dine. Unexpectedly, hOCT2 did not show any famotidine transport (Fig. 5A). Due to the detection limit of HPLC analysis, the uptake experiments were performed at 1 mM. It is possible that saturation of the transporter make it difficult to detect the transport of famotidine by hOCT2. However, as expected and as opposed to famotidine, cimetidine transport by hOCT2 was detected. Arndt et al. (2001) reported that some organic cations, such as quinine and tetrapentylammonium, inhibited rOCT2 but that these compounds seemed to be not translocated via rOCT2. Therefore, it is likely that famotidine inhibits hOCT2 but is hardly transported by hOCT2 differently from cimetidine. Recently, Lee et al. (2002) reported that famotidine was not transported by the TEA-sensitive organic cation transport systems across the basolateral membrane in LLC-PK_1 cells. Inasmuch as LLC-PK_1 cells retain basolateral organic cation transporter of the kidney (Saito et al., 1992), the report of Lee et al. (2002) is consistent with our results. These data suggest that hOCT2 is involved in the basolateral transport of cimetidine but not of famotidine in the human kidney.

Recently, cimetidine was reported to be a substrate for hOAT1 and hOAT3 in addition to hOCT2 (Cha et al., 2001; Burckhardt et al., 2003), and our present results are consistent with these reports. Gisclon et al. (1989) reported that probenecid, a classic inhibitor of organic anion, decreased the renal clearance of cimetidine. It was implied that organic anion transporters were concerned with renal secretion of cimetidine. However, in the report of Gisclon et al. (1989), interaction between cimetidine and probenecid was transient and slight. Although further studies are needed to calculate the contribution of hOATs for cimetidine excretion, it is speculated that organic anion transporters play minor roles for renal secretion of cimetidine. On the other hand, Inotsume et al. (1990) reported that probenecid had a pronounced effect on renal tubular secretion of famotidine. The tubular secretory clearance of famotidine was decreased to one-tenth by coadministration of probenecid. As probenecid is well known as a potent inhibitor for hOAT3, their report is consistent with our present results that hOAT3, but not hOCT2, mediated famotidine uptake. Therefore, it is suggested that hOCT2 plays one of the important roles for renal uptake of cimetidine and hOAT3 for famotidine uptake, respectively.

Dowling et al. (2001) showed that tubular secretion of famotidine in human was not saturated at its unbound plasma concentrations up to about 10 μM. In the present study, the estimated Ki value of famotidine for hOAT3 was 179 μM (Fig. 6), suggesting that the famotidine transport by hOAT3 was not saturated at the therapeutic levels. Boom et al. (1996) reported that the apparent Michaelis–Menten constant (K_T) for tubular secretion of famotidine in vivo was 76 μM using the beagle dog. The K_T value in the report of Boom et al. is lower than the Ki value of famotidine for hOAT3 (179 μM) in this study. At present, it is difficult to discuss the difference between the K_T value of Boom et al. and our Ki value because of various factors such as species difference and experimental conditions.

It is well known that cimetidine reduces the renal secretion of procainamide (Somogyi et al., 1983), although famotidine had no effect on the pharmacokinetics of procainamide (Klotz et al., 1985). In this study, we assessed the inhibitory potency of cimetidine on hOCT2, the candidate responsible for the renal uptake of procainamide. The IC_{50} of the cimetidine for hOCT2 was around its therapeutic plasma concentration. Therefore, cimetidine may block the hOCT2 transport activity in vivo. On the other hand, the inhibitory potency of famotidine for hOCT2 was weaker than that of cimetidine, and the clinical plasma levels of famotidine (0.15±0.06 μM; Yoshimoto et al., 1994) were extremely lower than the IC_{50} for hOCT2. Therefore, famotidine cannot inhibit hOCT2 transport in the kidney at the therapeutic dose. In this study, the IC_{50} of famotidine for hOAT3 was also much higher than the clinical plasma concentration, and famotidine did not interact with hOAT1. Differently from cimetidine, famotidine is not likely to inhibit the tubular secretion of other drugs via these organic ion transporters, hOAT1, hOAT3 and hOCT2.

