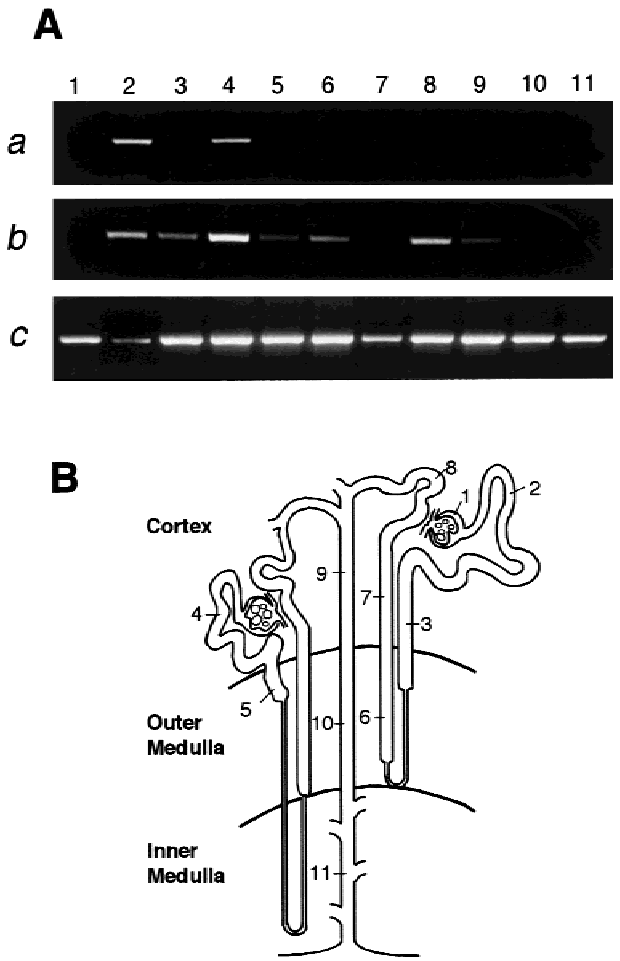

Fig. 1. Reverse transcriptaste polymerase chain reaction (RT-PCR) detection of rOCT1 and rOCT2 mRNA in microdissected nephron segments. (A) PCR amplification was performed using either 5 glomeruli or a 2-mm length of renal tubule. After microdissection, each dissected tubular segment was reverse-transcribed, and the cDNA synthesized was amplified using a set of primers for rOCT1 (a), rOCT2 (b) and GAPDH (c) as described in Materials and Methods. The PCR products were separated by electrophoresis through 2% agarose gels. (B) Schematic illustration of microdissected nephron segments. 1: glomerulus; 2: proximal convoluted tubule (superficial); 3: proximal straight tubule (superficial); 4: proximal convoluted tubule (juxtamedullary); 5: proximal straight tubule (juxtamedullary) 6: medullary thick ascending limb; 7: cortical thick ascending limb; 8: distal convoluted tubule, 9: cortical collecting duct; 10: outer medullary collecting duct; 11: inner medullary collecting duct.

Figure 4 shows the inhibition curves of several monoamines and endogenous organic cations for [^{14}C]tetraethylammonium uptake. Histamine had the most potent inhibitory effect on the uptake by rOCT2 transfectant, followed by serotonin > thiamine, dopamine > choline > adrenaline. Rat OCT2 showed higher affinities for serotonin and dopamine than rOCT1 by a factor of 1.7 and 1.9, respectively. The inhibitory effects on histamine and thiamine were comparable between rOCT1 and rOCT2.

The uptakes of structurally unrelated cationic compounds by the MDCK transfectant expressing rOCT1 or rOCT2 was assayed. As shown in Figure 5, the uptake of [^{14}C]tetraethylammonium, [^{3}H]1-methyl-4-phenylpyridinium,

