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Human organic anion transporter hOAT3 is a potent transporter of cephalosporin antibiotics, in comparison with hOAT1

Harumasa Ueo, Hideyuki Motohashi, Toshiya Katsura, Ken-ichi Inui *

Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Kyoto 606-8507, Japan

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Abstract

We examined the substrate specificity of human organic anion transporter (hOAT) 1 and hOAT3 for various cephalosporin antibiotics, cephaloridine, cefdinir, cefotiam, ceftibuten, cefaclor, ceftizoxime, cefoselis and cefazolin by using HEK293 cells stably transfected with hOAT1 or hOAT3 cDNA (HEK-hOAT1, HEK-hOAT3). Additionally, we examined the uptake of various compounds by these transfectants. The mRNA level of hOAT3 in HEK-hOAT3 was about three-fold that of hOAT1 in HEK-hOAT1. Functional expression of hOAT1 and hOAT3 was confirmed by the uptake of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate, respectively. *p*-[^{14}C]Aminohippurate, [^{3}H]estrone sulfate, [^{14}C]captopril, [^{3}H]methotrexate, [^{3}H]ochratoxin A, [^{3}H]leucovorin and [^{3}H]cimetidine were shown to be substrates for hOAT1 and hOAT3, and [^{3}H]dehydroepiandrosterone sulfate was shown to be a substrate for hOAT3. All cephalosporin anitibiotics tested were shown to inhibit the uptake of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate via hOAT1 and hOAT3, respectively, in a dose-dependent manner, and the IC_{50} values of these antibiotics, except for cefaclor, for the hOAT1-mediated uptake of *p*-[^{14}C]aminohippurate were within four-fold of those for the hOAT3-mediated uptake of [^{3}H]estrone sulfate. The uptake of cephaloridine, cefdinir and cefotiam by HEK-hOAT3 was 35–50-fold greater than that by control cells. Moreover, the accumulation of the other cephalolsporin antibiotics was significantly greater in HEK-hOAT3 than in control cells. In contrast, the uptake of these antibiotics by HEK-hOAT1 was within two-fold of that by control cells. In conclusion, hOAT3 plays a more important role than hOAT1 in the renal secretion of cephalosporin antibiotics.

Keywords: Organic anion transporter; Cephalosporin antibiotics; *p*-Aminohippurate; Estrone sulfate; Renal secretion; Transport

1. Introduction

In the kidney, organic anion transporters, which are expressed in the apical and basolateral membranes of tubular epithelial cells, are responsible for tubular secretion of organic anions including drugs, toxins and endogenous compounds [1–3]. Cloned human organic anion transporters (hOATs) have been shown to transport clinically important drugs, such as antiviral agents [4], nonsteroidal anti-inflammatory drugs (NSAIDs) [5] and diuretics [6]. We previously reported that mRNA levels of hOAT1 and hOAT3 were much higher than those of other organic ion transporters in the human kidney cortex, and that hOAT1 and hOAT3 were localized to the basolateral membrane of the proximal tubular cells [7]. These results suggest that hOAT1 and hOAT3 play important roles in the tubular uptake of various drugs from the circulation. Therefore, for elucidation of the mechanism behind the renal elimination of various drugs, it is important to characterize the substrate specificities of these transporters.

Most cephalosporin antibiotics are excreted into urine in nonmetabolized forms, and renal tubular secretion appears to be an important pathway for their renal clearance [8]. Several in vivo and in vitro studies have tried to elucidate the transport mechanisms of cephalosporins in the kidney [9–11]. From these findings, it was suggested that OATs would be involved in the renal secretion of cephalosporins. Actually, rat OAT1 transported cephalosporins [12].

Abbreviations: hOAT, human organic anion transporter; rOAT, rat organic anion transporter

* Corresponding author. Tel.: +81 75 751 3577; fax: +81 75 751 4207.
E-mail address: inui@kuhp.kyoto-u.ac.jp (K.-i. Inui).

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With regard to human OATs, Takeda et al. [13] reported that cephalosporin antibiotics interacted with hOATs. However, it has not been clarified whether hOATs are responsible for the tubular secretion of antibiotics. In our previous study, it was recognized that hOAT3 mediated the transport of cefazolin [14]. Moreover, renal excretion of cefazolin was significantly correlated with hOAT3 mRNA levels in patients with renal diseases, suggesting that hOAT3 plays an important role in the secretion of cefazolin in such patients [14]. It has remained to be elucidated whether hOAT1 or hOAT3 transports other cephalosporins.

In this study, we examined the transport of various cephalosporin antibiotics by hOAT1 and hOAT3 to characterize the substrate specificity of these transporters.

2. Materials and methods

2.1. Materials

p-[Glycyl-1-^{14}C]aminohippurate (1.9 GBq/mmol) and [1,2,6,7-^{3}H(*N*)]dehydroepiandrosterone sulfate, sodium salt (2.2 TBq/mmol) were obtained from NEN™ Life Science Products Inc. (Boston, MA). [6,7-^{3}H(*N*)]estrone sulfate, ammonium salt (2.1 TBq/mmol) was from Perkin-Elmer Life Sciences Inc. (Boston, MA). [^{14}C]captopril (115 MBq/mmol) was from Sankyo Co. (Tokyo, Japan). [3′,5′,7′-^{3}H(*N*)]methotrexate, disodium salt (851 GBq/mmol), [^{3}H(G)]ochratoxin A (666 GBq/mmol) and [3′,5′,7′,9′-^{3}H] (6*S*)-leucovorin, diammonium salt (962 GBq/mmol) were from Moravek Biochemicals Inc. (Brea, CA). [*N*-Methyl-^{3}H]Cimetidine (451 GBq/mmol) was from Amersham Biosciences (Uppsala, Sweden). Cefaclor, cephaloridine and ceftibuten (Shionogi Co., Osaka, Japan), cefdinir, ceftizoxime, cefoselis and cefazolin (Fujisawa Pharmaceutical Co., Osaka, Japan) and cefotiam (Takeda Chemical Industries, Osaka, Japan) were gifts from the respective suppliers. All other chemicals used were of the highest purity available.

