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Transport of guanidine compounds by human organic cation transporters, hOCT1 and hOCT2

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ABSTRACT

Although some guanidine compounds were reported as superior substrates for organic cation transporter (OCT)2 than OCT1, it was unclear whether this guanidino group was an important factor in determining the specificity of hOCT1 and hOCT2. Using HEK293 cells transfected with human (h)OCT1 or hOCT2 cDNA, we assessed the role of hOCT1 and/or hOCT2 in the transport of guanidine compounds such as uremic toxins and therapeutic agents. Guanidine, creatinine and aminoguanidine more markedly inhibited the uptake of [^{14}C]tetraethylammonium (TEA) by hOCT2 than by hOCT1. [^{14}C]TEA uptake by hOCT2, but not hOCT1, was *trans*-stimulated by unlabeled guanidine, methylguanidine, creatinine, aminoguanidine and phenylguanidine. In patients with renal failure, the impairment of hOCT2 might decrease the excretion of guanidine, methylguanidine, and creatinine as uremic toxins. The uptake of aminoguanidine, a candidate for an anti-diabetic agent, was enhanced by hOCT2 with the Michaelis constant (K_m) of 4.10 ± 0.35 mM. Metformin, which was also an anti-diabetic agent, and creatinine more potently inhibited the uptake of [^{14}C]aminoguanidine by hOCT2 than that by hOCT1. Aminoguanidine had little impact on the uptake of [^{14}C]metformin by hOCT1, but inhibited that by hOCT2 with the IC_{50} of 1.49 ± 0.14 mM. These results indicated that the specificity of hOCT1 and hOCT2 was not determined simply by guanidino group. Among guanidine compounds, aminoguanidine was identified as a new superior substrate for hOCT2.

1. Introduction

Organic cation transporters (OCTs) play an important role in the tissue distribution of a wide variety of positively charged molecules, including drugs and endogenous substrates. Human organic cation transporter 1 (hOCT1) is preferentially expressed in the liver, and mediates hepatic uptake of cationic compounds [1,2]. In contrast, hOCT2 is specifically expressed in the renal proximal tubules, and is considered to mediate the renal uptake of cationic compounds [3,4]. Functional studies suggested that these transporters were often similar in substrate specificity [4,5], but in recent years, the compounds with a guanidino group such as guanidine, creatinine, and metformin were reported to be better substrates for OCT2 than OCT1 in rat and/or human [6–8]. However, it was unclear whether a guanidino group was important in determining the affinity of the two transporters.

Some guanidine compounds have been known as uremic toxins [9–14]. Other guanidine compounds have been reported as anti-diabetic agents, in particular, aminoguanidine is positively charged at physiological pH, and its renal clearance was more than twice the glomerular filtration rate (GFR), suggesting the contribution of tubular secretion [15–17].

The aim of this study was to compare the specificity of hOCT1 and hOCT2 for several guanidine compounds, including uremic toxins and aminoguanidine (Fig. 1).

2. Materials and methods

2.1. Materials

[Ethyl-1-^{14}C]tetraethylammonium bromide (55 mCi/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA). [^{14}C]aminoguanidine (51 mCi/mmol) and [biguanidine-^{14}C]metformin hydrochloride (26 mCi/mmol) were purchased from Moravek Biochemicals, Inc. (Brea, CA). [^{3}H]1-methyl-4-phenylpyridium acetate (MPP) (2.7 TBq/mmol) was purchased from PerkinElmer Life and Analytical Sciences Waltham, MA). Creatinine and guanidine hydrochloride were obtained from Nacalai Tesque (Kyoto, Japan). N_{α}-Acetyl-L-arginine, aminoguanidine bicarbonate salt, 1-butylguanidine sulfate, creatine anhydrous, 1,1-dimethylguanidine sulfate salt, guanidinoacetic acid, guanidinosuccinic acid, guanidinovaleric acid hemihydrate, methylguanidine hydrochloride, N-propylguanidine sulfate, phenylguanidine carbonate salt, 1,1,3,3-tetramethylguanidine, and 1-

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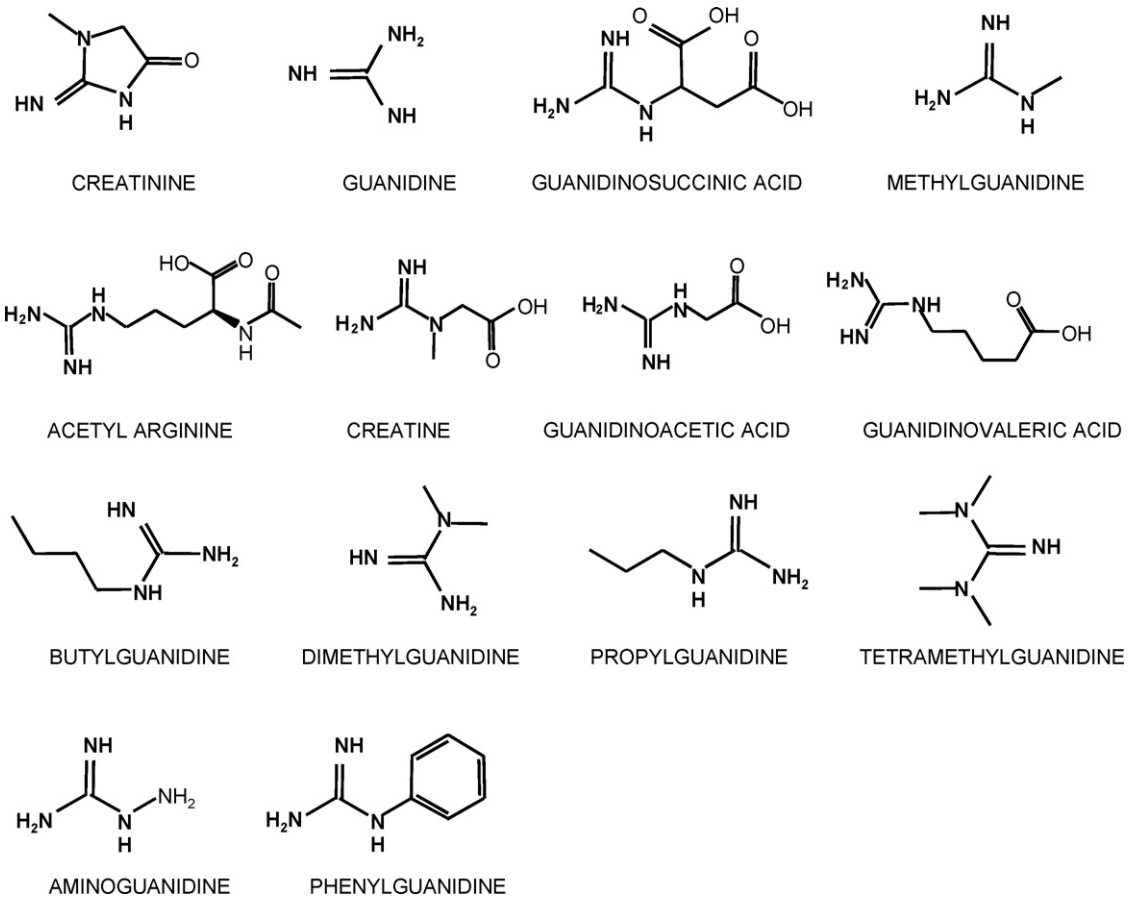