Several reports represented that pharmacokinetics of famotidine was related with renal function (Takabatake et al., 1985; Lin et al., 1988). Dosage adjustment of famotidine is necessary for the patients with renal insufficiency. In our previous studies, expression levels of renal drug transporters were altered in the impaired kidney. The expression level of rOCT2 was decreased in the 5/6 nephrectomized rats, but those of rOAT1, rOAT3 and rOCT1 were not influenced (Ji et al., 2002). Hyperuricemic rats represented the down-regulation of rOAT1, rOAT3 and rOCT2 but not of rOCT1 (Habu et al., 2003). Recently, we reported the alteration of organic ion transporters in the kidney of renal disease patients (Sakurai et al., 2004). In that report, elimination rate of cefazolin, which is substrate for hOAT3, was correlated with hOAT3 mRNA level, suggesting that the expression levels of renal drug transporter affect urinary drug excretion. It is interesting whether renal excretion of famotidine is affected by hOAT3 expression level in the patients with renal insufficiency.

In conclusion, this study represented the differences between famotidine and cimetidine in the interaction with human renal organic ion transporters, hOAT1, hOAT3 and hOCT2, and suggested that hOAT3 contributed to the renal tubular secretion of famotidine. Theses findings could be useful information to understand the renal handling of famotidine and to make optimum dosage regimens of the histamine H_2 receptor antagonists.

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Research Paper

Metformin Transport by Renal Basolateral Organic Cation Transporter hOCT2

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Purpose. Metformin, an antihyperglycemic agent, is eliminated by tubular secretion in addition to glomerular filtration in the human kidney. This study was performed to characterize metformin transport by human organic cation transporter 2 (hOCT2), the most abundant organic cation transporter in the basolateral membranes of the human kidney.
Methods. Accumulation of [^{14}C]metformin was assessed by the tracer experiments in the human embryonic kidney (HEK293) cells expressing hOCT2.
Results. The transport of [^{14}C]metformin was markedly stimulated in hOCT2-expressing cells compared with the vector-transfected cells. The accumulation of [^{14}C]metformin was concentrative and was dependent on the membrane potential, showing consistency with the characteristics of hOCT2. The apparent K_m and V_{max} values of [^{14}C]metformin transport by hOCT2-expressing HEK293 cells were 1.38 ± 0.21 mM and 11.9 ± 1.5 nmol mg protein^{-1} min^{-1}, respectively. The order of the potencies of unlabeled biguanides to inhibit [^{14}C]metformin transport by hOCT2 was phenformin > buformin > metformin. Furthermore, [^{14}C]metformin transport was inhibited slightly or moderately by cationic drugs such as procainamide and quinidine at respective therapeutic concentrations.
Conclusions. Metformin is transported by the basolateral organic cation transporter hOCT2 in the human kidney. hOCT2 could play a role in the drug interactions between metformin and some cationic drugs.

KEY WORDS: hOCT2; human kidney; metformin; organic cation transporter; renal tubular secretion.

INTRODUCTION

Biguanide agents have been used for the treatment of type 2 diabetes mellitus since the late 1950s. These drugs are useful in primary therapy for type 2 diabetes mellitus with obesity or hyperlipidemia and can also be used for add-on therapy in patients with diabetes uncontrolled by sulfonylureas and diet (1–2). The mechanisms of the pharmacological action of metformin involve the decreased hepatic glucose production without an effect on the release of insulin and the increased glycogenesis and lactate production. The most life-threatening adverse effect of biguanides is lactic acidosis, because there is a risk of overproduction of lactate through inhibition of mitochondrial respiration and increased anaerobic glycolysis (1). A risk of decreased use of lactate through inhibition of gluconeogenesis by biguanides has been also documented (1). Although phenformin was removed from the market because of its association with lactic acidosis, the relative risk is much lower for metformin than for phenformin. Moreover, the risk of lactic acidosis caused by metformin is less than the risk of severe hypoglycemia induced by sulfonylurea drugs (3).

Metformin is mainly excreted into urine, almost entirely in an unchanged form. The renal clearance of metformin (440–454 ml/min) is much higher than the glomerular filtration rate in humans (4–5), suggesting a significant contribution of tubular secretion in addition to glomerular filtration. As biguanides consist of two molecules of guanidine linked together by the removal of an ammonia group, they are protonated at physiologic pH. Organic cation transporters have been suggested to mediate tubular secretion of metformin (6–7); however, the molecular mechanisms underlying the renal tubular secretion of biguanides have not been clarified.