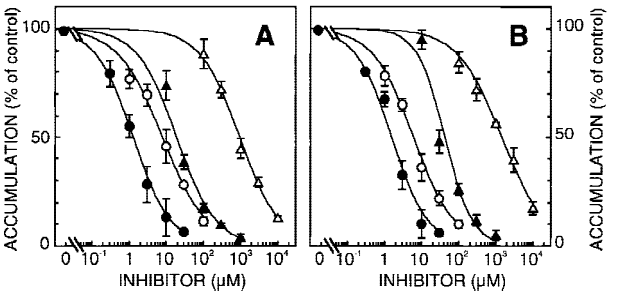

Fig. 2. Effects of n-tetraalkylammonium compounds on [^{14}C]tetraethylammonium uptake by Madin–Darby canine kidney (MDCK)-rOCT1 and MDCK-rOCT2. Monolayers of MDCK-rOCT1 (A) and MDCK-rOCT2 (B) in 1.0-cm^2 chambers were incubated for 15 min at 37°C with 50 μM [^{14}C]tetraethylammonium in the presence of tetrapentylammonium (●), tetrabutylamminuim (○), tetrapropylammonium (▲), or tetramethylammonium (△) added to the basolateral side (pH 7.4). After incubation, the radioactivity of solubilized cells was measured. Each point represents the mean ± standard error of the mean of three independent experiments.

[^{3}H]cimetidine and [^{14}C]guanidine was markedly stimulated in both rOCT1 and rOCT2 transfectants as compared to MDCK cells.

To investigate whether other inhibitors might also be substrates of rOCT1 and rOCT2, *trans*-stimulation experiments were performed. We preincubated the transfectants with concentrations equivalent to approximately 1.5-fold the IC_{50} value of unlabeled compounds and observed the *trans*-stimulation of [^{14}C]tetraethylammonium influx by preincubated transfectants. As shown in Figure 6, after preincubation of MDCK-rOCT1 and MDCK-rOCT2 with tetraethylammonium or N^1-methylnicotinamide, [^{14}C]tetraethylammonium uptake by both transfectants was significantly enhanced ($^*P < 0.05$). The uptake by rOCT transfectants was significantly enhanced after preincubation with procainamide, *N*-acetylprocainamide. or disopyramide. This *trans*-stimulation effect was not observed on preincubation of either transfectant with quinidine.

trans-Stimulation experiments were also performed with amiloride, chlorpheniramine, imipramine, and various monoamines. As shown in Figure 7, [^{14}C]tetraethylammonium uptake by rOCT1 and rOCT2 transfectants was significantly enhanced by preincubation with amiloride, histamine, adrenaline, serotonin, or dopamine. In contrast, preincubation with chlorpheniramine did not induce any significant changes in [^{14}C]tetraethylammonium uptake whereas preincubation with imipramine significantly decreased [^{14}C]tetraethylammonium uptake, in both transfectants.

DISCUSSION

The results of the present study indicated that the distributions of rOCT1 and rOCT2 mRNA along the rat nephron were different and that the two transporters have similar but not identical affinities for various compounds, including exogenous and endogenous cationic compounds.

To clarify the physiologic roles of rOCT1 and rOCT2 in the renal handling of organic cations, their precise distribution and localization in the nephron should be determined. Organic cation transporters in the basolateral and brush-border membranes have been well characterized functionally

Table I. The IC_{50} and Apparent K_i Values of Various Organic Cations for [^{14}C]Tetraethylammonium Uptake by rOCT1 and rOCT2

Organic cations	The IC_{50} or apparent K_i (in parentheses) values for [^{14}C]tetraethylammonium uptake (μM)		IC_{50} rOCT1/ IC_{50} rOCT2
	rOCT1	rOCT2	
Tetraalkylammoniums			
Tetrapentylammonium	1.3 ± 0.29	1.7 ± 0.16	0.79
Tetrabutylammonium	7.9 ± 1.4	5.7 ± 0.56	1.4
Tetrapropylammonium	23 ± 3.1	39 ± 0.74[b]	0.59
Tetraethylammonium	(47)[a]	(52)[a]	0.90
Tetramethylammonium	1138 ± 344	1674 ± 239	0.68
Neurotoxin and monoamines			
1-Methl-4-phenylpyridinium	(0.77)[a]	(1.7)[a]	0.45
Nicotine	(64)[a]	(50)[a]	1.3
Histamine	324 ± 59	363 ± 108	0.89
	(141 ± 25)	(171 ± 51)	
Serotonin	897 ± 42	513 ± 5.2[b]	1.7
	(390 ± 18)	(242 ± 2.5)	
Dopamine	1385 ± 62	733 ± 58[b]	1.9
	(603 ± 27)	(347 ± 28)	
Adrenaline	3678 ± 156	2519 ± 618	1.5
	(1600 ± 68)	(1191 ± 292)	
Endogenous cations			
Thiamine	736 ± 70	646 ± 112	1.1
N^1-Methylnicotinamide	(669)[a]	(403)[a]	1.7
Choline	1452 ± 108	1450 ± 58	1.0
Guanidine	(724)[a]	(713)[a]	1.0
Antiarrhythmic drugs			
N-Acetylprocainamide	20 ± 3.1	11 ± 0.63[b]	1.9
	(8.8 ± 1.4)	(5.0 ± 0.30)	
Quinidine	(15)[a]	(19)[a]	0.79
Disopyramide	142 ± 19	64 ± 1.3[b]	2.2
	(62 ± 8.0)	(30 ± 0.63)	
Procainamide	109 ± 10	76 ± 5.2[b]	1.4
	(47 ± 4.5)	(36 ± 2.4)	
Cationic drugs			
Imipramine	16 ± 8.2	9.9 ± 1.4	1.6
Amiloride	13 ± 0.60	13 ± 0.80	1.1
	(6.9 ± 3.6)	(4.7 ± 0.66)	
Cimetidine	(5.7)[a]	(9.4)[a]	0.61
Chlorpheniramine	14 ± 3.2	26 ± 1.8[b]	0.55
Diphenhydramine	24 ± 0.56	32 ± 2.2[b]	0.74