Fig. 1. Chemical structures of guanidine compounds.

methyl-4-phenylpyridium iodide were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All other compounds used were of the highest purity available.

2.2. Cell culture

HEK 293 cells (ATCC CRL-1573, American Type Culture Collection, Manassas, VA) 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, and used as host cells. The transfectant stably expressing hOCT1 and hOCT2 were established as described previously [7,8]. The HEK293 cells transiently transfected with pCMV6-XL4 plasmid vector DNA (OriGene Technologies, Rockville, MD) containing hOCT1, hOCT2 or hOCT3-cDNA were prepared as described previously [7,18]. The cell monolayers were used at day 3 of culture for uptake experiments. In the present study, cells were used between the 78th and 90th passages.

2.3. Uptake experiments

The cellular uptake of cationic compounds was measured with monolayer cultures of HEK293 cells grown on poly-D-lysine-coated 24-well plates [7,19]. The protein content of the solubilized cells was determined by the method of Bradford [20], using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA) with bovine-globulin as a standard. For the *cis*-inhibition study, the uptake of [^{14}C]tetraethylammonium (TEA), [^{14}C]aminoguanidine, or [^{14}C]metformin was achieved by adding various concentrations of unlabeled inhibitors to the incubation medium. IC_{50} values were calculated from the inhibition plots based on the equation, $V = V_0/[1 + ([I]/IC_{50})^n]$, by a nonlinear least square regression analysis with Kaleidagraph Version 4.00 (Synergy Software, Reading, PA, USA). V and V_0 were the uptake rates of [^{14}C]TEA, [^{14}C]aminoguanidine, or [^{14}C]metformin in the presence and absence of inhibitor, respectively. [I] is the concentration of inhibitor, and n is the Hill coefficient. For the

Table 1
The apparent IC_{50} values of guanidine compounds for [^{14}C]TEA uptake by hOCT1 and hOCT2.

Guanidine compounds	IC_{50} values for [^{14}C]TEA uptake (mM)	
	hOCT1	hOCT2
Uremic toxins[a]		
Creatinine	N/A	6.06 ± 0.98
Guanidine	N/A	3.03 ± 0.42
Guanidinosuccinic acid	1.54 ± 0.15	1.47 ± 0.20
Methylguanidine	2.36 ± 0.06	1.53 ± 0.31
Acetyl arginine	N/A	N/A
Creatine	N/A	N/A
Guanidinoacetic acid	N/A	N/A
Guanidinovaleric acid	0.66 ± 0.03	1.18 ± 0.14*
Butylguanidine	0.21 ± 0.02	0.12 ± 0.01*
Dimethylguanidine	0.54 ± 0.09	0.36 ± 0.02
Propylguanidine	0.36 ± 0.04	0.29 ± 0.02
Tetramethylguanidine	0.48 ± 0.08	0.78 ± 0.13
Aminoguanidine	N/A	0.80 ± 0.11
Phenylguanidine	0.23 ± 0.03	0.26 ± 0.02

See experimental conditions in the legend of Fig. 2. The apparent IC_{50} values were calculated from inhibition plots (Fig. 2) by nonlinear regression analysis as described in Section 2. The data represent the mean ± S.E. of three independent experiments. N/A, not available. *$P < 0.05$, significantly different from the IC_{50} value of hOCT1.

[a] [18].

trans-inhibition study, the cells were preincubated with either the incubation medium (control) or the incubation medium plus the indicated concentration of unlabeled compounds for 30 min. The cells were rinsed twice with 1 mL of ice-cold incubation medium before the uptake experiments. The concentration dependence of the transport of aminoguanidine by hOCT1 and hOCT2 was analyzed using the Michaelis–Menten equation; $V = V_{max}\cdot[S]/(K_m + [S]) + K_{diff}\cdot[S]$, where V is the transport rate, V_{max} is the maximum transport rate, [S] is the concentration of aminoguanidine, K_m is the Michaelis constant and K_{diff} is a diffusion constant. The accumulation of [^{14}C]aminoguanidine by hOCT1- and hOCT2-HEK293 cells was measured at various concentrations (0.1–10 mM) for 2 min at 37 °C (pH 7.4).

2.4. Statistical analysis

Data were expressed as the mean ± S.E. Data were analyzed statistically using the non-paired Student's *t* test or one-way analysis of variance (ANOVA) and Dunnett's multiple comparison procedure.