Organic cation transporters in the kidney, liver, intestine, brain, and placenta play essential physiologic and pharmacological roles in the handling of cationic drugs and endogenous organic ions. Human organic cation transporter 1 (hOCT1) is expressed primarily in the liver (8–9) and is likely responsible for the hepatic uptake of various cationic drugs. In contrast to hOCT1, we demonstrated that human organic cation transporter 2 (hOCT2) is the most abundant organic cation transporter in the basolateral membranes of human kidney (10). Moreover, hOCT2 as well as its orthologue in the rat (rOCT2) were localized at the basolateral membranes of renal proximal tubules and were suggested to contribute to the secretion of organic cations, such as tetraethylammonium,

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ABBREVIATIONS: hOCT1, human organic cation transporter 1; hOCT2, human organic cation transporter 2; rOCT1, rat organic cation transporter 1; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

1-methyl-4-phenylpyridinium, cimetidine, and guanidine (10–13). Recently, rat (r) OCT1 as well as mouse (m) OCT1 was revealed to transport biguanides (14,15). It was also demonstrated that tissue distribution of metformin was decreased in the liver, duodenum, jejunum, ileum, but not in the kidney and colon, in the mOCT1 gene-knockout mice. Moreover, Dresser *et al.* (16) reported that metformin and phenformin induced currents and *trans*-stimulated [^{3}H]1-methyl-4-phenylpyridinium uptake in *Xenopus* oocytes expressing hOCT1 and hOCT2. However, information has been limited regarding the characteristics of metformin transport by hOCT2 and hOCT2-mediated drug interactions between metformin and concomitantly administered drugs. In the current study, we characterized the transport of [^{14}C]metformin using human embryonic kidney (HEK293) cells stably expressing hOCT2. We also assessed drug interactions between [^{14}C]metformin and various cationic drugs.

MATERIALS AND METHODS

Materials

[Biguanidine-^{14}C]metformin hydrochloride (26 mCi/mmol) was purchased from Moravek Biochemicals, Inc. (Brea, CA, USA). [Ethyl-1-^{14}C]tetraethylammonium bromide (55 mCi/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). Metformin, phenformin, and 1-methyl-4-phenylpyridinium iodide were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Buformin was purchased from Wako Pure Chemicals (Osaka, Japan). Tetraethylammonium bromide and cimetidine were obtained from Nacalai Tesque Inc. (Kyoto, Japan). All other compounds used were of the highest purity available.

Cell Culture and Transfection

To construct the transfectant stably expressing hOCT2, HEK 293 cells (ATCC CRL-1573, American Type Culture Collection, Manassas, VA, USA), a transformed cell line derived from human embryonic kidney, were transfected with 0.8 μg of total plasmid DNA (pCMV-XL4:pBK-CMV vector = 2:1) per well. At 24 h after transfection, the cells split between 1:15 and 1:30 were cultured in complete medium consisting of Dulbecco's modified Eagle's medium with 10% fetal bovine serum in an atmosphere of 5% CO_2/95% air at 37°C containing G418 (0.5 mg/ml) (Wako Pure Chemical Industries, Osaka, Japan). Then, 14 to 21 days after transfection, single colonies were selected. G418-resistant colonies were analyzed by RT-PCR for expression of hOCT2 mRNA (17).

For uptake experiments, the cells were seeded onto poly-D-lysine–coated 24-well plates at a density of 2.0×10^5 cells per well. The cell monolayers were used at day 3 of culture for uptake experiments. In this study, HEK293 cells between passages 70 and 83 were used.

Uptake Experiments

Cellular uptake of cationic compounds was measured with monolayer cultures of HEK293 cells grown on poly-D-lysine–coated 24-well plates (17). The incubation medium for uptake experiments contained 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, and 5 mM HEPES (pH 7.4). The composition of the high K^+ incubation medium was 3 mM NaCl, 145 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, and 5 mM HEPES (pH 7.4). When indicated, 9.2 mM $BaCl_2$ was added to the incubation medium. The pH of the medium was adjusted with NaOH or HCl. The cells were preincubated with 0.2 ml of incubation medium for 10 min at 37°C. The medium was then removed, and 0.2 ml of incubation medium containing [^{14}C]metformin or [^{14}C]tetraethylammonium was added. The medium was aspirated off at the end of the incubation, and the monolayers were rapidly rinsed twice with 1 ml of ice-cold incubation medium. The cells were solubilized in 0.5 ml of 0.5 N NaOH, and then the radioactivity in aliquots was determined by liquid scintillation counting. The protein content of the solubilized cells was determined by the method of Bradford (18), using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) with bovine γ-globulin as a standard. The concentration dependence of metformin transport by hOCT2 was analyzed using the Michaelis-Menten equation; $V = V_{max} \cdot [S]/(K_m + [S]) + K_d \cdot [S]$, where V is the transport rate, V_{max} is the maximum transport rate, [S] is the concentration of metformin, K_m is the Michaelis constant, and K_d is a diffusion constant. For the *cis*-inhibition study, the uptake of [^{14}C]metformin 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, Reading, PA, USA) (12). V and V_0 are the uptake rates of [^{14}C]metformin in the presence and absence of inhibitor, respectively. I is the concentration of inhibitor, and n is the Hill coefficient.

