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Novel Membrane Transporter OCTN1 Mediates Multispecific, Bidirectional, and pH-Dependent Transport of Organic Cations[1]

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ABSTRACT

In the present study, functional characteristics of organic cation transporter (OCTN)1, which was cloned as the pH-dependent tetraethylammonium (TEA) transporter when expressed in mammalian human embryonic kidney (HEK)293 cells, were further investigated using *Xenopus* oocytes as well as HEK293 cells as gene expression systems. When OCTN1-derived complementary RNA was injected into *Xenopus* oocytes, pH-dependent transport of [^{14}C]TEA was observed as the same in HEK293 cells. In contrast, a replacement of sodium ions with potassium ions in the surrounding medium did not cause any change in [^{14}C]TEA uptake in *Xenopus* oocytes expressed with OCTN1. In addition, when OCTN1 was expressed in HEK293 cells, efflux of TEA from the cells was pH dependent, with an accelerated rate at acidic external medium pH. Accordingly, membrane potential or sodium ions are suggested to have no influence on [^{14}C]TEA transport and the transport activity of OCTN1 is directly affected by pH itself. Furthermore, addition of the unlabeled TEA in external medium enhanced the efflux of preloaded [^{14}C]TEA. These observations suggest that OCTN1 is a pH-dependent and bidirectional TEA transporter. OCTN1-mediated [^{14}C]TEA uptake was inhibited by various organic cations such as cimetidine, procainamide, pyrilamine, quinidine, quinine, and verapamil. In addition, uptakes of cationic compounds such as [^{3}H]pyrilamine, [^{3}H]quinidine, and [^{3}H]verapamil and zwitterionic L-[^{3}H]carnitine were increased by expression of OCTN1 in *Xenopus* oocytes. Accordingly, OCTN1 was functionally demonstrated to be a multispecific and pH-dependent organic cation transporter, which presumably functions as a proton/organic cation antiporter at the renal apical membrane and other tissues.

Renal organic cation transporters are responsible for the secretion of endogenous amines as well as various drugs. Studies using isolated renal membrane vesicles suggested that organic cations are transported by distinct mechanisms in brush-border and basolateral membranes. The first step in organic cation secretion, uptake across the basolateral membrane, involves membrane potential-dependent transporters (Koepsell, 1998; Zhang et al., 1998). This transport process is independent of sodium ion or proton gradient and appears to be due to the recently cloned organic cation transporters (OCT), OCT1 and OCT2 (Koepsell, 1998). The second step of renal secretion, the transport across the brush-border membrane, is performed at least by three transport systems: a multispecific proton/organic cation antiporter that transports tetraethylammonium (TEA), a more specific proton/organic cation antiporter that transports guanidine (Miyamoto et al., 1989; Chun et al., 1997), and the P-glycoprotein (Thiebaut et al., 1987; Fojo et al. 1987; Tanigawara et al., 1992; Schinkel et al., 1994). By means of membrane transport experiments using isolated membrane vesicles, TEA, *N*-methylnicotinamide (NMN), choline, procainamide, cimetidine, and aminocephalosporins have been demonstrated to be substrates of the multispecific proton/organic cation antiporter (Koepsell, 1998), which may be partially driven by the sodium/proton exchanger (Takano et al., 1984; Wright and Wunz, 1987). Furthermore, interaction between TEA and levofloxacin on the apical proton/organic cation antiport system in LLC-PK1 cells has been observed (Ohtomo et al., 1996), suggesting that the transporter with broad substrate specificity may also be involved in organic cation transport at the renal apical membrane. Although Gründemann et al. (1997) suggested that porcine OCT2 is an apical-type transporter on the basis of its susceptibility to specific transport inhibitors and the pH dependence of TEA uptake in OCT2-transfected human embryonic kidney (HEK)293 cells, Okuda

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ABBREVIATIONS: TEA, tetraethylammonium; NMN, N^1-methylnicotinamide.