2.2. Cell culture and transfection

HEK 293 cells (American Type Culture Collection CRL-1573), a transformed cell line derived from human embryonic kidney, were cultured in complete medium consisting of Medium 199 (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (Thermo. Electron Co., Waltham, MA) (Invitrogen) in an atmosphere of 5% CO_2, 95% air at 37 °C. hOAT1 and hOAT3 cDNAs were subcloned into pBK-CMV plasmid vector (Stratagene, La Jolla, CA). HEK 293 cells were transfected with hOAT1 cDNA, hOAT3 cDNA or empty vector using LipofectAMINE 2000 (Invitrogen) according to the manufacturer's instructions. G418 (Nacalai Tesque, Kyoto, Japan) (0.5 mg/ml)—resistant cells were removed. Cells expressing hOAT1 (HEK-hOAT1) were selected by measuring *p*-[^{14}C]aminohippurate uptake, and cells expressing hOAT3 (HEK-hOAT3) were selected by measuring [^{3}H]estrone sulfate uptake. Cells transfected with empty vector (HEK-pBK) were used as control cells. These transfectants were maintained in complete medium with G418 (0.5 mg/ml).

2.3. Quantification of mRNA expression of hOATs in HEK-hOAT1 and HEK-hOAT3

Total RNA was extracted from HEK-hOAT1 or HEK-hOAT3 using an RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and reverse-transcribed using SuperScriptsTMII RT (Invitrogen, Grand Island, NY). For quantification of the amounts of hOAT1 and hOAT3 mRNA, the real-time PCR method was carried out using the ABI PRISM 7700 sequence detector (Applied Biosystems, Foster, CA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was also quantified as an internal control with GAPDH Control Reagent (Applied Biosystems).

2.4. Uptake of various compounds by HEK-hOAT1 or HEK-hOAT3

Uptake experiments were performed as described previously [15] with some modifications. HEK-hOAT1 and HEK-hOAT3 were seeded on poly-D-lysine-coated 24-well plates at a density of 2×10^5 cells/well for the uptake of *p*-[^{14}C]aminohippurate, [^{3}H]estrone sulfate, [^{14}C]captopril, [^{3}H]methotrexate, [^{3}H]ochratoxin A, [^{3}H]leucovorin, [^{3}H]cimetidine and [^{3}H]dehydroepiandrosterone sulfate. At 48 h after seeding, the uptake of these compounds by HEK-hOAT1 or HEK-hOAT3 was examined. The composition of the incubation medium was as follows (in mM): 145 NaCl, 3 KCl, 1 $CaCl_2$, 0.5 $MgCl_2$, 5 D-glucose and 5 HEPES (pH 7.4). The cells were preincubated with 0.2 ml of the incubation medium for 10 min at 37 °C. After the preincubation, the medium was replaced with 0.2 ml of incubation medium containing each anionic compound. At the end of the incubation period, the medium was aspirated, and then cells were washed two times with 1 ml of ice-cold incubation medium. The cells were lysed in 0.25 ml of 0.5N NaOH solution, and the radioactivity in aliquots was determined in 3 ml of ACSII (Amersham International, Buckingham shire, UK). The protein contents of the solubilized cells were determined by the method of Bradford [16] using the Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA) with bovine γ-globulin as a standard.

2.5. Uptake of cephalosporin antibiotics by HEK 293 cells stably expressing hOAT1 or hOAT3

For the experiments on the uptake of cephalosporin antibiotics, HEK-hOAT1 and HEK-hOAT3 were seeded

on 6-cm poly-D-lysine-coated dishes at a density of 2×10^6 cells/dish. At 48 h after seeding, the uptake of cephalosporin antibiotics was examined. The cells were preincubated with 2 ml of the incubation medium for 10 min at 37 °C. After this preincubation, the medium was replaced with 2 ml of incubation medium containing various cephalosporin antibiotics. At the end of the incubation period, the medium was aspirated, and then cells were washed three times with 5 ml of ice-cold incubation medium. To measure the accumulation of cephalosporin antibiotics, the cells were scraped and homogenized with 1 ml of water. Protein levels were determined with 5 μl of the homogenate. For the determination of cephalosporin antibiotics, to 0.9 ml of the homogenate, 100 μl of water and 20 μl of phosphoric acid were added and mixed for 30 s, then 1.0 ml of the sample was loaded onto an Oasis HLB cartridge (Waters Corporation, Milford, MA) preconditioned with 1 ml each of methanol and water. The column was washed with 1 ml of 5% methanol and cephalosporin antibiotic was eluted from the column with 1 ml of methanol. The eluate was evaporated to dry at 45–50 °C and resuspended in 200 μl of mobile phase buffer. The solution was filtered through a 0.45-μm polyvinylidene fluoride filter. The concentration of cephalosporin antibiotic was measured by use of a high performance liquid chromatograph (LC-10AT, LC-10AD, Shimadzu Co., Kyoto, Japan) equipped with a UV spectrophotometric detector (SPD-10AV, SPD-10A, Shimadzu) under the following conditions: column, Zorbax ODS column 4.6 mm inside diameter × 250 mm (Du Pont, Wilmington, DE); mobile phase, 30 mM citric acid buffer in methanol at 85:15 for cefdinir, ceftibuten, cefaclor, ceftizoxime and cefoselis, 30 mM phosphate buffer (pH 5.2) in methanol at 83:17 for cephaloridine and cefazolin, 30 mM phosphate buffer (pH 6.5) in methanol at 78:22 for cefotiam; flow rate, 0.8 ml/min; wave length, 254 nm for cephaloridine, cefotiam, ceftibuten, ceftizoxime and cefoselis, 288 nm for cefdinir, 266 nm for cefaclor, 272 nm for cefazolin; injection volume, 50 μl; temperature, 40 °C.