3. Results

3.1. Inhibitory effects of guanidine compounds on TEA uptake by hOCT1 and hOCT2

To compare the specificity of hOCT1 and hOCT2, the inhibitory effects of several guanidine compounds on the uptake of [^{14}C]TEA

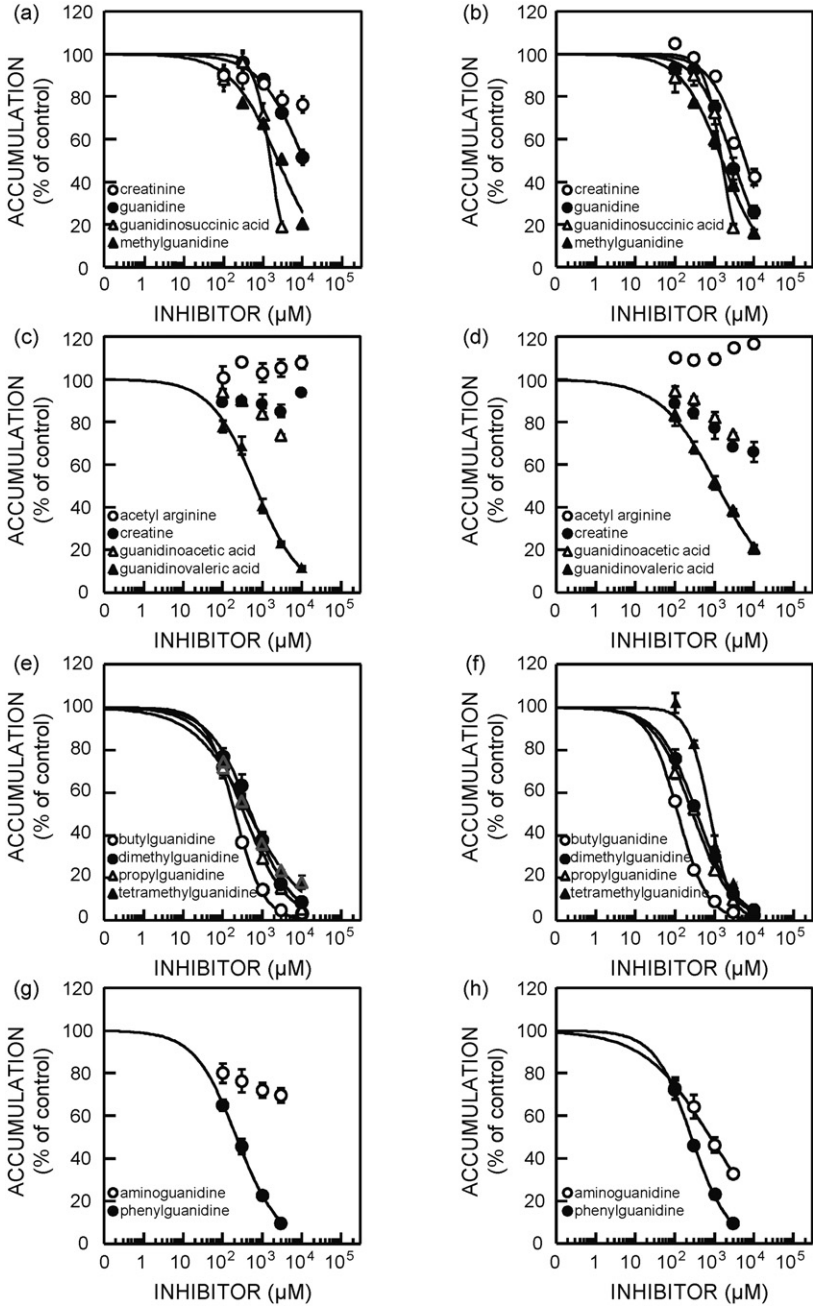

Fig. 2. Effects of guanidine compounds on [^{14}C]TEA uptake by hOCT1 (a, c, e and g) and hOCT2 (b, d, f and h). HEK293 cells transfected with hOCT1 and hOCT2 were incubated at 37 °C for 2 min with 5 μM [^{14}C]TEA (pH 7.4) in the presence of (a and b); creatinine (open circle), guanidine (closed circle), guanidinosuccinic acid (open triangle), or methylguanidine (closed triangle), (c and d); acetyl arginine (open circle), creatine (closed circle), guanidinoacetic acid (open triangle), or guanidinovaleric acid (closed triangle), (e and f); butylguanidine (open circle), dimethylguanidine (closed circle), propylguanidine (open triangle), or tetramethylguanidine (closed triangle), (g and h); aminoguanidine (open circle), or phenylguanidine (closed circle). Each point represents the mean ± S.E. of three independent experiments.

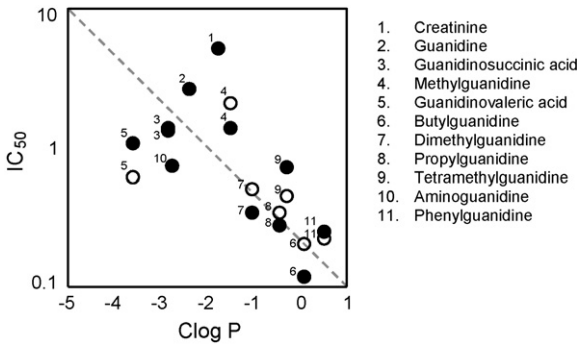

Fig. 3. Relationship between the calculated hydrophobicity (*C* log *P*) of guanidine compounds and IC_{50} values for inhibition of [^{14}C]TEA uptake by hOCT1 and hOCT2. The relationship between the calculated hydrophobicity (*C* log *P*) of guanidine compounds and the measured IC_{50} values for inhibition of [^{14}C]TEA uptake in hOCT1-HEK293 (open circle) and hOCT2-HEK293 (closed circle) cells. See experimental conditions in the legend of Fig. 2. The apparent IC_{50} values were calculated from inhibition plots (Fig. 2) by nonlinear regression analysis as described in Section 2. Octanol/water partition coefficients (log *P*) were calculated using Chem Draw Ultra 7.0 software.