Statistical Analyses

Data were analyzed statistically by one-way analysis of variance followed by Dunnett's test. p values of less than 0.05 were considered to be significant.

RESULTS

Concentration Dependence of [^{14}C]Metformin Transport by hOCT2

To examine whether metformin is transported by hOCT2, we evaluated the uptake of [^{14}C]metformin by HEK293 cells stably expressing hOCT2. Figure 1 illustrates the time-course of [^{14}C]metformin uptake by HEK 293 cells transfected with hOCT2 and empty vector (Fig. 1). The uptake of [^{14}C]metformin increased time-dependently, and was linear for up to 2 min. Figure 2 shows the concentration dependence of [^{14}C]metformin uptake by hOCT2-expressing cells. The uptake of [^{14}C]metformin by these cells was saturated at high concentrations. The apparent Michaelis-Menten constant (K_m) value of [^{14}C]metformin uptake by hOCT2-transfected cells, estimated by subtracting the nonsaturable component of [^{14}C]metformin transport in the presence of 1-methyl-4-phenylpyridinium (5 mM) was 1.38 ± 0.21 mM. The maximal uptake rate (V_{max}) value of the [^{14}C]metformin uptake by hOCT2-transfected cells was 11.9 ± 1.5 nmol mg protein^{-1} min^{-1} (mean ± SE of three separate experiments using three monolayers).

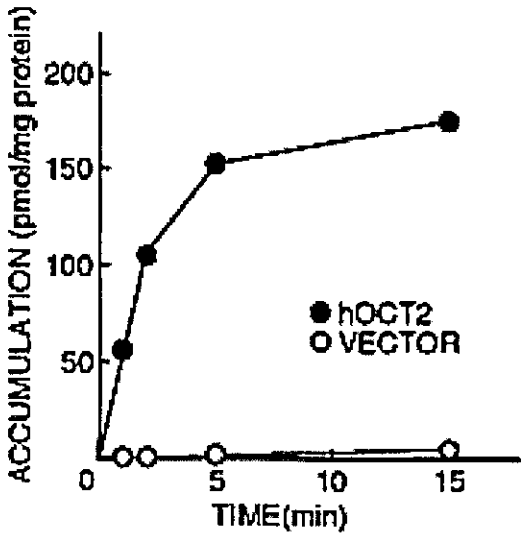

Fig. 1. Time course of [^{14}C]metformin uptake by HEK293 cells stably expressing hOCT2. HEK 293 cells transfected with hOCT2 (●) or pCMV6-XL4 vector (○) were incubated for the specified periods (1, 2, 5, and 15 min) at 37°C with 0.2 ml of 10 μM [^{14}C]metformin (pH 7.4). Each point represents the mean ± SE of three monolayers from a typical experiment.