The uptake of [^{14}C]tetraethylammonium (50 μM) was achieved by adding various concentrations of unlabeled inhibitors to the incubation medium. The IC_{50} values were calculated from inhibition plots based on the equation with nonlinear regression analysis as described in Materials and Methods. The apparent K_i values of the inhibitors that *trans*-stimulation effect were observed by were calculated from the IC_{50} values as described in Materials and Methods. The coefficients of correlation were more than 0.93 when all inhibition plots were fitted. The data represent the mean ± SEM of three independent experiments.

[a] The apparent K_i values obtained from the previous paper (19).

[b] $P < 0.05$, different from the IC_{50} value of rOCT1.

(1–4). We reported that both rOCT1 and rOCT2 localized to the basolateral membrane of renal tubular cells (14). Furthermore, by immunofluorescence microscopy, rOCT1 was shown to be concentrated in the proximal tubules in the renal cortex, whereas rOCT2 was abundant in the proximal tubules in the outer stripe of the outer medulla (14). Therefore, rOCT1 and rOCT2 could mediate translocation of organic cations in the proximal convoluted and straight tubules (Fig. 1), where the organic cation transport system has been well characterized. Several reports indicate that dopamine D_{1A} receptor was localized to the proximal tubule, distal tubule, and cortical collecting duct (23), and renal dopamine plays an important role in the regulation of salt balance (24). Although the functions of rOCT2 in other nephron segments, including thick ascending limbs, distal convoluted tubule, and cortical collecting duct, remain unknown (Fig. 1), it might be involved physiologically in renal tubular handling, which modulates transepithelial flux of sodium ion.

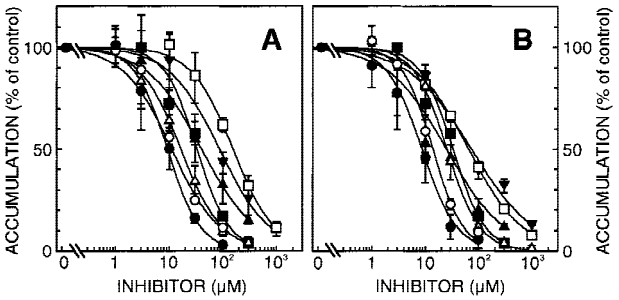

Fig. 3. Effects of cationic drugs on [^{14}C]tetraethylammonium uptake by Madin–Darby canine kidney (MDCK)-rOCT1 and MDCK-rOCT2. Monolayers of MDCK-rOCT1 (A) and MDCK-rOCT2 (B) in 1.0-cm^2 chambers were incubated for 15 min at 37°C with 50 μM [^{14}C]tetraethylammonium in the presence of imipramine (●), amiloride (○), *N*-acetylprocainamide (▲), chlorpheniramine (△), diphenhydramine (■), disopyramide (□), or procainamide (▼) added to the basolateral side (pH 7.4). After incubation, the radioactivity of solubilized cells was measured. Each point represents the mean ± standard error of the mean of three independent experiments.