et al. (1996) found a negligible pH-dependent transport of rat OCT2 in complementary RNA (cRNA)-injected *Xenopus* oocytes and suggested that it is a basolateral, not an apical, membrane transporter. Human OCT2 was also cloned recently and was implicated in pH-independent, membrane potential-dependent transport at the luminal surface of distal tubules (Gorboulev et al., 1997), whereas immunohistochemical studies indicated that rat OCT1 and OCT2 are localized at the basolateral membrane of proximal tubules (Koepsell, 1998). Accordingly, the OCT1 and OCT2 family is presumed to function for either reabsorption at the brush-border membrane or secretion at the basolateral membrane of organic cationic compounds. So far, the proton/organic cation antiporter in the renal apical membrane has not been fully characterized at the molecular level.

We recently determined the primary structure of an organic cation transporter, human OCTN1 (Tamai et al., 1997). Human OCTN1 encodes a 551-amino acid protein with 11 putative transmembrane domains and is present at several adult tissues, including kidney and skeletal muscle. The degrees of similarity of human OCTN1 with rat OCT1 (Gründemann et al., 1994), rat OCT2 (Okuda et al., 1996), human OCT1 (Zhang et al., 1997), human OCT2 (Gorboulev et al., 1997), and rat OCT3 (Kekuda et al., 1998) in amino acid sequences were 32%, 33%, 31%, 33%, and 36%, respectively. Accordingly, human OCTN1 was considered to be a new member of the organic cation transporter family. The OCTN1-induced TEA uptake in HEK293 cells was pH dependent and the apparent K_m was nearly identical with that of pH-dependent TEA uptake in renal brush-border membrane vesicles of rat (Takano et al., 1984; Wright and Wunz, 1987; Maeda et al., 1993; Tamai et al., 1997). Therefore, although its subcellular localization has not yet been established, OCTN1 is thought to be responsible for the efflux of organic cations across the renal epithelial brush-border membrane. However, Kekuda et al. (1998) reported that rat OCT3-mediated TEA transport was markedly influenced by extracellular pH in HeLa cells, and when OCT3 was expressed in *Xenopus* oocytes, the induced TEA transport was electrogenic. This phenomenon could be explained by the change of membrane potential due to the change in extracellular pH. Accordingly, the appearance of the pH-dependent transport of OCTN1 does not necessarily represent directly pH- and/or proton-gradient-dependent transport, and that apparent pH dependence might be affected by the experimental conditions, including the gene expression method used.

The purpose of the present study was to confirm the driving force for OCTN1-mediated transport and to investigate further the functional properties of OCTN1-mediated transport of several cationic compounds by using two gene expression systems, *Xenopus* oocytes and HEK293 cells, to establish whether the transport characteristics are influenced by the experimental methods.

Experimental Procedures

Materials. [1-^{14}C]TEA bromide (2.4 mCi/mmol), *p*-[glycyl-1-^{14}C]aminohippuric acid (53.1 mCi/mmol), and [*N*-methyl-^{3}H]verapamil hydrochloride (78.6 Ci/mmol) were purchased from New England Nuclear (Boston, MA). L-[Methyl-^{3}H]carnitine hydrochloride and [pyridinyl-5-^{3}H]pyrilamine (28 Ci/mmol) were purchased from Amersham Pharmacia Biotech UK, Ltd. (Buckinghamshire, England). [9-^{3}H]Quinidine (15 Ci/mmol) was from ARC Inc. (St. Louis, MO). All other chemicals were purchased from Sigma (St. Louis, MO) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Transport Experiments in *Xenopus* Oocytes. Oocytes from *Xenopus laevis* were obtained by manual dissection in medium A (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, and 5 mM HEPES adjusted to pH 7.6 with NaOH) and defolliculated in modified Barth's solution (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 100 U/ml penicillin G, 100 mg/ml streptomycin, and 10 mM HEPES adjusted to pH 7.5 with NaOH).