(a typical substrate for the organic cation transporter) were examined (Table 1, Fig. 2). The inhibitory effects of guanidinosuccinic acid and methylguanidine were comparable between hOCT1 and hOCT2. Guanidine and creatinine had stronger inhibitory effects on [^{14}C]TEA uptake by hOCT2 than by hOCT1, whereas guanidinovaleric acid inhibited hOCT1 more than hOCT2. Guanidinoacetic acid tended to inhibit the uptake of [^{14}C]TEA by both hOCT1 and hOCT2, while creatine tended only to inhibit the hOCT2. Acetyl arginine did not inhibit [^{14}C]TEA uptake by either transporters. The inhibition curves of alkyl guanidine compounds for [^{14}C]TEA uptake showed that alkyl guanidine compounds had potent inhibitory effects on both hOCT1 and hOCT2, and that only butylguanidine had moderately higher affinity for hOCT2 than hOCT1. Phenylguanidine had an inhibitory effect on [^{14}C]TEA uptake by both hOCT1 and hOCT2, while aminoguanidine had a much greater inhibitory effect on hOCT2 than hOCT1. Fig. 3 plots the relationship between the log of the measured IC_{50} values (Table 1) and the calculated log *P* values (*C* log *P*) of guanidine compounds.

3.2. Trans-stimulation effects of guanidine compounds on TEA uptake by hOCT1 and hOCT2

To examine whether these guanidine compounds were substrates of hOCT1 and hOCT2, *trans*-stimulation experiments were performed. The transfectants were preincubated with a concentration equivalent to approximately 3-fold the IC_{50} value of the unlabeled guanidine compounds, or else with 10 mM if the IC_{50} value was not available (Table 1) [5]. Then, the [^{14}C]TEA uptake by the preincubated transfectants was measured. Fig. 4 shows the

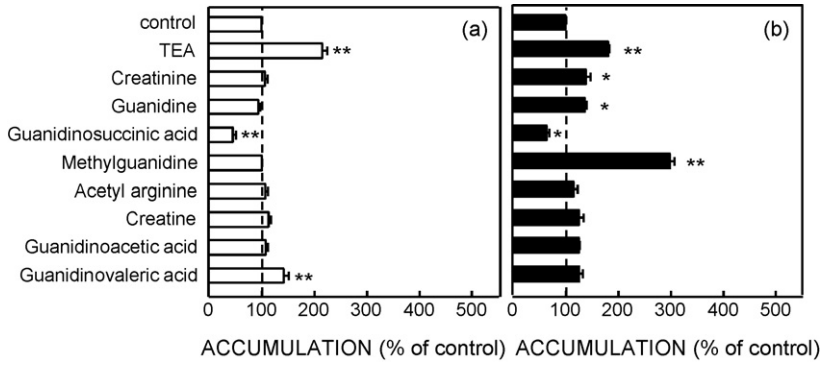

Fig. 4. *Trans*-stimulation effects of guanidine compounds as uremic toxins on [^{14}C]TEA uptake by hOCT1 (a) and hOCT2 (b). HEK-hOCT1 and HEK-hOCT2 cells were incubated for 2 min at 37 °C with 5 μM [^{14}C]TEA after preincubation with incubation medium (control) or incubation medium containing TEA (5 mM), creatinine (10 mM, hOCT1; 20 mM, hOCT2), guanidine (10 mM, hOCT1; 9 mM, hOCT2), guanidinosuccinic acid (5 mM, hOCT1; 4 mM, hOCT2), methylguanidine (7 mM hOCT1; 5 mM, hOCT2), acetyl arginine (10 mM), creatine (10 mM), guanidinoacetic acid (10 mM), and guanidinovaleric acid (2 mM, hOCT1; 4 mM, hOCT2) for 30 min at 37 °C, respectively. Data are expressed as a percentage of the control value. Control values for HEK-hOCT1 and HEK-hOCT2 were 25.6 ± 1.4 and 14.5 ± 1.2 pmol/mg protein/2 min, respectively. Each column represents the mean ± S.E. of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from the control.

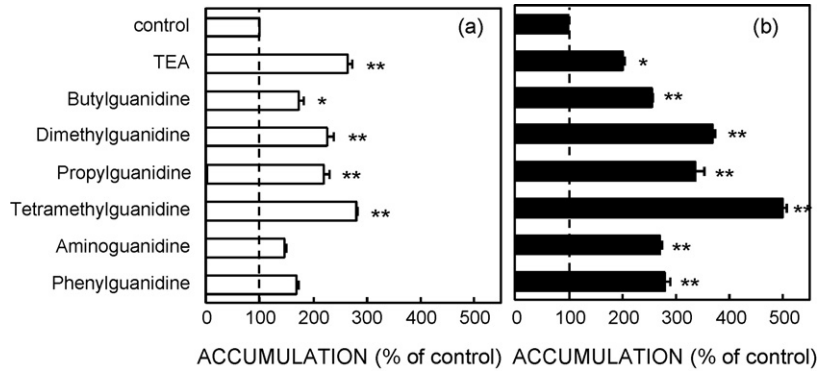

Fig. 5. *Trans*-stimulation effects of alkyl guanidine compounds, aminoguanidine and phenylguanidine on [^{14}C]TEA uptake by hOCT1 (a) and hOCT2 (b). HEK-hOCT1 and HEK-hOCT2 cells were incubated for 2 min at 37 °C with 5 μM [^{14}C]TEA after preincubation with incubation medium (control) or incubation medium containing TEA (5 mM), butylguanidine (0.6 mM, hOCT1; 0.4 mM, hOCT2), dimethylguanidine (2 mM hOCT1; 1 mM, hOCT2), propylguanidine (1 mM, hOCT1; 0.9 mM, hOCT2), tetramethylguanidine (1 mM, hOCT1; 2 mM, hOCT2), aminoguanidine (10 mM, hOCT1; 2 mM hOCT2) and phenylguanidine (0.7 mM, hOCT1; 0.8 mM, hOCT2) for 30 min at 37 °C, respectively. Data are expressed as a percentage of the control value. Control values for HEK-hOCT1 and HEK-hOCT2 were 25.9 ± 1.4 and 11.0 ± 0.2 pmol/mg protein/2 min, respectively. Each column represents the mean ± S.E. of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from the control.