We performed a comparative characterization of the substrate affinities of rOCT1 and rOCT2 systematically under carefully controlled conditions. Because both OCT1 and OCT2 are thought to be localized to basolateral membranes of renal tubular cells (13,14,19,25), MDCK transfectants allowed us to assess the functional properties of the transporters localized to the basolateral membranes in a physiologically convincing manner.

In the present study, we observed that the inhibitory patterns of *n*-tetraalkylammonium compounds were closely related with the length of their alkyl side chain (Fig. 2). In general, an increase in the length of the alkyl side chain is associated with an increase in hydrophobicity. In fact, double logarithmic plots showed a good correlation between the IC_{50} values and the lipophilicities of the *n*-tetraalkylammonium compounds examined (data not shown). A similar correlation is generally observed for these compounds in a inhibition of organic cation transport (26). Recently, Zhang *et al.* (27) found using HeLa cells transfected with hOCT1 that a longer alkyl chain was associated with a higher affinity of the *n*-

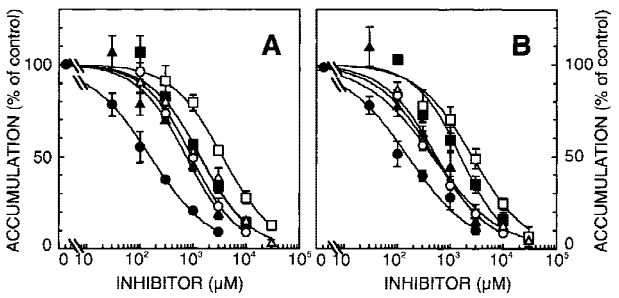

Fig. 4. Effects of monoamines and endogenous cations on [^{14}C]tetraethylammonium uptake by Madin–Darby canine kidney (MDCK)-rOCT1 and MDCK-rOCT2. Monolayers of MDCK-rOCT1 (A) and MDCK-rOCT2 (B) in 1.0-cm^2 chambers were incubated for 15 min at 37°C with 50 μM [^{14}C]tetraethylammonium in the presence of histamine (●), serotonin (○), thiamine (▲), dopamine (△), choline (■), or adrenaline (□) added to the basolateral side (pH 7.4). After incubation, the radioactivity of solubilized cells was measured. Each point represents the mean ± standard error of the mean of three independent experiments.

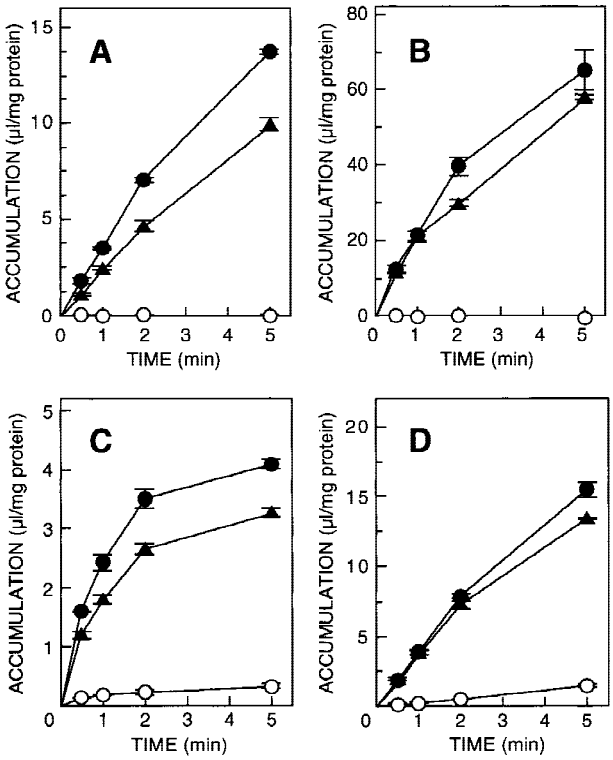

Fig. 5. Uptake of cationic compounds by Madin–Darby canine kidney (MDCK)-rOCT1 and MDCK-rOCT2. Monolayers of MDCK (○), MDCK-rOCT1 (●), and MDCK-rOCT2 (▲) in 1.0-cm^2 chambers were incubated for specified periods at 37°C with 50 μM [^{14}C]tetraethylammonium (A), 100 nM [^{3}H]1-methyl-4-phenylpyridinium (B), 100 nM [^{3}H]cimetidine (C), or 50 μM [^{14}C]guanidine (D) added to the basolateral side (pH 7.4). Unlabeled incubation medium was added to the apical side (pH 7.4). After incubation, the radioactivity of solubilized cells was measured. Each point represents the mean ± standard error of the mean of three monolayers from a typical experiment.