Human OCTN1 cDNA (Tamai et al., 1997) was subcloned into the *Hin*dIII/*Eco*RI sites of the expression vector pcDNA3 (Invitrogen, San Diego, CA). The construct pcDNA3/OCTN1 was then linearized with *Eco*RI and used for in vitro transcription after capping. cRNA was dissolved in water at a concentration of 0.2 μg/μl and injected (50 nl) into oocytes, which were used for the assay of transport activity after cultivation for 3 or 4 days. To measure uptake of organic cations, oocytes (5–10/individual time point or condition) were incubated in 250 μl of transport medium (100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES/NaOH, pH 7.5) containing a radiolabeled compound at 25°C. The uptake was terminated by washing the oocytes three times with 15 ml of ice-cold transport buffer. Washed oocytes were transferred to vials containing 100 μl of 5% SDS to be solubilized and the associated radioactivity was measured with a liquid scintillation counter. When transport was measured at an acidic or alkaline pH, 2-(*N*-morpholino) ethanesulfonic acid, or Tris was used instead of HEPES to maintain the desired pH, respectively.

Uptake and Efflux Experiments by Transient Expression in HEK293 Cells. The construct pcDNA3/OCTN1 was used to transfect HEK293 cells (Japanese Cancer Research Resources Bank, Tokyo, Japan) according to the calcium phosphate precipitation method. HEK293 cells were routinely grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (Gibco, Grand Island, NY), penicillin, and streptomycin in a humidified incubator at 37°C under 5% CO_2. After 24 h cultivation of HEK293 cells in 10-cm dishes, pcDNA3/OCTN1 or pcDNA3 vector alone was transfected by adding 10 μg of the plasmid DNA per dish. At 48 h after transfection, the cells were harvested and suspended in transport medium containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, and 25 mM HEPES (pH 7.4).

In uptake experiments, cell suspension and a solution of a radiolabeled test compound in the transport medium were each incubated at 37°C for 20 min, then transport was initiated by mixing them. At appropriate times, 200-μl aliquots of the mixture were withdrawn and the cells were separated from the transport medium by centrifugal filtration through a layer of mixture of silicon oil and liquid paraffin with a density of 1.03. Each cell pellet was solubilized in 3 N KOH, neutralized with HCl, and the associated radioactivity was measured by means of a liquid scintillation counter. In the [^{14}C]TEA efflux assay, HEK293 cells were preloaded with 0.5 μCi of [^{14}C]TEA at 37°C for 10 min, then pelleted by centrifugation at 10,000 rpm for 10 s, and efflux was initiated by resuspending the cells in transport medium. At the indicated time, efflux was terminated by centrifugal filtration as described above. Cellular protein content was measured according to the method of Bradford (1976) using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) and BSA as the standard.

Data Analysis. All data were expressed as mean ± S.E.M. and statistical analysis was performed by the use of Student's *t* test. The criterion of significance was taken to be $P < .05$.

Results

Expression of [^{14}C]TEA Uptake in *Xenopus* Oocytes Injected with Human OCTN1 cRNA. To confirm the expression of functional organic cation transport activity of human OCTN1, we investigated the OCTN1-mediated

[^{14}C]TEA transport in a *Xenopus* oocyte heterologous expression system. The uptake of [^{14}C]TEA in OCTN1 cRNA-injected oocytes was significantly higher than that in water-injected oocytes on day 2 and was further increased on days 3 and 4 after injection of the cRNA (Fig. 1). Subsequent uptake studies were carried out more than 3 days after cRNA injection. Although the data are not shown, uptake of [^{14}C]TEA] by *Xenopus* oocytes was linearly increased till 90 min and saturable with apparent K_m and V_{max} values of 0.195 ± 0.033 mM and 18.5 ± 1.45 pmol/60 min/oocyte, respectively.