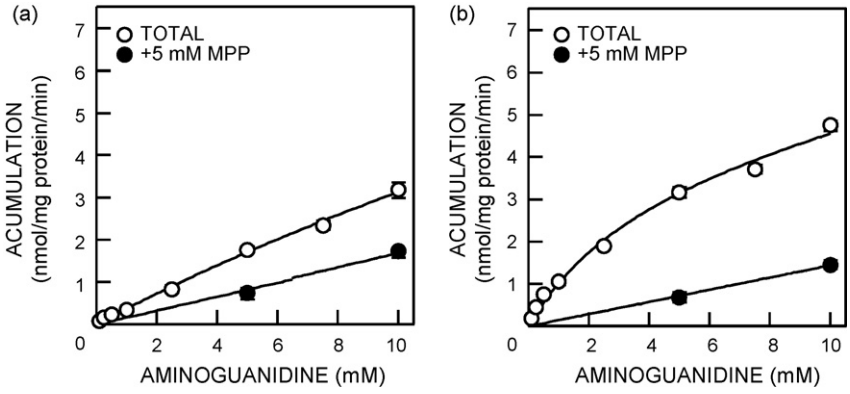

Fig. 6. Concentration dependence of [^{14}C]aminoguanidine transport by hOCT1 (a) and hOCT2 (b). hOCT1 and hOCT2 transfectants were incubated at 37 °C for 2 min with various concentrations of [^{14}C]aminoguanidine (0.1, 0.25, 0.5, 1, 2.5, 5, 7.5 and 10 mM) in the absence (open circle) or presence (closed circle) of 5 mM 1-methyl-4-phenylpyridinium (pH 7.4). Each point represents the mean ± S.E. of three independent experiments.

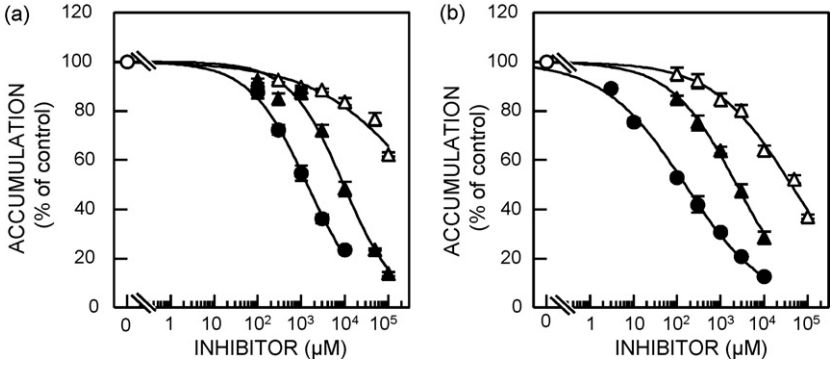

Fig. 7. Effects of TEA, creatinine and metformin on [^{14}C]aminoguanidine transport by hOCT1 (a) and hOCT2 (b). HEK 293 cells transfected with hOCT1 and hOCT2 were incubated at 37 °C for 2 min with 10 μM [^{14}C]aminoguanidine (pH 7.4) in the absence (open circle) or presence of TEA (closed circle), creatinine (open triangle), or metformin (closed triangle). Each point represents the mean ± S.E. of three independent experiments.

trans-stimulation effects of endogenous guanidine compounds as uremic toxin. Preincubation with unlabeled guanidine, methylguanidine, and creatinine significantly increased the uptake of [^{14}C]TEA by hOCT2 but not hOCT1. Meanwhile, preincubation with guanidinovaleric acid increased the [^{14}C]TEA uptake by hOCT1 but not hOCT2. As shown in Fig. 5, we examined the *trans*-stimulation effects of the other guanidine compounds. Preincubation with unlabeled butylguanidine, propylguanidine, dimethylguanidine, and tetramethylguanidine increased the uptake of [^{14}C]TEA by both transfectants. On the other hand, the preincubation with aminoguanidine and phenylguanidine significantly enhanced the [^{14}C]TEA uptake by hOCT2 but not hOCT1.

3.3. Uptake of aminoguanidine by hOCT1 and hOCT2

To obtain more information about the substrate specificity of hOCT2, the transport characteristics of [^{14}C]aminoguanidine was compared between hOCT1 and hOCT2. Fig. 6 shows the concentration dependence of [^{14}C]aminoguanidine uptake by hOCT1 and hOCT2. The uptake by hOCT2 was greater than that by hOCT1. The uptake was saturated at high concentrations in hOCT2-expressing cells, although no such saturation was observed in hOCT1-expressing cells. The apparent Michaelis–Menten constant (K_m) for the uptake of [^{14}C]aminoguanidine by hOCT2 was 4.10 ± 0.35 mM. The maximal uptake rate (V_{max}) in hOCT2-expressing cells was 4.40 ± 0.42 nmol/mg protein/min (mean ± S.E. of three separate experiments). Next, we examined the inhibitory effects of TEA, creatinine and metformin on the uptake of [^{14}C]aminoguanidine by hOCT1 and hOCT2 (Fig. 7). Fig. 7a and b shows the inhibition curves of TEA, creatinine and metformin in hOCT1- and hOCT2-expressing cells, respectively. Although TEA, creatinine and metformin inhibited the uptake of [^{14}C]aminoguanidine by both hOCT1 and hOCT2 in a dose-dependent manner, the uptake by hOCT2 was more inhibited. We calculated the IC_{50} values of these cationic compounds from the inhibition plots as described in Section 2 (Table 2). We also examined the inhibitory effect of aminoguanidine on the uptake of [^{14}C]metformin (Fig. 8). Aminoguanidine had little impact on the uptake of [^{14}C]metformin by hOCT1, and the IC_{50} value of aminoguanidine was not estimated (Fig. 8a). However, aminoguanidine inhibited the [^{14}C]metformin uptake by hOCT2 with the IC_{50} of 1.49 ± 0.14 mM (Fig. 8b).

To confirm aminoguanidine as a new substrate selective for hOCT2, the influence of *cis*-inhibition and *trans*-stimulation of aminoguanidine on the [^{3}H]MPP transport by hOCT3 was

Table 2
The apparent IC_{50} values of cationic compounds for [^{14}C]aminoguanidine uptake by hOCT1 and hOCT2.