tetraalkylammonium compounds for hOCT1 but a slower rate of transport. The IC_{50} value appears to be a useful parameter for distinguishing substrate specificities, but not for identifying transported or nontransported substrates, of rOCT1 and rOCT2. Among the *n*-tetraalkylammonium compounds examined, rOCT2 showed a moderately low specificity for tetrapropylammonium and tetramethylammonium, but not for other compounds, when compared with rOCT1 (Fig. 2, Table I). These results suggested that rOCT1 and rOCT2 differ in the hydrophobic interaction and/or binding affinity for quaternary alkylammonium compounds.

Among the cationic compounds examined (including monoamines and antiarrhythmics), rOCT2 had a moderately high substrate affinity for serotonin, dopamine, procainamide, and disopyramide as compared to rOCT1 (Table I). Busch *et al.* (8) clearly demonstrated that hOCT2 transports monoamine neurotransmitters such as norepinephrine, serotonin, histamine and dopamine. When hOCT2 was expressed in *Xenopus* oocytes, apparent K_m values of 1.9 mM (noradrenaline), 1.3 mM (histamine), 0.39 mM (dopamine), and 0.08 mM (serotonin) were estimated for the neurotransmitters. Gründemann *et al.* (17) reported apparent K_m values of 2.1 mM (dopamine), 4.4 mM (noradrenaline), 1.9 mM (adrenaline), and 3.6 mM (serotonin) human embryonic kid-

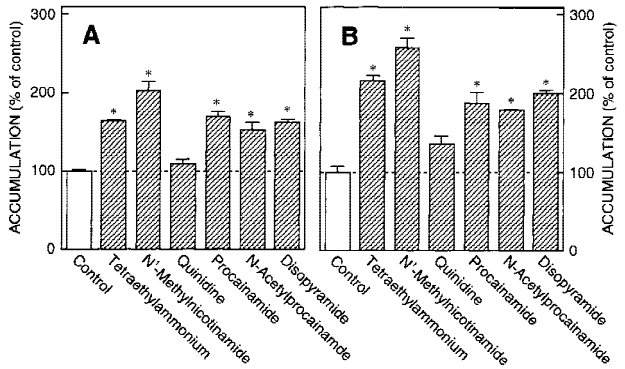

Fig. 6. *trans*-Stimulation effects of antiarrhythmic drugs on [^{14}C]tetraethylammonium uptake by MDCK-rOCT1 and MDCK-rOCT2. Monolayers of MDCK-rOCT1 (A) and MDCK-rOCT2 (B) in 1.0-cm^2 chambers were incubated for 15 min at 37°C with 50 μM [^{14}C]tetraethylammonium after preincubation with incubation medium (control) or incubation medium containing tetraethyammonium (150 μM, rOCT1; 150 μM, rOCT2), N^1-methynicotinamide (2 mM, rOCT1; 1.5 mM, rOCT2), quinidine (45 μM, rOCT1; 60 μM, rOCT2), procainamide (150 μM, rOCT1; 60 μM, rOCT2), *N*-acetylprocainamide (12 μM, rOCT1; 10 μM, rOCT2), and disopyramide (200 μM, rOCT1; 75 μM, rOCT2) for 60 min at 37°C, respectively. Data are expressed as a percentage of control value. Control values for MDCK-rOCT1 and MDCK-rOCT2 were 825 ± 16 and 474 ± 33 pmol/mg protein/15 min, respectively. Each column represents the mean ± standard error of the mean of three monolayers from a typical experiment. $*P < 0.05$, significantly different from control.