Effect of Membrane Potential on [^{14}C]TEA Uptake in *Xenopus* Oocytes. We examined pH dependence of TEA uptake by OCTN1 expressed in *Xenopus* oocytes. When the pH in the transport medium was acidic (pH 6.0), OCTN1-mediated uptake of [^{14}C]TEA was decreased to about 40% of those at neutral and alkaline pH, whereas no significant change in the uptake of [^{14}C]TEA was observed in water-injected oocytes. Furthermore, comparable uptakes were observed at neutral and alkaline pH (data not shown). The observation was comparable with that observed in TEA transport by OCTN1 expressed in HEK293 cells (Tamai et al., 1997).

Figure 2 shows the effect of membrane potential on [^{14}C]TEA uptake by OCTN1 examined by cRNA-injected *Xenopus* oocytes. The uptake of [^{14}C]TEA was not significantly altered upon replacement of Na^+ with K^+. Accordingly, a lack of membrane potential dependent transport of TEA by OCTN1 was suggested.

Effect of Extracellular pH on Efflux of [^{14}C]TEA in HEK293 cells. Because it is important to know the driving force for TEA transport by OCTN1 to differentiate the physiological role on this transporter from those of the previously known OCT1 and OCT2, we further examined the effect of extracellular pH on TEA efflux from OCTN1-transfected HEK293 cells (Table 1). Efflux of [^{14}C]TEA was initiated by incubating the cells preloaded with 150 μM [^{14}C]TEA in the fresh medium at various pHs, and the efflux was evaluated from the remaining amount of [^{14}C]TEA in the cells. Table 1 shows the time course of the amount of remaining [^{14}C]TEA in the cells as the percentage of initial. The remaining amount of [^{14}C]TEA at acidic pH 6.0 was minimum and increased with the increase of pH up to pH 8.0 over 5 min, demonstrating an enhanced efflux at acidic pH.

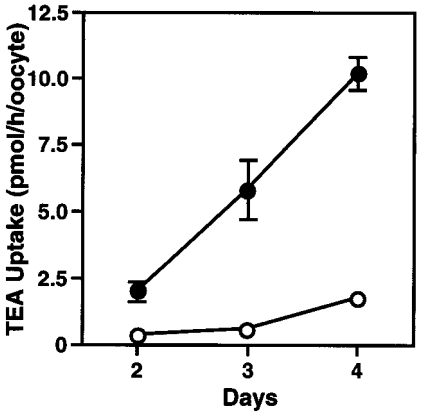

Fig. 1. Effect of cultivation time of oocytes after cRNA injection on uptake of [^{14}C]TEA for 60 min. Uptake of [^{14}C]TEA (60 μM) in OCTN1 cRNA (10 ng)-injected (●) and water (50 nl)-injected (○) oocytes was measured at 25°C in transport medium (pH 7.4). Results are shown as means ± S.E. of 5 to 10 oocytes. When S.E. was smaller than symbols, it was not shown.

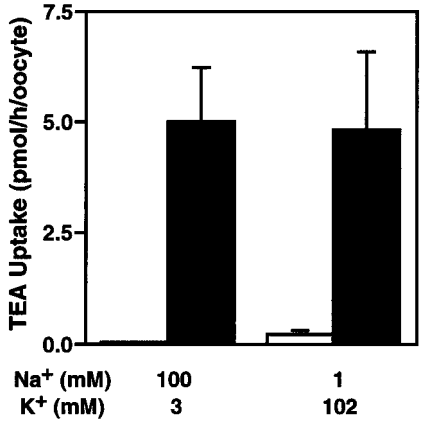

Fig. 2. Effect of membrane potential on [^{14}C]TEA uptake in OCTN1 cRNA-injected oocytes. Uptakes of [^{14}C]TEA (60 μM) in OCTN1 cRNA (10 ng)-injected (filled columns) and water (50 nl)-injected (open columns) oocytes were measured for 60 min at 25°C in the presence or absence of Na^+ in transport medium (pH 7.4). Results are shown as means ± S.E. of 5 to 10 oocytes. When S.E. was smaller than symbols, it was not shown.