Inhibitors	IC_{50} values for [^{14}C]aminoguanidine uptake (mM)	
	hOCT1	hOCT2
TEA	1.39 ± 0.06	0.16 ± 0.02
Creatinine	N/A	42.4 ± 2.6
Metformine	9.48 ± 0.56	2.37 ± 0.20

See experimental conditions in the legend of Fig. 7. The apparent IC_{50} values were calculated from inhibition plots (Fig. 7) by nonlinear regression analysis as described in Section 2. The data represent the mean ± S.E. of three independent experiments. N/A, not available.

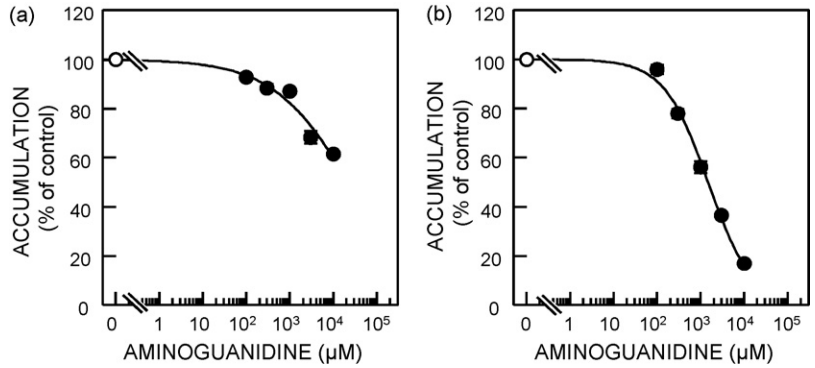

Fig. 8. Effects of aminoguanidine on [^{14}C]metformin transport by hOCT1 (a) and hOCT2 (b). HEK 293 cells transfected with hOCT1 and hOCT2 were incubated at 37 °C for 2 min with 10 μM [^{14}C]metformin (pH 7.4) in the absence (open circle) or presence of aminoguanidine (closed circle). Each point represents the mean ± S.E. of three independent experiments.

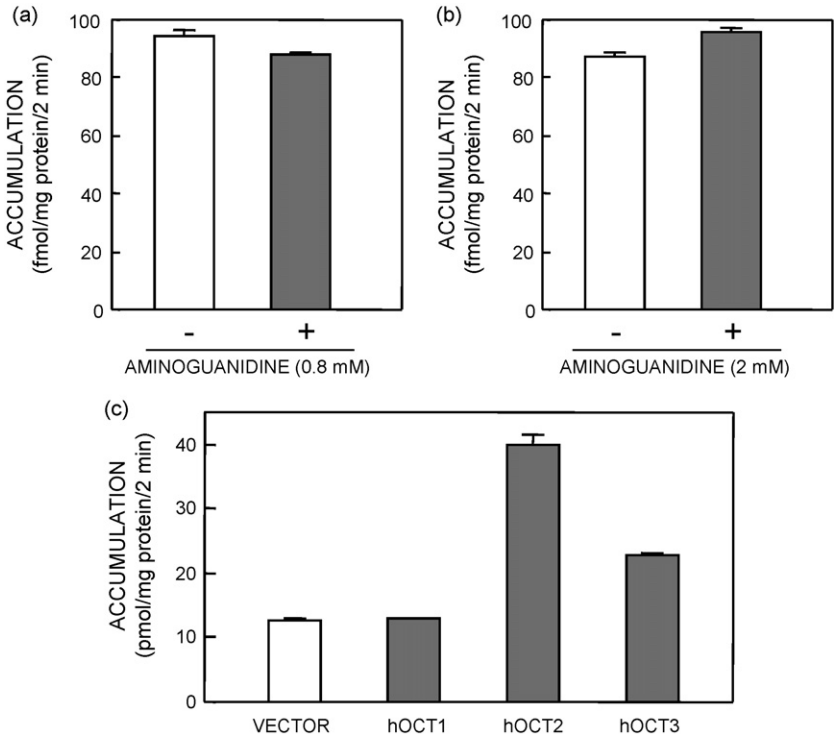

Fig. 9. Influence of *cis*-inhibition (a) and *trans*-stimulation (b) of aminoguanidine on the [^{3}H]MPP transport by hOCT3, and [^{14}C]aminoguanidine transport by hOCT1, hOCT2 and hOCT3 (c). (a) HEK293 cells transiently expressing hOCT3 were incubated at 37 °C for 2 min with 13.7 nM [^{3}C]MPP (pH 7.4) in the absence (−) or presence (+) of aminoguanidine (0.8 mM). (b) HEK293 cells transiently expressing hOCT3 were incubated for 2 min at 37 °C with 13.7 nM [^{3}H]MPP after preincubation with incubation medium (−) or incubation medium containing aminoguanidine (2 mM) (+) for 30 min at 37 °C. (c) HEK293 cells transfected with empty vector, hOCT1, hOCT2 or hOCT3 were incubated for 2 min at 37 °C with 5 μM [^{14}C]aminoguanidine (pH 7.4). Each column represents the mean ± S.E. of three monolayers.

examined in comparison with hOCT2 (Fig. 9a and b). The hOCT3-mediated uptake of [^{3}H]MPP was little affected by aminoguanidine in both conditions of *cis*-inhibition and *trans*-stimulation. In addition, the transport of [^{14}C]aminoguanidine by hOCT2 was the highest among three OCT isoforms (Fig. 9c).

4. Discussion

Previous reports suggested that guanidine and creatinine, which had a guadinino group, were predominantly transported by OCT2 rather than OCT1 [6,7]. We tested the hypothesis that the guanidino group was a decisive factor in being recognized by hOCT2, but could not found such selectivity simply by this group. At the same time, we discovered that aminoguanidine was a new superior substrate for hOCT2 than hOCT1.