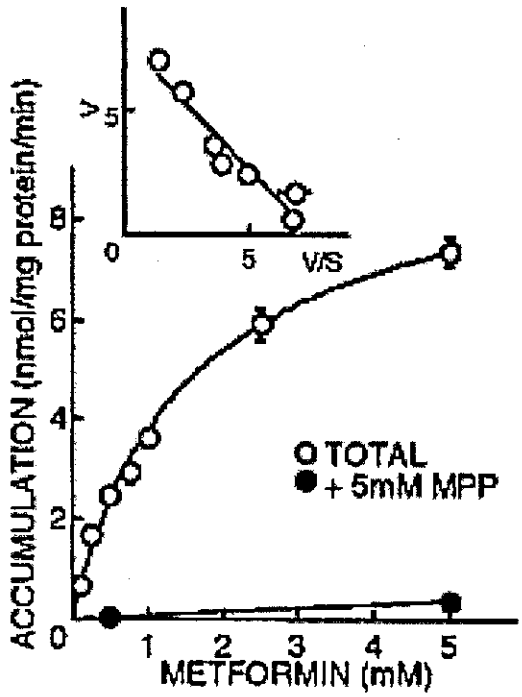

Fig. 2. Concentration dependence of [^{14}C]metformin transport by hOCT2. hOCT2 transfectants were incubated at 37°C for 2 min with various concentrations of [^{14}C]metformin (100, 250, 500, 750, 1000, 2500, and 5000 μM) in the absence (○) or presence (●) of 5 mM 1-methyl-4-phenylpyridinium (pH 7.4). Each point represents the mean ± SE of three monolayers from a typical experiment. Inset: Eadie-Hofstee plots of metformin uptake after correction for non-saturable components. V, uptake rate (nmol mg protein^{-1} min^{-1}); S, metformin concentration (mM).

Effect of Membrane Potential on the Transport of [^{14}C]Metformin and [^{14}C]Tetraethylammonium by hOCT2

Next, we examined the effect of membrane potential on the accumulation of [^{14}C]metformin and [^{14}C]tetraethylammonium in HEK293 cells expressing hOCT2 (Fig. 3). The accumulation of [^{14}C]metformin in hOCT2-expressing cells decreased in the presence of high K^+ (145 mM) medium (Fig. 3A) similarly as observed for [^{14}C]tetraethylammonium (Fig. 3B). Furthermore, the accumulation of [^{14}C]metformin and [^{14}C]tetraethylammonium *via* hOCT2 decreased in the presence of Ba^{2+} (9.2 mM), a nonselective blocker of K^+ channels. The mean percent of control values ± SE of [^{14}C]metformin accumulation obtained from three separate experiments using three monolayers were 24.8 ± 2.8% and 59.0 ± 9.9% for high K^+ and Ba^{2+} media, respectively ($p < 0.01$ vs. control). The values ± SE of [^{14}C]tetraethylammonium accumulation were 17.8 ± 2.7% and 39.7 ± 9.6% for high K^+ and Ba^{2+} media, respectively ($p < 0.01$ vs. control).

Inhibition of hOCT2-Mediated Transport of [^{14}C]Metformin by Cationic Drugs

To assess the potencies of cationic drugs to cause drug-interactions with hOCT2-mediated metformin transport, we examined the inhibitory effects of several cationic compounds on the uptake of [^{14}C]metformin by the hOCT2-expressing cells (Fig. 4). Then, we calculated the IC_{50} values of cationic compounds from the inhibition plots as described in "Materials and Methods." Tetraethylammonium (a typical substrate for the renal organic cation transporter), 1-methyl-4-phenylpyridinium (a cationic neurotoxin), procainamide and quinidine (antiarrhythmic drugs), trimethoprim (an antibiotic), and cimetidine and ranitidine (H_2 receptor antagonists) inhibited the uptake of [^{14}C]metformin by hOCT2-expressing cells in a dose-dependent manner. As summarized in Table I, 1-methyl-4-phenylpyridinium showed the most potent inhibitory effect, whereas procainamide and cimetidine had moderate inhibitory effects on the transport of metformin by hOCT2. Furthermore, biguanides inhibited the transport of [^{14}C]metformin by hOCT2 in the following order: phenformin > buformin > metformin.

DISCUSSION

In the current study, we characterized [^{14}C]metformin transport using hOCT2-expressing HEK293 cells, and assessed drug interactions between metformin and cationic drugs using [^{14}C]metformin as a tracer. [^{14}C]metformin uptake was markedly enhanced in HEK293 cells stably transfected with hOCT2 (Fig. 1). The uptake of [^{14}C]metformin was dependent on membrane potential (Fig. 3), being consistent with the functional characteristics of OCT2 (13,19). To our knowledge, this is the first demonstration showing direct evidence of metformin transport by hOCT2. Considering that hOCT2 is the dominant organic cation transporter expressed in the basolateral membranes of the human renal cortex (10), hOCT2 should play a relevant role in the transport of metformin across basolateral membranes in the human kidney.