2.6. Statistical analysis

Data were analysed statistically using nonpaired *t*-tests.

3. Results

3.1. Construction of HEK-hOAT1 and HEK-hOAT3

In a previous study, we performed the characterization of the uptake of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate in HEK 293 cells transfected with hOAT1 and hOAT3 cDNA, respectively, using transient expression systems [14]. In this study, we constructed HEK 293 cells stably expressing hOAT1 or hOAT3. First, mRNA levels of hOAT1 and hOAT3 were investigated by real-time PCR. The mRNA level of hOAT1 in HEK-hOAT1 was quantified to be 64.9 amol/μg total RNA, and that of hOAT3 in HEK-hOAT3 was 225.6 amol/μg total RNA. The functional expression of hOAT1 and hOAT3 was assessed by the uptake of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate by HEK-hOAT1 and HEK-hOAT3, respectively. Fig. 1 shows that HEK-hOAT1 and HEK-hOAT3 exhibited time-dependent uptake of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate, respectively. As shown in Fig. 2, a concentration-dependent uptake of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate by HEK-hOAT1 and HEK-hOAT3, respectively, was observed. Using a nonlinear least squares regression analysis, kinetic parameters were calculated according to the Michaelis-Menten equation in three separate experiments. Apparent Michaelis-Menten constants (K_m) for the uptake of *p*-[^{14}C]aminohippurate by HEK-hOAT1 and of [^{3}H]estrone sulfate by HEK-hOAT3 were

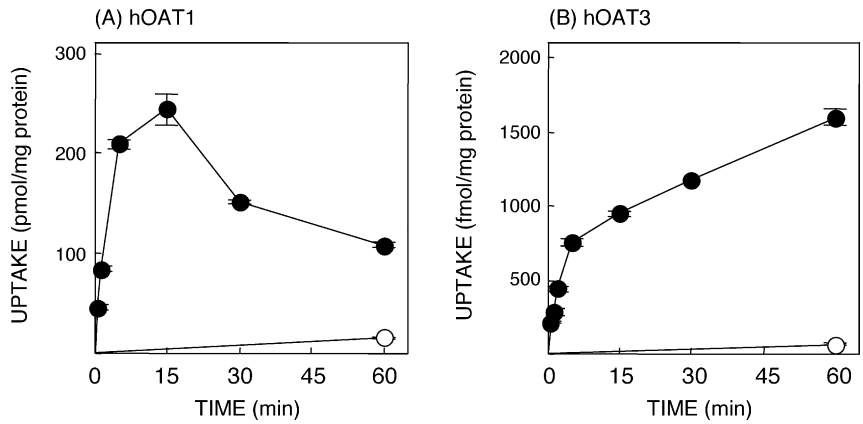

Fig. 1. Time course of *p*-[^{14}C]aminohippurate (A) and [^{3}H]estrone sulfate (B) accumulation in HEK-hOAT1 and HEK-hOAT3, respectively. (A) *p*-[^{14}C]Aminohippurate accumulation in HEK-pBK (○) or HEK-hOAT1 (●). The cells were incubated with 5 μM *p*-[^{14}C]aminohippurate for the periods indicated at 37 °C. (B) [^{3}H]Estrone sulfate accumulation in HEK-pBK (○) or HEK-hOAT3 (●). The cells were incubated with 20 nM [^{3}H]estrone sulfate for the periods indicated at 37 °C. After the incubation, the radioactivity of solubilized cells was measured. Each point represents the mean ± S.E. of three monolayers from a typical experiment.

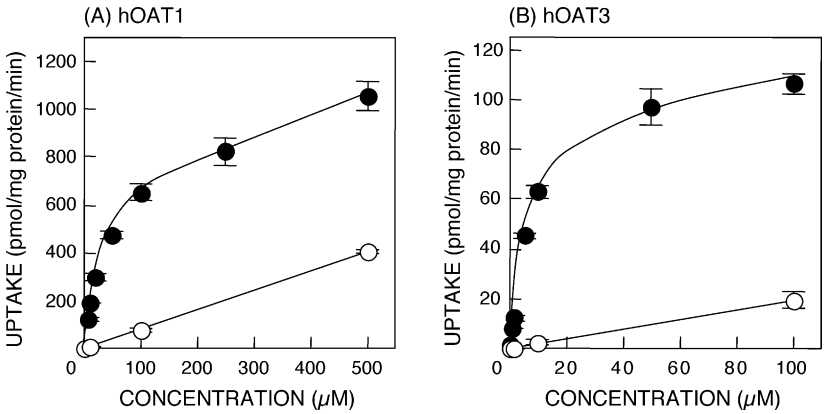

Fig. 2. Concentration dependence of *p*-[^{14}C]aminohippurate (A) and [^{3}H]estrone sulfate (B) accumulation in HEK-hOAT1 and HEK-hOAT3, respectively. HEK-hOAT1 and HEK-hOAT3 were incubated with various concentrations of *p*-[^{14}C]aminohippurate or [^{3}H]estrone sulfate in the absence (●) or presence (○) of unlabeled 5 mM *p*-aminohippurate (A) or 1 mM estrone sulfate (B) for 1 min at 37 °C. After the incubation, the radioactivity of solubilized cells was measured. Each point represents the mean ± S.E. of three monolayers from a typical experiment.