TABLE 1

Effect of pH, unlabeled TEA, and quinidine on efflux of [^{14}C]TEA from OCTN1-transfected HEK293 cells

Efflux of [^{14}C]TEA was measured after preloading OCTN1-expressed HEK293 cells with [^{14}C]TEA (150 μM) for 10 min. Each value represents mean ± S.E. of three or four determinations, and results were shown as percentage of remaining amount of initial [^{14}C]TEA in cells at indicated efflux time. Concentration of unlabeled TEA and quinidine in efflux medium was 1 mM.

External pH	20 s	3 min	5 min
6.0 (Control)	54.0 ± 4.88	45.3 ± 1.27	36.3 ± 0.50
6.6	60.1 ± 1.63	49.3 ± 1.87	37.7 ± 0.27[a]
7.4	64.3 ± 2.81	59.1 ± 1.61[a]	51.3 ± 1.32[a]
8.0	67.9 ± 0.93[a]	69.2 ± 1.15[a]	61.8 ± 2.00[a]
6.0 + TEA	55.5 ± 1.94	35.5 ± 1.30[b]	28.1 ± 0.77[b]
6.0 + Quinidine	63.2 ± 1.28	63.1 ± 1.70[a]	58.2 ± 0.96[a]

[a,b] Significantly higher ([a]) or lower ([b]) than value of control at each time point ($p < .05$).

Inhibition of [^{14}C]TEA Uptake by Various Cationic and Anionic Compounds in HEK293 Cells. The effect of various endogenous compounds and xenobiotics on the OCTN1-mediated [^{14}C]TEA uptake in HEK293 cells is shown in Table 2. Various cationic compounds, such as choline, clonidine, cimetidine, nicotine, procainamide, pyrilamine, quinine, quinidine, and verapamil, had significant inhibitory effects ($p < .05$). Furthermore, zwitterionic compounds such as L- and D-carnitine and aminocephalosporin, cephaloridine and quinolone antibacterial agents, levofloxacin, and ofloxacin, were also significantly inhibitory, whereas other organic cations, such as NMN, guanidine, putrescine, spermine, and spermidine were not inhibitory. Cationic amino acids (L-arginine and L-lysine) and anionic compounds (*p*-aminohippurate and benzylpenicillin) were not inhibitory either.

Transport of Various Organic Cationic Compounds. The substrate selectivity of OCTN1 was assessed by measuring the uptake of radiolabeled compounds by *Xenopus* oocytes expressing OCTN1, because HEK293 cells exhibited significantly high background level of uptake. As shown in Fig. 3, although background uptakes were still rather high because of the binding, nonmediated diffusion and/or native transport activity of *Xenopus* oocytes, uptakes of [^{3}H]quinidine, [^{3}H]pyrilamine, [^{3}H]verapamil, and L-[^{3}H]carnitine, as well

TABLE 2

Inhibitory effect of various compounds on uptake of [^{14}C]TEA by OCTN1-transfected HEK293 cells

Uptake of [^{14}C]TEA (60 μM) was measured for 3 min at 37°C in transport medium (pH 7.4) containing each inhibitor. Each value represents mean ± S.E. of three determinations. Data were obtained by subtraction of uptake by mock-transfected HEK293 cells from that by OCTN1-transfected HEK293 cells.