The accumulation of [^{14}C]metformin in hOCT2-expressing cells was saturated at high concentrations (Fig. 2). Although the driving force of both compounds by hOCT2 seems to be common, that is, the membrane potential (Fig. 3), the apparent affinity of metformin to hOCT2 (K_m = 1.38 mM) was much lower than that of tetraethylammonium

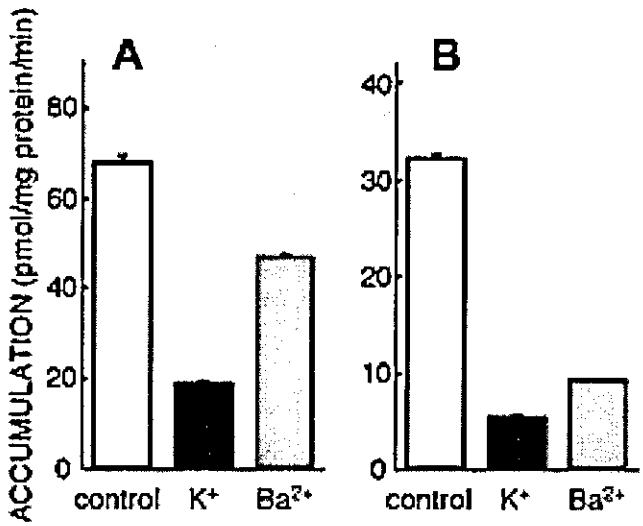

Fig. 3. Effect of membrane potential on [^{14}C]metformin (A) and [^{14}C]tetraethylammonium (B) transport by hOCT2. The cells transfected with hOCT2 were incubated with respective buffers at 37°C for 2 min with 10 μM [^{14}C]metformin (A) or 5 μM [^{14}C]tetraethylammonium (B) (pH 7.4). Each column represents the mean ± SE of three monolayers from a typical experiment.

(K_m = 431 μM) (13). Because the maximum plasma concentration of metformin was reported to be 9–12 μM after a single oral administration of metformin HCl (850 mg) in patients with type 2 diabetes mellitus (20–22) and up to 15 μM and 25 μM in healthy elderly patients and patients with moderate chronic renal impairment, respectively (23), the transport of metformin by hOCT2 should not saturate at therapeutic concentrations. Moreover, these results seem to be

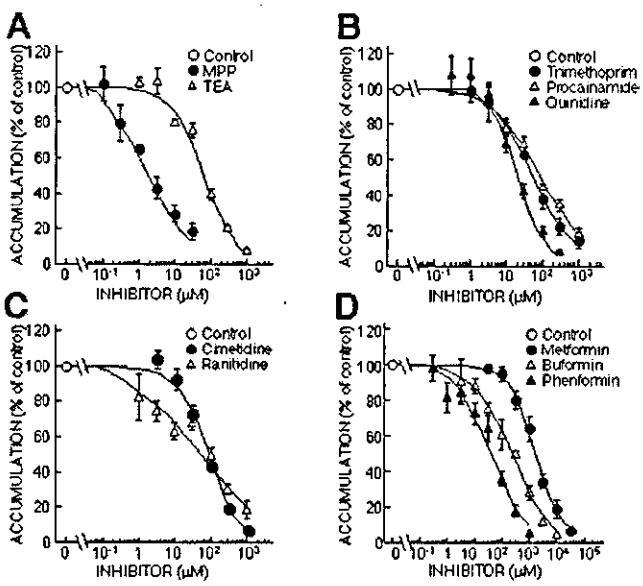

Fig. 4. Effects of cationic compounds on [^{14}C]metformin transport by hOCT2. HEK 293 cells transfected with hOCT2 were incubated at 37°C for 2 min with 10 μM [^{14}C]metformin (pH 7.4) in the presence of (A) 1-methyl-4-phenylpyridinium (MPP, ●) or tetraethylammonium (TEA, △); (B) trimethoprim (●), procainamide (△), or quinidine (▲); (C) cimetidine (●) or ranitidine (△); (D) metformin (●), buformin (△), or phenformin (▲). Each point represents the mean ± SE of three to six separate experiments using three monolayers.