28.0 ± 1.9 and 6.3 ± 2.2 μM (n = 3, mean ± S.E.), respectively, which were consistent with previous reports [17–19]. Maximal uptake rate (V_{max}) values for HEK-hOAT1 and HEK-hOAT3 were 553.2 ± 70.9 and 102.7 ± 19.7 pmol/mg protein/min (n = 3, mean ± S.E.), S.E.), respectively.

In addition, we measured the uptake of various compounds by these transfectants. Results of these uptake experiments are shown in Fig. 3. A remarkable increase in the uptake of *p*-[^{14}C]aminohippurate, [^{14}C]captopril, [^{3}H]ochratoxin A and [^{3}H]leucovorin, and slight increase (within two-fold) in the uptake of [^{3}H]estrone sulfate, [^{3}H]methotrexate and [^{3}H]cimetidine were observed in HEK-hOAT1 compared to control cells. A remarkable increase in the uptake of *p*-[^{14}C]aminohippurate, [^{3}H]estrone sulfate, [^{14}C]captopril, [^{3}H]methotrexate, [^{3}H]ochratoxin A, [^{3}H]leucovorin, [^{3}H]cimetidine and [^{3}H]dehydroepiandrosterone sulfate was observed in HEK-hOAT3 compared to control cells.

3.2. Inhibitory effects of cephalosporin antibiotics on hOAT1 and hOAT3

To determine the affinity of cephalosporins for hOAT1 and hOAT3, we examined the inhibitory effects of these drugs on the uptake of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate by HEK-hOAT1 and HEK-hOAT3, respectively. As shown in Fig. 4, cephalosporin anitibiotics inhibited the uptake of organic anions by HEK-hOAT1 and HEK-hOAT3 in a dose-dependent manner. The IC_{50} values were estimated by nonlinear regression analysis of the competition curves with a one-compartment model with the following equation: $V = 100 \times IC_{50}/(IC_{50} + [I]) + A$, where V the uptake amount (% of control), [I] the concentration of cephalosporin antibiotic and A is the non-specific organic anion uptake (% of control). The findings are summarized in Table 1.

3.3. Characterization of the uptake of cephalosporin antibiotics by hOAT1 and hOAT3

To investigate whether hOAT1 and hOAT3 transport cephalosporin antibiotics, we measured the accumulations of these drugs in HEK-hOAT1 and HEK-hOAT3. As shown in Fig. 5, the uptake of cephaloridine, cefdinir and cefotiam by HEK-hOAT3 was 35–50-fold higher than that by control cells. Moreover, the accumulation of ceftibuten, cefaclor, ceftizoxime, cefoselis and cefazolin was significantly greater in HEK-hOAT3 than in control cells. Those cephalosporin antibiotics whose accumulation was significantly greater in HEK-hOAT1 than control cells were cephaloridine, cefdinir, ceftibuten and ceftizoxime. The uptake of these antibiotics by HEK-hOAT1 was within two-fold of that by control cells.

Fig. 6 shows the time course of the uptake of cephaloridine, cefotiam or cefazolin by HEK-hOAT1 or HEK-hOAT3. The accumulation of cephaloridine, cefotiam and cefazolin in HEK-hOAT3 increased markedly in a time-dependent manner. The accumulation of these antibiotics in HEK-hOAT1 was comparable to that in control cells.

Table 1
The IC_{50} values of various cephalosporin antibiotics for the uptake of *p*-aminohippurate and estrone sulfate by hOAT1 and hOAT3, respectively

Cephalosporin	IC_{50} (μM)	
	hOAT1	hOAT3
Cephaloridine	2470.0 ± 339.3	626.4 ± 66.7
Cefdinir	691.8 ± 222.9	271.5 ± 46.5
Cefotiam	639.7 ± 63.0	212.6 ± 26.9
Ceftibuten	563.1 ± 50.1	247.3 ± 74.0
Cefaclor	1095.6 ± 95.9	120.2 ± 7.2
Ceftizoxime	3598.6 ± 368.6	956.7 ± 29.7
Cefoselis	2600.5 ± 439.3	2925.1 ± 27.1
Cefazolin[a]	100.6 ± 25.3	116.6 ± 13.0

The values represent the means ± S.E. of three separate experiments.
[a] Values are from Ref. [14].