Inhibitor	Concentration	Relative Uptake
	mM	*% of control*
Control		100
Choline	5	73.2 ± 3.6*
Cimetidine	1	50.6 ± 1.7*
Clonidine	1	70.9 ± 4.7*
Guanidine	5	89.6 ± 8.3
NMN	5	101 ± 1.8
Nicotine	1	71.6 ± 3.6*
Procainamide	1	15.6 ± 0.7*
Putrescine	5	111 ± 10.0
Pyrilamine	1	0.0 ± 2.73*
Spermidine	5	109 ± 7.2
Spermine	5	104 ± 7.2
Quinine	1	24.6 ± 1.1*
Quinidine	1	15.2 ± 2.1*
TEA	1	18.4 ± 1.3*
Verapamil	1	8.8 ± 4.2*
Ofloxacin	1	61.4 ± 5.3*
Levofloxacin	1	63.6 ± 1.4*
L-Carnitine	1	32.5 ± 3.3*
D-Carnitine	1	53.9 ± 0.5*
Cephaloridine	1	35.7 ± 4.5*
L-Arginine	5	111 ± 2.3
L-Lysine	5	93.8 ± 2.5
p-Aminohippuric acid	5	103 ± 8.4
Benzylpenicillin	5	113 ± 3.3

* Significantly different from the control uptake ($p < .05$).

as [^{14}C]TEA, by OCTN1 cRNA-injected oocytes were apparently increased in comparison with those by water-injected oocytes ($p < .05$). In contrast, the uptake of [^{14}C]benzylpenicillin by OCTN1 cRNA-injected oocytes was not increased.

Effect of Extracellular TEA and Quinidine on Efflux of [^{14}C]TEA in HEK293 Cells. To examine the specificity and direction of TEA transport, the effect of substrate/inhibitor of OCTN1 on the efflux of [^{14}C]TEA from HEK293 cells was examined. When unlabeled TEA was included in the external medium, the intracellular [^{14}C]TEA decreased faster than control, whereas in the presence of extracellular quinidine the remaining amount of [^{14}C]TEA was kept high (Table 1). Effects of TEA and quinidine are explained by the enhancement of [^{14}C]TEA efflux by an exchange transport with unlabeled TEA and inhibition of [^{14}C]TEA efflux by quinidine, which is taken up by the HEK293 cells during efflux study rather than the enhancement by exchange transport, respectively. These observations suggest that efflux of [^{14}C]TEA from the HEK293 cells is mediated by OCTN1 in a cation exchange mechanism.

Discussion

Recently, we cloned a novel organic cation transporter, human OCTN1, which is strongly expressed in kidney and resulted in pH-dependent TEA uptake when expressed in HEK293 cells (Tamai et al., 1997). In the present study, we further investigated the precise functional properties of organic cation transport by OCTN1 using two gene expression systems, *Xenopus* oocytes and HEK293 cells.

OCTN1 cRNA-injected oocytes showed significantly enhanced transport of [^{14}C]TEA as compared with water-injected oocytes. The K_m for OCTN1-mediated TEA uptake obtained in *Xenopus* oocytes (0.195 mM) was close to those of the previously characterized OCTN1-mediated TEA uptake in HEK293 cells (0.436 mM; Tamai et al., 1997) and in isolated brush-border membrane vesicles from rat and rabbit (0.15 ~ 0.8 mM; Takano et al., 1984; Wright and Wunz, 1987; Maeda et al., 1993). In addition, similar pH dependence in [^{14}C]TEA uptake by OCTN1 cRNA-injected oocytes to that by OCTN1-transfected HEK293 cells was observed (Tamai et al., 1997). However, the uptake of [^{14}C]TEA in OCTN1 cRNA-injected oocytes was not significantly affected upon replacement of Na^+ with K^+ (Fig. 2). The absence of potassium replacement effect is quite distinct from the apparent pH- and membrane potential-dependent transport as observed in OCT3 (Kekuda et al., 1998) and, therefore, suggests that the apparent pH-dependent transport of TEA by OCTN1 is not accounted for by the change of membrane potential. Moreover, [^{14}C]TEA efflux from OCTN1-transfected HEK293 cells was stimulated by an increase in the proton concentration of the external medium (Table 1). Accordingly, the transport of TEA by OCTN1 is presumably driven directly with a movement of proton but not with an inside-negative membrane potential difference as discussed in our previous observations obtained in a mammalian gene expression system (Tamai et al., 1997). The absence of further increase of TEA uptake at an alkaline pH compared with neutral pH is apparently comparable with the observation in TEA uptake by renal brush-border membrane vesicles (Maegawa et al., 1988). pH dependence observed in OCTN1-mediated TEA uptake might be ascribed to at least two underlying mechanisms, including activation by outwardly proton gradient and by the presence of functionally optimal protonated form of the transporter protein.