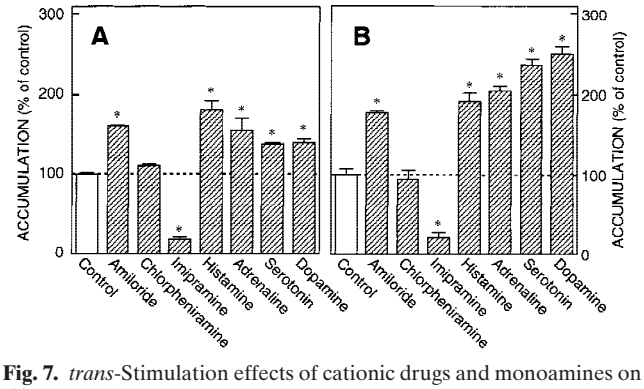

Fig. 7. *trans*-Stimulation effects of cationic drugs and monoamines on [^{14}C]tetraethylammonium uptake by MDCK-rOCT1 and MDCK-rOCT2. Monolayers of MDCK-rOCT1 (A) and MDCK-rOCT2 (B) in 1.0-cm^2 chambers were incubated for 15 min at 37°C with 50 μM [^{14}C]tetraethylammonium after preincubation with incubation medium (control) or incubation medium containing amiloride (20 μM, rOCT1; 15 μM, rOCT2), chlorpheniramine (15 μM, rOCT1; 40 μM, rOCT2), imipramine (13 μM, rOCT1; 15 μM, rOCT2), histamine (350 μM, rOCT1; 300 μM, rOCT2), adrenaline (4.5 mM, rOCT1; 3 mM, rOCT2), serotonin (1.2 mM, rOCT1; 650 μM, rOCT2), and dopamine (2.4 mM, rOCT1; 850 μM, rOCT2) for 60 min at 37°C, respectively. Data are expressed as a percentage of control value. Control values for MDCK-rOCT1 and MDCK-rOCT2 were 887 ± 10 and 518 ± 19 pmol/mg protein/15 min, respectively. Each column represents the mean ± standard error of the mean of three monolayers from typical experiment. $*P < 0.05$, significantly different from control.

ney 293 cells using stably transfected with rOCT2. Notably, there was a marked difference in the K_m value for serotonin. The K_m value for dopamine was also five-fold greater in rOCT2 than hOCT2. These disparities may have been due to species differences and/or differences in experimental conditions including the expression systems. Therefore, a comparison of the substrate specificity of different transporters should be carried out under the same experimental conditions. The present results showed that rOCT2 had a 1.5-fold higher substrate affinity for dopamine and serotonin than rOCT1 (Table I). The two transporters had comparable substrate affinities for histamine. OCT2 has been assumed to play a physiological role in the central nervous system by regulating interstitial concentrations of monoamine neurotransmitters (8). On the other hand, OCT1 has been suggested to be a high capacity transporter responsible for the first step in the excretion of these monoamines (28). The differences in apparent K_i values obtained in the present study would be related to the distinct functions of OCT1 and OCT2 in maintaining the homeostasis of bioactive monoamines.

Gründemann *et al.* (29) reported that clear-cut differences between rOCT1 and rOCT2 were observed for the transport of cimetidine, creatinine, guanidine, histamine, choline, and tetraethylammonium, respectively. Furthermore, these substrates exhibited key differences in solute recognition and turnover. In contrast, our findings are not necessarily comparable to those of Gründemann *et al*. As shown in Fig. 5, rOCT1 showed the ability to transport guanidine as well as rOCT2, and both transporters appeared to have a similar affinity for guanidine (Table I). In addition, Gründemann *et al.* (29) indicated that rOCT1 transports choline, although rOCT2 does not. In contrast, we have found that rOCT1 and rOCT2 have a similar affinity for choline (Table I), and both transporters mediated significant uptake of choline (unpublished observation). Furthermore, using rOCT2-expressing oocytes, Budimann *et al.* (30) reported that choline evoked changes in electrical current measured by the patch clamp technique. Considering these findings, it seems clear that rOCT2 mediates transport of choline. The reason why Gründemann *et al.* could not detect transport of choline by rOCT2, and obtained the different K_i values for guanidine between rOCT1 and rOCT2 remains unknown. The different systems used for the expression of the transporter and/or uptake experiments might be related to this issue.

In the present study, we performed *trans*-stimulation studies to investigate whether various inhibitors might be substrates of OCT1 or OCT2. *trans*-Stimulation is a frequently used as the method to investigate whether two molecules share a common transport pathway. As shown in Figure 6, procainamide, *N*-acetylprocainamide and disopyramide induced tetraethylammonium uptake by OCT transfectants. These results suggested that those antiarrhythmic drugs are translocated not only by OCT2 but also by OCT1. In contrast, *trans*-stimulation effects were not observed by preincubation of either transfectant with concentrations representing five times the IC_{50} values of quinidine and chlorpheniramine (data not shown). Moreover, imipramine demonstrated an apparent *trans*-inhibition effect (Fig. 7), suggesting that OCT1 and OCT2 loaded with imipramine have slower turnover rates than those of the unloaded transporters.