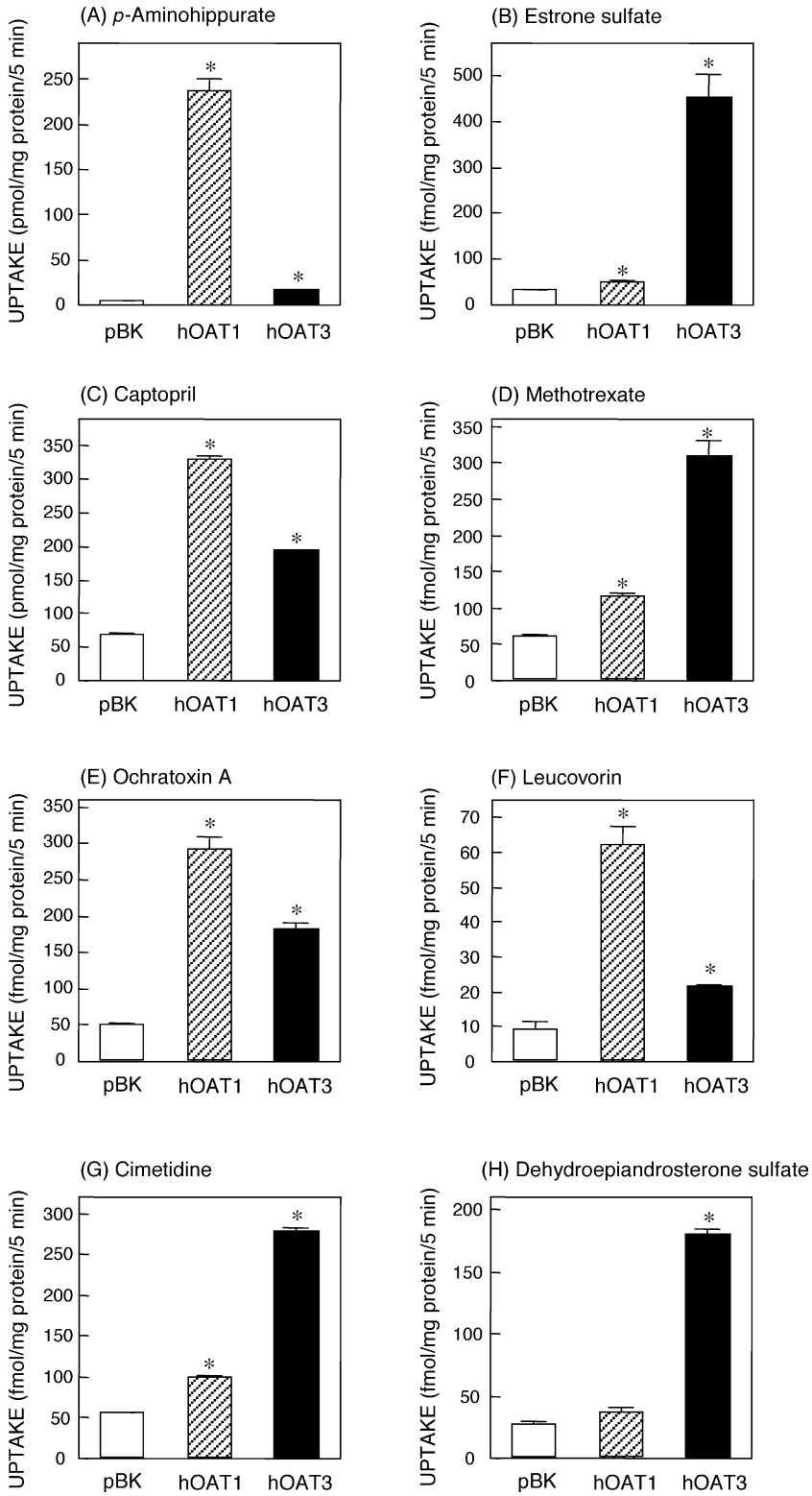

Fig. 3. Uptake of various anionic compounds by HEK-hOAT1 or HEK-hOAT3. HEK-pBK, HEK-hOAT1 and HEK-hOAT3 were incubated with 5 μM *p*-[^{14}C]aminohippurate (A), 20 nM [^{3}H]estrone sulfate (B), 160 μM [^{14}C]captopril (C), 40 nM [^{3}H]methotrexate (D), 20 nM [^{3}H]ochratoxin A (E), 40 nM [^{3}H]leucovorin (F), 80 nM [^{3}H]cimetidine (G) or 20 nM [^{3}H]dehydroepiandrosterone sulfate (H) for 5 min at 37 °C. After the incubation, the radioactivity of solubilized cells was measured. Each column represents the mean ± S.E. of three monolayers from a typical experiment. $^{*}p < 0.05$, significant differences from control.