To determine the specificity of OCTN1, we examined the effect of various compounds on the initial uptake of [^{14}C]TEA and the uptake of several organic cations that exhibited inhibitory effects on TEA uptake (Table 2 and Fig. 3). The initial uptake of [^{14}C]TEA was significantly inhibited by a number of classical inhibitors on organic cation transport (cimetidine, procainamide, quinine, quinidine, and choline), other organic cationic compounds (clonidine, nicotine, pyrilamine, and verapamil) and zwitterionic compounds such as L-carnitine, aminocephalosporin (cephaloridine), and quinolone antibacterial agents (levofloxacin and ofloxacin), whereas p-aminohippuric acid and benzylpenicillin (typical substrates of organic anion transporter), L-arginine, L-lysine, and the endogenous polyamines spermine, spermidine, and putrescine had no inhibitory effect (Table 2). Moreover, we observed the increased uptake of several radiolabeled cationic compounds by expression of OCTN1 in *Xenopus* oocytes, suggesting that these cationic compounds as well as TEA are transported by OCTN1 and that the transporter may be multispecific. These data are consistent with previous observations on proton/organic cation transport in renal brush-border membrane vesicles in the human and other species. The absence of inhibition by guanidine suggested that OCTN1 is a pH-dependent transporter that has specificity for TEA and is different from the guanidine transporter in the renal proximal tubule.

TEA transport via OCTN1 was not inhibited by 5 mM NMN, suggesting a low affinity of NMN for OCTN1, although many previous studies have demonstrated that TEA and