In conclusion, the results of the present study suggested that rOCT1 and rOCT2 possess similar but not identical multispecificities for various endogenous and exogenous compounds, and are expressed differentially along the rat nephron. Therefore, the two transporters could serve distinct physiologic and pharmacologic functions in renal handling of

cationic compounds by mediating basolateral uptake into tubular cells.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan and a grant from the Smoking Research Foundation. Yumiko Urakami is a Research Fellow of the Japan Society for the Promotion of Science.

REFERENCES

1. J. B. Pritchard and D. S. Miller. Mechanisms mediating renal secretion of organic anions and cations. *Physiol. Rev.* **73**:765–796 (1993).
2. K. J. Ullrich. Specificity of transporters for 'organic anion and organic cation' in the kidney. *Biochim. Biophys. Acta* **1197**:45–62 (1994).
3. H. Koepsell. Organic cation transporters in intestine, kidney, liver, and brain. *Annu. Rev. Physiol.* **60**:243–266 (1998).
4. K. Inui, S. Masuda, and H. Saito. Cellular and molecular aspects of drug transport in the kidney. *Kidney Int.* **58**:944–958 (2000).
5. D. Gründemann, V. Gorboulev, S. Gambaryan, M. Veyhl, and H. Koepsell. Drug excretion mediated by a new prototype of polyspecific transporter. *Nature* **372**:549–552 (1994).
6. M. Okuda, H. Saito, Y. Urakami, M. Takano, and K. Inui. cDNA cloning and functional expression of a novel rat kidney organic cation transporter, OCT2. *Biochem. Biophys. Res Commun.* **224**: 500–507 (1996).
7. R. Kekuda, P. D. Prasad, X. Wu, H. Wang, Y.-J. Fei, F. H. Leibach, and V. Ganapathy. Cloning and functional characterization of a potential-sensitive, polyspecific organic cation transporter (OCT3) most abundantly expressed in placenta. *J. Biol. Chem.* **273**:15971–15979 (1998).
8. A. E. Busch, U. Karbach, D. Miska, V. Gorboulev, A. Akhoundova, C. Volk, P. Arndt, J. C. Ulzheimer, M. S. Sonders, C. Baumann, S. Waldegger, F. Lang, and H. Koepsell. Human neurons express the polyspecific cation transporter hOCT2, which translocates monoamine neurotrasmitters, amantadine, and memantine. *Mol. Pharmacol.* **54**:342–352 (1998).
9. M. Okuda, Y. Urakami, H. Saito, and K. Inui. Molecular mechanisms of organic cation transport in OCT2-expressing Xenopus oocytes. *Biochim. Biophys. Acta.* **1417**:224–231 (1999).
10. A. E. Busch, S. Quester, J. C. Ulzheimer, S. Waldegger, V. Gorboulev, P. Arndt, F. Lang, and H. Koepsell. Electrogenic properties and substrate specificity of the polyspecific rat cation transporter rOCT1. *J. Biol. Chem.* **271**:32599–32604 (1996).
11. G. Nagel, C. Volk, T. Friedrich, J. C. Ulzheimer, E. Bamberg, and H. Koepsell. A reevaluation of substrate specificity of the rat cation transporter rOCT1. *J. Biol. Chem.* **272**:31953–31956 (1997).
12. F. Martel, T. Vetter, H. Russ, D. Gründemann, I. Azevedo, H. Koepsell, and E. Shömig. Transport of small organic cations in the rat liver: the role of the organic cation transporter OCT1. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **354**:320–326 (1996).
13. U. Karbach, J. Kricke, F. Meyer-Wentrup, V. Gorboulev, C. Volk, D. Loffing-Cueni, B. Kaissling, S. Bachmann, and H. Koepsell. Localization of organic cation transporters OCT1 and OCT2 in rat kidney. *Am. J. Physiol.* **279**:F679–F687 (2000).