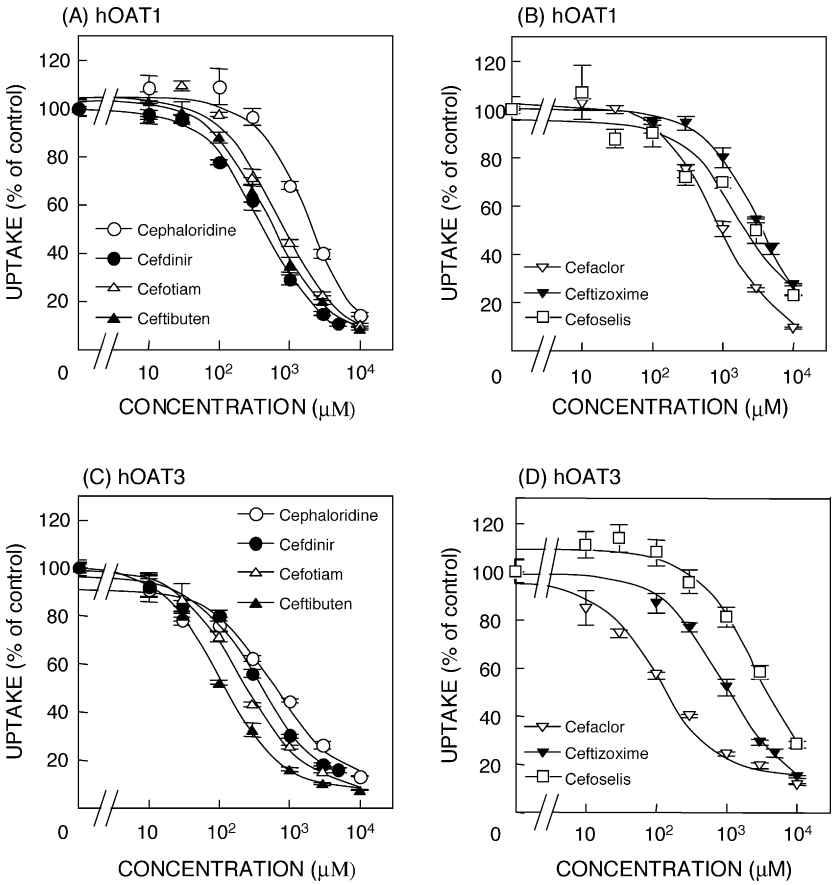

Fig. 4. Effects of cephalosporin antibiotics on the uptake of *p*-[^{14}C]aminohippurate (A and B) and [^{3}H]estrone sulfate (C and D) by HEK-hOAT1 and HEK-hOAT3, repectively. HEK-hOAT1 and HEK-hOAT3 was incubated with 5 μM *p*-[^{14}C]aminohippurate (A and B) and 20 nM [^{3}H]estrone sulfate (C and D), respectively, for 1 min at 37 °C in the presence of various concentrations of cephaloridine (○), cefdinir (●), cefotiam (△), ceftibuten (▲), cefaclor (▽), ceftizoxime (▼) or cefoselis (□). After the incubation, the radioactivity of solubilized cells was measured. Each point represents the mean ± S.E. of three monolayers from a typical experiment.

4. Discussion

Renal organic anion transporters, hOAT1 and hOAT3, mediate the basolateral uptake of various drugs in proximal tubules. In our previous studies, Uwai et al. [12] reported that rOAT1 transported cephalosporin antibiotics, cefazolin, cefotiam and cefalexin, and Sakurai et al. [14] reported that hOAT3 plays an important role in the renal secretion of cefazolin in patients with renal diseases. The present study examined the transport of various cephalosporin antibiotics via hOAT1 and hOAT3. In this study, for all cephalosporin antibiotics tested, particularly, cephaloridine, cefdinir and cefotiam, the uptake by HEK-hOAT3 was greater than that by HEK-hOAT1. The mRNA level of hOAT3 in HEK-hOAT3 was about three-fold higher than that of hOAT1 in HEK-hOAT1, and we have previously reported that the level of hOAT3 mRNA is about three-fold higher than that of hOAT1 mRNA in human kidney [7]. Therefore, we suggest that the uptake by HEK-hOAT1 and HEK-hOAT3 reflects to some extent the basolateral uptake via hOAT1 and hOAT3 in renal epithelial cells. The present results showed that hOAT3 plays a major role in the basolateral uptake of various cephalosporin antibiotics, as well as cefazolin [14], into epithelial cells from the blood.

We examined the transport of various compounds via hOAT1 and hOAT3. It has been suggested that hOAT3 can mediate the transport of organic anions with bulky side groups, compared with hOAT1 [18], and in the current study, the remarkable increase in the uptake of [^{3}H]estrone sulfate and [^{3}H]dehydroepiandrosterone sulfate was observed in HEK-hOAT3 compared to control cells, while little or no increase was observed in HEK-hOAT1. Nevertheless, the difference in substrate specificity between hOAT1 and hOAT3 remains to be properly elucidated. We previously reported that hOAT1 mRNA levels are significantly lower in the kidneys of patients with renal diseases than in the normal kidney cortex, whereas hOAT3 mRNA levels are not significantly reduced, and suggested that each transporter undergoes a different effect in the impaired kidney [14]. By combining information about the alteration in the expression level and substrate specificity of a transporter, we could at least in part contribute to establishment of the administration schedule in cases of renal disease.