Several guanidine compounds were reported to accumulate in blood with renal insufficiency, some being described as uremic toxins [9–14]. Guanidinosuccinic acid and methylguanidine had the two highest scores for the uremic concentration (C_U)/normal concentration (C_N) ratio, and there were also significant differences between the C_U and C_N of guanidine and creatinine [14]. The plasma concentrations of many cationic drugs increase with renal failure. It has been considered that the tubular secretion of organic cations is impaired and the elevated plasma level of alpha1 acid glycoprotein prevents the renal excretion in renal failure [21–25]. Based on the present results, it is also possible that the uremic guanidine compounds inhibit the excretion of cationic drugs mediated by hOCT.

Fig. 3 shows the relationship between the inhibitory patterns and the $C\log P$ values of guanidine compounds. In guanidine

compounds, hydrophobicity was not the major factor in determining the affinity for hOCT as it was, for example, in n-tetraalkylammonium [5,26,27].

In the *trans*-stimulation study, we showed that the [^{14}C]TEA uptake by hOCT2, but not hOCT1, were increased by preincubation with unlabeled guanidine, methylguanidine, creatinine, aminoguanidine and phenylguanidine. Possibly, these compounds are transported by hOCT2 and the dysfunction of hOCT2 with renal failure decreases the excretion of guanidine, methylguanidine, and creatinine as uremic toxins.

Because the three uremic guanidine compounds, guanidinosuccinic acid, methylguanidine and guanidinovaleric acid inhibited [^{14}C]TEA uptake by hOCT1 as well as hOCT2, the pharmacokinetics of the cationic drugs may be affected in the patients with renal failure. The [^{14}C]TEA uptake by hOCT1 was *trans*-stimulated by guanidinovaleric acid, suggesting the hOCT1-mediated transport of guanidinovaleric acid compensating the impaired renal function. It might relate to the fact that the serum level of guanidinovaleric acid in the patients with renal insufficiency was similar to normal values [10,13].

Among 14 guanidine compounds, aminoguanidine was found to be a selective substrate for hOCT2 compared to hOCT1 and hOCT3. A guanidine compound agmatine (1-amino-4-guanidobutane) was reported as a substrate for hOCT2 and hOCT3, but not for hOCT1 [28], while guanidine was transported by rOCT2, but not by rOCT1 and hOCT3 [6]. Therefore, aminoguanidine as well as agmatin and guanidine can be a good probe to examine the transport activity of hOCT2 in comparison with hOCT1 and hOCT3.

The apparent affinity of aminoguanidine for hOCT2 was similar to that of creatinine (K_m = 4.0 mM) [7] and lower than that of metformin (K_m = 1.4 mM) [19]. Aminoguanidine, which inhibits many diabetes-related complications, remains under therapeutic testing [16,17,29]. Because aminoguanidine was excreted into urine by tubular secretion as well as glomerular filtration and hOCT2 was the most abundant organic cation transporter in the basolateral membranes of human kidney [3,15], the secretion of aminoguanidine may be predominantly mediated by hOCT2.

In ACTION I trial (*A* Clinical *T*rial *I*n *O*vert *N*ephropathy of Type *1* Diabetics), which included patients with type 1 diabetes mellitus [17], aminoguanidine reduced significantly secondary measures of outcome such as proteinuria and had additional effects on diabetic retinopathy and circulating lipid levels. However, the reduction in the primary end point of time to doubling of the serum creatinine concentration was not statistically significant. Although creatinine clearance is often used for the estimation of GFR, creatinine is also excreted via tubular secretion mediated by hOCT2 [7,30,31]. Aminoguanidine might inhibit the transport of creatinine by hOCT2 and increase the serum concentration of creatinine without inducing renal impairment. Therefore, the other parameters whose elimination was unaffected by aminoguanidine should have been used.

Although creatinine and metformin were also excreted into urine through transport by hOCT2 [7,19], their IC_{50} values for aminoguanidine uptake by hOCT2 (creatinine, 42.4 mM; metformin, 2.37 mM) were much higher than the physiological concentrations of creatinine (about 45–85 μM for male and 30–60 μM for female) and metformin (about 15–25 μM) (Table 2, Fig. 7) [24,32–34]. Therefore, the transport of aminoguanidine mediated by hOCT2 is not likely to be affected by creatinine and metformin, and diabetic patients whose plasma creatinine concentrations are increased or who use metformin may be able to use aminoguanidine safely. It was reported that the maximum aminoguanidine concentration was only 40 μM, during the interdialytic period [15]. It is also probable that aminoguanidine has little effect on the transport of metformin mediated by hOCT2, at the physiological concentrations (Fig. 8).

In this study, we demonstrated that many guanidine compounds examined had relatively equal affinity to hOCT1 and hOCT2 and could not found the selectivity for hOCT2 simply by guanidino group. Among guanidine compounds, we newly discovered that aminoguanidine had greater affinity for hOCT2 than hOCT1, in addition to guanidine and creatinine. Therefore hOCT2 could function as a transporter for aminoguanidine at the basolateral membranes of renal proximal tubules. These findings will be helpful to elucidate the specificity of hOCT2, and clarify the pharmacokinetics of aminoguanidine.