14. M. Sugawara-Yokoo, Y. Urakami, H. Koyama, K. Fujikura, S. Masuda, H. Saito, T. Naruse, K. Inui, and K. Takata. Differential localization of organic cation transporters rOCT1 and rOCT2 in the basolateral membrane of rat kidney proximal tubules. *Histochem. Cell Biol.* **114**:175–180 (2000).
15. V. Gorboulev, J. C. Ulzheimer, A. Akhoundova, I. Ulzheimer-Teuber, U. Karbach, S. Quester, C. Baumann, F. Lang, A. E. Busch, and H. Koepsell. Cloning and characterization of two human polyspecific organic cation transporters. *DNA Cell Biol.* **16**: 871–881 (1997).
16. L. Zhang, M. J. Dresser, A. T. Gray, S. C. Yost, S. Terashita, and K. M. Giacomini. Cloning and functional expression of a human liver organic cation transporter. *Mol. Pharmacol.* **51**:913–921 (1997).
17. D. Gründemann, S. Köster, N. Kiefer, T. Breidert, M. Engelhardt, F. Spitzenberger, N. Obermüller, and E. Schömig. Transport of monoamine transmitters by the organic cation transporter type 2, OCT2. *J. Biol. Chem.* **273**:30915–30920 (1998).
18. L. Zhang, M. E. Schaner, and K. M. Giacomini. Functional characterization of an organic cation transporter (hOCT1) in a transiently transfected human cell line (HeLa). *J. Pharmacol. Exp. Ther.* **286**:354–361 (1998).
19. Y. Urakami, M. Okuda, S. Masuda, H. Saito, and K. Inui. Functional characteristics and membrane localization of rat multispecific organic cation transporters, OCT1 and OCT2, mediating tubular secretion of cationic drugs. *J. Pharmacol. Exp. Ther.* **287**: 800–805 (1998).
20. Y. Urakami, M. Okuda, S. Saito, and K. Inui. Hormonal regulation of organic cation transporter OCT2 expression in rat kidney. *FEBS Lett.* **473**:173–176 (2000).
21. S. Masuda, H. Saito, H, Nonoguchi, K. Tomita, and K. Inui. mRNA distribution and membrane localization of the OAT-K1 organic anion transporter in rat renal tubules. *FEBS Lett.* **407**: 127–131 (1997).
22. M. M. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
23. D. P. O'Connell, S. J. Botkin, S. I. Ramos, D. R. Sibley, M. A. Ariano, R. A. Felder, and R. M. Carey. Localization of dopamine D_{1A} receptor protein in rat kidneys. *Am. J. Physiol.* **268**:F1185–F1197 (1995).
24. F. Ibarra, A. Aperia, L. Svensson, A. Eklöf, and P. Greengard. Bidirectional regulation of Na^+, K^+-ATPase activity by dopamine and an α–adrenergic agonist. *Proc. Natl. Acad. Sci. USA* **90**:21–24 (1993).
25. H. Koepsell, V. Gorboulev, and P. Arndt. Molecular pharmacology of organic cation transporters in kidney. *J. Membr. Biol.* **167:**103–117 (1999).
26. W. H. Dantzler, S. H. Wright, V. Chatsudthipong, and O. H. Brokl. Basolateral tetraethylammonium transport in intact tubules: specificity and *trans*-stimulation. *Am. J. Physiol.* **261**:F386–F392 (1991).
27. L. Zhang, W. Gorset, M. J. Dresser, and K. M. Giacomini. The interaction of n-tetraalkylammonium compounds with a human organic cation transporter, hOCT1. *J. Pharmacol. Exp. Ther.* **288**: 1192–1198 (1999).
28. A. E. Busch, S. Quester, J. C. Ulzheimer, V. Gorboulev, A. Akhoundova, S. Waldegger, F. Lang, and H. Koepsell. Monoamine neurotransmitter transport mediated by the polyspecific cation transporter rOCT1. *FEBS Lett.* **395**:153–156 (1996).
29. D. Gründemann, G. Liebich, N. Kiefer, S. Köster, and E, Schömig. Selective substrates for non-neuronal monoamine transporters. *Mol. Pharmacol.* **56**:1–10 (1999).
30. T. Budimann, E. Bamberg, H. Kopsell, and Nagel, G. Mechanism of electrogenic cation transport by the cloned organic cation transporter 2 from rat. *J. Biol. Chem* **257**:29413–29420 (2000).