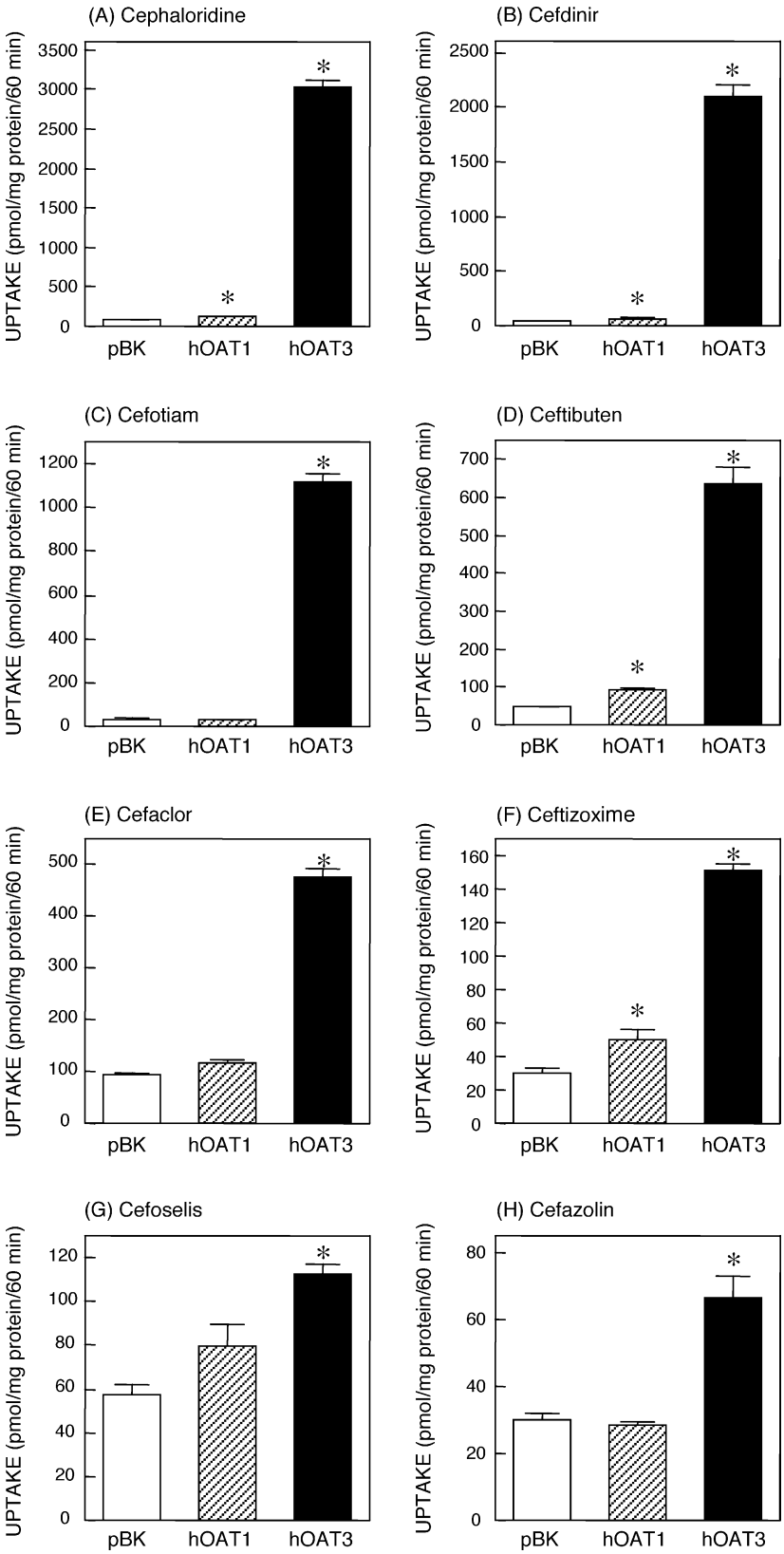

Fig. 5. Uptake of cephalosporin antibiotics by HEK-hOAT1 and HEK-hOAT3. HEK-pBK, HEK-hOAT1 and HEK-hOAT3 were incubated for 60 min at 37 °C with 500 μM cephaloridine (A), cefdinir (B), cefotiam (C), ceftibuten (D), cefaclor (E), ceftizoxime (F), cefoselis (G) or cefazolin (H). After the incubation, the accumulation of these antibiotics in the cells was measured by use of a high performance liquid chromatograph. Each column represents the mean ± S.E. of three monolayers from a typical experiment. $^{*}p < 0.05$, significant differences from control.

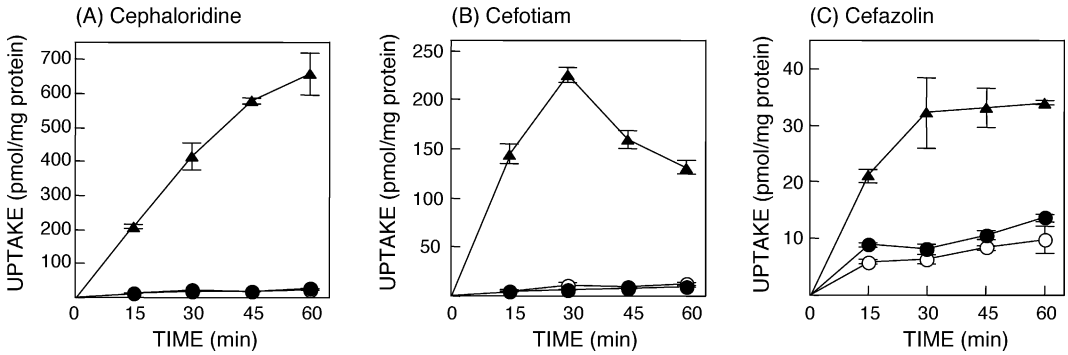

Fig. 6. Time course of cephaloridine (A), cefotiam (B) and cefazolin (C) accumulation in HEK-hOAT1 and HEK-hOAT3. HEK-pBK (○), HEK-hOAT1 (●) and HEK-hOAT3 (▲) were incubated for the indicated periods at 37 °C with 100 μM cephaloridine (A), 200 μM cefotiam (B) or 200 μM cefazolin (C). After the incubation, the accumulation of these antibiotics in the cells was measured by use of a high performance liquid chromatograph. Each point represents the mean ± S.E. of three monolayers from a typical experiment.