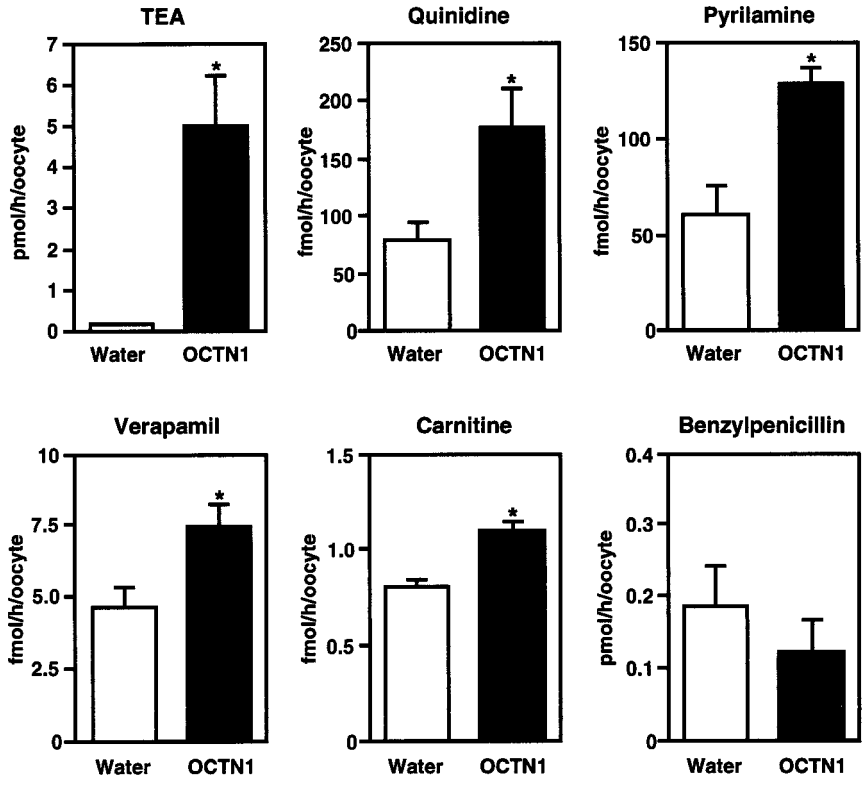

Fig. 3. Transport of various organic cations by OCTN1. Uptakes of [^{14}C]TEA (60 μM), L-[^{3}H]carnitine (10 nM), [^{3}H]quinidine (65 nM), [^{3}H]pyrilamine (35 nM), [^{3}H]verapamil (10 nM), and [^{14}C]benzylpenicillin (20 μM) in water-injected (open columns) or OCTN1 cRNA-injected (closed columns) oocytes were measured for 60 min at 25°C in transport buffer (pH 7.5). Each value represents means ± S.E. of 7 oocytes. When S.E. was smaller than symbols, it was not shown. *Significantly different from the uptake by water-injected oocytes by Student's t test ($p < .05$).

NMN appear to share the same proton/organic cation transport system (Zhang et al., 1998). However, Koepsell (1998) suggested that the affinity of the proton/TEA antiporter for NMN is low in renal proximal tubules, where a K_i value of 8.3 mM for NMN was obtained (David et al., 1995). The difference among these results may be ascribed to experimental artifact, species differences, or the existence of multiple cation antiporters. Because of its broad substrate specificity, OCTN1 is likely to play a significant role as a xenobiotic transporter and may serve as a pharmacologically important multispecific cationic drug transporter.

Efflux of [^{14}C]TEA was accelerated in the presence of the unlabeled TEA in the external medium, whereas quinidine caused a reduction of the efflux rate (Table 1). As clearly shown in Table 1 and Fig. 3, quinidine as well as TEA is a substrate of OCTN1 and has strong inhibitory potency to [^{14}C]TEA transport by OCTN1. The uptake efficiency of TEA by OCTN1 expressed in *Xenopus* oocytes is about 32 times less than that of quinidine when evaluated by the cell per medium concentration ratios calculated from the results shown in Fig. 3. Accordingly, it is expected that quinidine added in the external medium accumulates extensively and rapidly into the intracellular space during the efflux of [^{14}C]TEA and competitively inhibits efflux of [^{14}C]TEA at the internal side of the cells. On the other hand, TEA accumulation does not occur in the extensive amount during efflux study and it hardly inhibits the efflux of [^{14}C]TEA from the cells. Accordingly, an enhancement of efflux by the unlabeled TEA may represent countertransport effect, suggesting that OCTN1-mediated TEA transport is symmetrical and is explained by cation/cation exchange mechanism.

Wu et al. (1998) and we (Tamai et al., 1998) have successfully cloned OCTN2, an isoform of OCTN1. Wu et al. (1998) reported a pH-dependent TEA transport via OCTN2, whereas we found that OCTN2 predominantly transports L-[^{3}H]carnitine in a sodium-dependent manner in OCTN2-transfected HEK293 cells, with only a low activity of TEA transport (Tamai et al., 1998). Furthermore, physiological importance of OCTN2 as the carnitine transporter was demonstrated in our recent study on carnitine-deficient patients and mice (Nezu et al., 1999). In the present study, [^{14}C]TEA transport via OCTN1 was significantly inhibited by L- and D-carnitine and L-carnitine was transported by OCTN1. Accordingly, the common physiological role of OCTN1 and OCTN2 might be transport of carnitine in several tissues, although carnitine transport by OCTN1 will need to be functionally characterized further.

In conclusion, OCTN1 was demonstrated to be a multispecific, bidirectional, and pH-dependent organic cation transporter that is presumably energized by proton antiport mechanism in renal epithelial cells, although its subcellular localization is not clear at present. In addition, the broad substrate specificity of OCTN1 suggests a crucial role of the transporter in facilitating the elimination of xenobiotics. Furthermore, expression of OCTN1 in oocytes and HEK293 cells is expected to be useful for the development of in vitro assay systems for drug elimination and drug/drug interaction studies.

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