Table I. The Apparent IC_{50} Values of Various Cationic Compounds for [^{14}C]Metformin Uptake by hOCT2

Compounds	Apparent IC_{50} values for [^{14}C]metformin uptake (μM)
MPP	2.99 ± 1.15
Quinidine	17.4 ± 5.7
Phenformin	55.3 ± 12.5
Trimethoprim	60.0 ± 19.0
TEA	66.6 ± 2.3
Cimetidine	72.6 ± 11.4
Ranitidine	74.6 ± 32.2
Procainamide	79.8 ± 7.5
Buformin	203 ± 63
Metformin	1840 ± 370

See experimental conditions in the legend of Fig. 4. The apparent IC_{50} values were calculated from inhibition plots (Fig. 4) by nonlinear regression analysis as described in "Materials and Methods." The data represent the mean ± SE of three to six separate experiments using three monolayers. MPP, 1-methyl-4-phenylpyridinium; TEA, tetraethylammonium.

comparable with the reports by Sambol *et al.* (20) that renal clearance of metformin was not changed by single dosings between 850 mg and 2550 mg, respectively.

In the current study, we demonstrated the inhibition of hOCT2-mediated [^{14}C]metformin transport by various cationic drugs, and then calculated respective IC_{50} values (Fig. 4 and Table I). Of the organic cations tested, quinidine or procainamide inhibited [^{14}C]metformin transport with IC_{50} values of 17.4 μM and 79.8 μM, respectively, which were comparable to the plasma concentrations of these drugs. It is reported that the plasma concentration of *N*-acetylprocainamide, a major metabolite of procainamide, also inhibit renal organic cation transporters (24). Moreover, the plasma procainamide concentration was elevated, when the drug was administered concomitantly with cimetidine (25). Therefore, we consider that concomitant administration of quinidine or procainamide with metformin should decrease the tubular secretion of metformin by interrupting hOCT2.

Somogyi *et al.* (7) reported the presence of drug interactions between metformin and cimetidine, where renal clearance of metformin was reduced by cimetidine, while cimetidine disposition was not altered by concomitantly administered metformin. According to the fact that apparent K_m value of metformin transport by hOCT2 was about 10-fold larger than that of cimetidine (K_m = 145 μM, unpublished observation), it seems to be reasonable that renal disposition of metformin, but not of cimetidine, was decreased by the drug interaction between these drugs. Dresser *et al.* (16) reported that IC_{50} value of metformin on hOCT2-mediated [^{3}H]cimetidine transport was 1700 μM. Because the value was approximately 100-fold higher than the therapeutic concentration of metformin, we consider that their results may indicate the unchanged disposition of cimetidine by concomitant administration of metformin, which was observed by Somogyi *et al.* (7).

The transport of [^{14}C]metformin was inhibited dose-dependently by cimetidine. This phenomenon apparently seems to be comparable to the observation by Somogyi *et al.* (7) that cimetidine inhibited the tubular secretion of metformin, and thereby increased its plasma concentration. The IC_{50} value of cimetidine for metformin transport (72.6 ± 11.4 μM)

was moderately higher than the plasma concentration of cimetidine, i.e., a single oral dose of 200 mg of cimetidine to the patients with normal renal function gave C_{max} values of between 2.3 μM and 6.8 μM (26). These data suggest that contribution of hOCT2 in the drug interaction between metformin and cimetidine would be minimum. However, it is known that systemic clearance of cimetidine decreases in the patients with renal dysfunction (27) and in the elderly (28), and thereby increases plasma concentrations of cimetidine. Therefore, the role of hOCT2 in the drug interactions between metformin and cimetidine could be relatively large in the patients with decreased renal function.

In the current study, the order of the potencies of biguanides to inhibit [^{14}C]metformin transport was phenformin > buformin > metformin. Dresser *et al.* (16) also demonstrated that the potency of phenformin to inhibit [^{3}H]cimetidine transport by hOCT2 was higher than that of metformin, suggesting consistency with the data in the present study. Interestingly, Wang *et al.* (14,15) also reported the order of affinity of biguanides to the rOCT1 was phenformin > buformin > metformin, suggesting similar substrate spectrum between OCT1 and OCT2. This phenomenon seems to be reasonable, because substrate spectrums were similar between OCT1 and OCT2 as demonstrated by us (11,12). Because the information regarding the relationship between plasma concentration of biguanides and blood lactose levels is limited (21), the role of hOCT2 in the biguanides-induced lactic acidosis should be clarified in subsequent studies.

In conclusion, hOCT2 is involved in the basolateral membrane transport of metformin in the human kidney. hOCT2 could also play a relevant role in the drug interaction between metformin and some cationic drugs.

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