Uwai et al. [20] reported that probenecid markedly inhibited the transport of *p*-aminohippurate via rOAT1, although was not transported via rOAT1. Zhang et al. [21] showed that HIV protease inhibitors including indinavir, nelfinavir, ritonavir and saquinavir are potent inhibitors of hOCT1; however, they are poor substrates for hOCT1-mediated transport. In the current study, the IC_{50} values of cephaloridine, cefdinir and cefotiam for the hOAT1-mediated uptake of *p*-[^{14}C]aminohippurate were within four-fold of those for the hOAT3-mediated uptake of [^{3}H]estrone sulfate. On the other hand, the uptake of these antibiotics by HEK-hOAT3 (35–50-fold higher than that by control cells) was remarkably greater than that by HEK-hOAT1 (within 1.6-fold of that by control cells). In addition, although the IC_{50} value of cephaloridine was six-fold higher than that of cefazolin for hOAT3-mediated organic anion uptake, cephaloridine uptake was 46-fold higher than cefazolin uptake in HEK-hOAT3. The results suggested that these marked differences in transport activity are due to the efficacy of the translocation process. These previous findings and the current study have indicated that it is necessary to investigate not only substrate affinity but also substrate transport when characterizing the substrate specificity of a transporter.

Cephaloridine produces acute renal failure in humans and animals [22]. In the kidney, cephaloridine is rapidly transported into the epithelial cells, but undergoes minimal subsequent movement into the lumen [23], and the accumulation of drug in the tubular cells attributable to this process plays a role in the mechanism of renal failure [24]. It has been reported that rOAT1 and rOAT3 are, at least in part, responsible for the basolateral uptake of cephaloridine and therefore cephaloridine-induced renal failure [25,26]. In a previous study, the K_i values of cephaloridine for the rOAT1 and rOAT3-mediated uptake of organic anions were similar (1.32 mM for rOAT1 and 1.14 mM for rOAT3) and it was suggested that both rOAT1 and rOAT3 participate in the transport of cephaloridine and in cephaloridine-induced renal failure, although the uptake of cephaloridine by these transporters was not compared [26]. In the current study, the uptake of cephaloridine by HEK-hOAT3 was remarkably higher than that by HEK-hOAT1, demonstrating that hOAT3 plays a more important role in cephaloridine-induced renal failure than hOAT1.

Lu et al. [27] reported that the *p*-aminohippurate transporter PAHT (synonym: hOAT1) [28], displayed an overshoot characterized by a time-dependent, saturable accumulation of substrate, followed by a gradual return to the baseline. In the current study, the uptake of *p*-[^{14}C]aminohippurate by HEK-hOAT1 also displayed this phenomenon (Fig. 1). OAT1 has been known as an organic anion/dicarboxylate exchanger [29], and it has been suggested that the overshoot is consistent with exchange-mediated secondary active transport in which an outwardly directed gradient for a cytosolic exchange partner (likely α-ketoglutarate) is depleted during the uptake experiment because of an abundance of the external exchange partner [27]. Recently, it was revealed that OAT3 is also an anion/dicarboxylate exchanger [30], and in the current study, the uptake of cefotiam by HEK-hOAT3 also displayed an overshoot. This is the first example of this phenomenon in hOAT3-mediated uptake after hOAT3 was recognized as an exchanger. In the uptake of cephaloridine by HEK-hOAT3, no overshoot was observed, although the uptake rate of cephaloridine was higher than that of cefotiam (2.08 μl/mg protein / 15 min versus 0.72 μl/mg protein/15 min). Tune and Hsu [31] reported that cephaloridine reduces mitochondrial carnitine transport through competitive inhibition, and the zwitterionic region of this drug, like carnitine, is responsible for this ability. Given their study, it is suggested that cephaloridine was accumulated in mitochondria following its transport into the cytoplasm. Cytoplasmic concentration of cephaloridine may be low because intracellular cephaloridine was mainly in mitochondria. Therefore, the efflux of the antibiotic via hOAT3 was not occurred. We assume that this phenomenon also occurs in the proximal tubular cells, and participates in cephaloridine-induced renal failure.

We previously speculated that hOAT3 should play an important role in the secretion of cefazolin in patients with

renal diseases [14]. The current study demonstrated that other cephalosporin antibiotics are also secreted mainly via hOAT3. The uptake of cefoselis by HEK-hOAT3 was lower than the uptake of the other antibiotics, except for cefazolin, by HEK-hOAT3, and Sakamoto et al. [32] showed that cefoselis is mainly excreted by glomerular filtration. Accordingly, it should be that transport via hOAT3 is important for secretion in renal epithelial cells. It was reported that tubular secretion accounted for 50–80% of all the cefazolin excreted in patients with a glomerular filtration rate above 25 ml/min [33]. However, in the current study, the uptake of cefazolin by HEK-hOAT3 was lower than the uptake of the other antibiotics by HEK-hOAT3. The IC_{50} value of cefazolin for hOAT3 is remarkably lower than that of cefoselis for hOAT3 (116.6 μM versus 2925.1 μM). The protein-binding ratio of cefazolin (87%) is remarkably higher than that of cefoselis (8.8%) [32,33]. To elucidate the renal handling of drugs, it may be necessary to take such factors into consideration. We propose that information on transport via hOAT3 is, at least in part, useful for determining whether drugs can be secreted or not in renal epithelial cells.

In conclusion, hOAT3 plays a more important role than hOAT1 in the renal secretion of cephalosporin antibiotics. Furthermore, our findings provide useful information about the difference in substrate specificity between hOAT1 and hOAT3, and will contribute to further investigation of the renal handling of various drugs.

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