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References

[1] Gorboulev V, Ulzheimer JC, Akhoundova A, Ulzheimer-Teuber I, Karbach U, Quester S, et al. Cloning and characterization of two human polyspecific organic cation transporters. DNA Cell Biol 1997;16:871–81.
[2] Zhang L, Dresser MJ, Gray AT, Yost SC, Terashita S, Giacomini KM. Cloning and functional expression of a human liver organic cation transporter. Mol Pharmacol 1997;51:913–21.
[3] Motohashi H, Sakurai Y, Saito H, Masuda S, Urakami Y, Goto M, et al. Gene expression levels and immunolocalization of organic ion transporters in the human kidney. J Am Soc Nephrol 2002;13:866–74.
[4] Inui K, Masuda S, Saito H. Cellular and molecular aspects of drug transport in the kidney. Kidney Int 2000;58:944–58.
[5] Urakami Y, Okuda M, Masuda S, Akazawa M, Saito H, Inui K. Distinct characteristics of organic cation transporters, OCT1 and OCT2, in the basolateral membrane of renal tubules. Pharm Res 2001;18:1528–34.
[6] Grundemann D, Liebich G, Kiefer N, Koster S, Schomig E. Selective substrates for non-neuronal monoamine transporters. Mol Pharmacol 1999;56:1–10.
[7] Urakami Y, Kimura N, Okuda M, Inui K. Creatinine transport by basolateral organic cation transporter hOCT2 in the human kidney. Pharm Res 2004;21:976–81.
[8] Kimura N, Masuda S, Tanihara Y, Ueo H, Okuda M, Katsura T, et al. Metformin is a superior substrate for renal organic cation transporter OCT2 rather than hepatic OCT1. Drug Metab Pharmacokinet 2005;20:379–86.
[9] De Deyn P, Marescau B, Lornoy W, Becaus I, Lowenthal A. Guanidino compounds in uraemic dialysed patients. Clin Chim Acta 1986;157:143–50.
[10] De Deyn PP, Marescau B, Cuykens JJ, Van Gorp L, Lowenthal A, De Potter WP. Guanidino compounds in serum and cerebrospinal fluid of non-dialyzed patients with renal insufficiency. Clin Chim Acta 1987;167:81–8.
[11] Kishore BK, Kallay Z, Tulkens PM. Clinico-biochemical aspects of guanidine compounds in uraemic toxicity. Int Urol Nephrol 1989;21:223–32.
[12] Marescau B, Nagels G, Possemiers I, De Broe ME, Becaus I, Billiouw JM, et al. Guanidino compounds in serum and urine of nondialyzed patients with chronic renal insufficiency. Metabolism 1997;46:1024–31.
[13] Torremans A, Marescau B, Kranzlin B, Gretz N, Billiouw JM, Vanholder R, et al. Biochemical validation of a rat model for polycystic kidney disease: comparison of guanidino compound profile with the human condition. Kidney Int 2006;69:2003–12.
[14] Vanholder R, De Smet R, Glorieux G, Argiles A, Baurmeister U, Brunet P, et al. Review on uremic toxins: classification, concentration, and interindividual variability. Kidney Int 2003;63:1934–43.
[15] Foote EF, Look ZM, Giles P, Keane WF, Halstenson CE. The pharmacokinetics of aminoguanidine in end-stage renal disease patients on hemodialysis. Am J Kidney Dis 1995;25:420–5.
[16] Edelstein D, Brownlee M. Mechanistic studies of advanced glycosylation end product inhibition by aminoguanidine. Diabetes 1992;41:26–9.
[17] Bolton WK, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K, et al. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. Am J Nephrol 2004;24:32–40.
[18] Yokoo S, Masuda S, Yonezawa A, Terada T, Katsura T, Inui K. Significance of organic cation transporter 3 (SLC22A3) expression for the cytotoxic effect of oxaliplatin in colorectal cancer. Drug Metab Dispos 2008;36:2299–306.
[19] Kimura N, Okuda M, Inui K. Metformin transport by renal basolateral organic cation transporter hOCT2. Pharm Res 2005;22:255–9.
[20] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 1976;72:248–54.

[21] Gibson TP, Matusik EJ, Briggs WA. N-Acetylprocainamide levels in patients with end-stage renal failure. Clin Pharmacol Ther 1976;19:206–12.
[22] Piafsky KM, Borga O, Odar-Cederlof I, Johansson C, Sjoqvist F. Increased plasma protein binding of propranolol and chlorpromazine mediated by disease-induced elevations of plasma alpha1 acid glycoprotein. N Engl J Med 1978;299:1435–9.
[23] Larsson R, Bodemar G, Norlander B. Oral absorption of cimetidine and its clearance in patients with renal failure. Eur J Clin Pharmacol 1979;15:153–7.
[24] Sambol NC, Chiang J, Lin ET, Goodman AM, Liu CY, Benet LZ, et al. Kidney function and age are both predictors of pharmacokinetics of metformin. J Clin Pharmacol 1995;35:1094–102.
[25] Martinez-Gomez MA, Sagrado S, Villanueva-Camanas RM, Medina-Hernandez MJ. Characterization of basic drug-human serum protein interactions by capillary electrophoresis. Electrophoresis 2006;27:3410–9.
[26] Zhang L, Gorset W, Dresser MJ, Giacomini KM. The interaction of n-tetraalkylammonium compounds with a human organic cation transporter, hOCT1. J Pharmacol Exp Ther 1999;288:1192–8.
[27] Bednarczyk D, Ekins S, Wikel JH, Wright SH. Influence of molecular structure on substrate binding to the human organic cation transporter, hOCT1. Mol Pharmacol 2003;63:489–98.
[28] Grundemann D, Hahne C, Berkels R, Schomig E. Agmatine is efficiently transported by non-neuronal monoamine transporters extraneuronal monoamine transporter (EMT) and organic cation transporter 2 (OCT2). J Pharmacol Exp Ther 2003;304:810–7.
[29] Makita Z, Vlassara H, Cerami A, Bucala R. Immunochemical detection of advanced glycosylation end products in vivo. J Biol Chem 1992;267:5133–8.
[30] Shannon JA. The renal excretion of creatinine in man. J Clin Invest 1935;14:403–10.
[31] Miller BF, Winkler AW. The renal excretion of endogenous creatinine in man. Comparison with exogenous creatinine and inulin. J Clin Invest 1938;17:31–40.
[32] Sambol NC, Chiang J, O'Conner M, Liu CY, Lin ET, Goodman AM, et al. Pharmacokinetics and pharmacodynamics of metformin in healthy subjects and patients with noninsulin-dependent diabetes mellitus. J Clin Pharmacol 1996;36:1012–21.
[33] Sirtori CR, Franceschini G, Galli-Kienle M, Cighetti G, Galli G, Bondioli A, et al. Disposition of metformin (N,N-dimethylbiguanide) in man. Clin Pharmacol Ther 1978;24:683–93.
[34] Pentikainen PJ, Neuvonen PJ, Penttila A. Pharmacokinetics of metformin after intravenous and oral administration to man. Eur J Clin Pharmacol 1979;16:195–202.