much lower than those by OAT1 (Kusuhara et al., 1999; Hasegawa et al., 2003). Consistent with these transport activities, kinetic analyses using rat kidney slices suggest that OAT1 plays a major role in the renal uptake of hydrophilic organic anions with a small molecular weight, such as *p*-aminohippurate (PAH), 2,4-dichlorophenoxyacetate (2,4-D), and uremic toxins (hippurate and indole acetate), whereas OAT3 mainly accounts for the uptake of benzylpenicillin (PCG), dehydroepiandrosterone sulfate (DHEAS), uremic toxins (indoxyl sulfate and 3-carboxy-4-methyl-5-propyl-2-furanopropionate), and, in part, that of estrone sulfate (ES), but makes only a limited contribution to the uptake of hydrophilic and small organic anions (Hasegawa et al., 2002, 2003; Sweet et al., 2002; Deguchi et al., 2004; Eraly et al., 2006).

The functional importance of human OATs in drug disposition has been extensively analyzed by in vitro studies using cDNA transfectant. There is a poor correlation in the transport activities of OAT3 between rats and humans, whereas there is a good correlation for OAT1 (Deguchi et al., 2004; Tahara et al., 2005b). Therefore, the renal uptake involving OAT3 may exhibit species difference. Indeed, the effect of probenecid on the renal clearance of famotidine is species-dependent. Probenecid markedly inhibits the secretion clearance of famotidine in humans (Inotsume et al., 1990), whereas it has no effect on the renal clearance in rats, even though the plasma concentration of probenecid is similar or rather higher than that in clinical studies (Lin et al., 1988). This is accounted for partly by the species difference in the transport activity of famotidine by rat and human OAT3 (r/hOAT3), greater for hOAT3 than rOat3, and partly by the rodent-specific expression of organic cation transporter 1 (Tahara et al., 2005a). These studies prompted us to establish an in vitro experimental system to evaluate the contribution of hOAT1 and hOAT3 using human kidney.

Kidney slices have been widely used to characterize renal uptake. The extracellular marker compounds, such as methoxy-inulin and sucrose, were below the limit of detection in the luminal space of the proximal tubules, whereas they could be detected in the extracellular space (Wedeen and Weiner, 1973). Therefore, the kidney slices allow limited access of drugs from the luminal space in the kidney slices but free access from the basolateral side, thereby allowing evaluation of basolateral uptake. In vitro studies of the uptake of drugs by mammalian kidney slices have proved useful for examining uptake mechanisms. Fleck et al. (2000, 2002) prepared kidney slices from human kidney and demonstrated the active accumulation of PAH and methotrexate, suggesting that human kidney slices also retain the activities of organic anion transporters. However, transport studies using human kidney tissue have not been thoroughly investigated focusing on the contribution of different transporters. The purpose of the present study is to compare the uptake of OAT1 and OAT3 substrate drugs by human kidney slices and to establish inhibitors to evaluate the contribution of OAT1 and OAT3.

Materials and Methods

Materials. [^{3}H]PAH (4.1 Ci/mmol), [^{3}H]DHEAS (60 Ci/mmol), and [^{3}H]ES (43.1 Ci/mmol) were purchased from PerkinElmer Life Science (Boston, MA). [^{14}C]PCG (59 mCi/mmol) and [^{3}H]2,4-D (20 Ci/mmol) were purchased from GE Healthcare Bio-Sciences (Waukesha, WI) and American Radiolabeled Chemicals (St. Louis, MO), respectively. Unlabeled PAH, DHEAS, ES, and 2,4-D were purchased from Sigma-Aldrich (St. Louis, MO), and unlabeled PCG and α-ketoglutarate (KG) were from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of analytical grade and commercially available.

Preparation of Human Kidney Slices and Uptake of Organic Anions by Human Kidney Slices. This study protocol was approved by the Ethics Review Boards at both the Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan and Tokyo Women's Medical University, Tokyo, Japan. All participants provided written informed consent.

Intact renal cortical tissues were obtained from 42 surgically nephrectomized patients with renal cell carcinoma at Tokyo Women's Medical University between October, 2003 and September, 2005. Samples of human kidney from subjects were stored in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) on ice immediately after kidney removal. After 30-min transportation, kidney slices were prepared as described below.

Uptake studies by human kidney slices were carried out following previous reports (Hasegawa et al., 2002, 2003). Kidney slices (300 μm thick) from intact human cortical tissue were kept in ice-cold buffer before use. The buffer for the present study consists of 120 mM NaCl, 16.2 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 10 mM NaH_2PO_4/Na_2HPO_4 adjusted to pH 7.5. One slice, weighing 3 to 10 mg, was selected and incubated at 37°C on a 12-well plate with 1 ml of oxygenated buffer in each well after preincubation of slices for 5 min at 37°C. After incubating for 15 min, slices were rapidly removed from the incubation buffer, washed twice in ice-cold buffer, blotted on filter paper, weighed, and dissolved in 1 ml of Soluene-350 (Packard Instruments, Downers Grove, IL) at 50°C for 12 h. The radioactivity in the specimens was determined in scintillation cocktail (Hionic Flour; Packard Instruments).

Quantification of mRNA of hOAT1 and hOAT3 in Human Renal Cortical Tissue. Total RNA was isolated from intact cortical tissue of human kidney using ISOGEN (NIPPON GENE, Tokyo, Japan) according to manufacturer's protocol, followed by DNase treatment (TaKaRa, Shiga, Japan). Total RNA was converted to cDNA using random 9-mers and avian myeloblastosis virus reverse transcriptase. Real-time quantitative PCR was performed using SYBR Green (TaKaRa) and a LightCycler system (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Nucleotide sequences of the primers for hOAT1 and hOAT3 and GAPDH are: hOAT1, forward, 5′-GGCACCTTGATTGGCTATGT-3′; hOAT1, reverse, 5′-AAAAGGCGCAGAGACCAGTA-3′; hOAT3, forward, 5′-GTCCATACGCTGGTGGTCTT-3′; hOAT3, reverse, 5′-GCTGAGCCTTTCTCCCTCTT-3′; GAPDH, forward, 5′-GAAGGTGAAGGTCGGAGTC-3′; and GAPDH, reverse, 5′-GAAGATGGTGATGGGATTTC-3′.

GAPDH was used as a housekeeping gene for the internal standards. An external standard curve was generated by dilution of the target PCR fragment, which was purified by agarose gel electrophoresis. The absolute concentration of external standard was measured by PicoGreen dsDNA Quantitation Reagent (Molecular Probes, Eugene, OR). Expression of hOAT1 and hOAT3 were normalized by the expression of GAPDH.

Transport Studies in hOAT1- and hOAT3-Transfected Cells. hOAT1- and hOAT3-transfected HEK293 cells were established as described previously (Deguchi et al., 2004). HEK293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin at 37°C with 5% CO_2 and 95% humidity. HEK293 cells were seeded on 12-well plates at a density of 1.2×10^5 cells/well. Cells were cultured for 48 h with the above-mentioned medium and for an additional 24 h with culture medium supplemented with 5 mM sodium butyrate before starting the transport studies.

Transport studies were carried out as described previously (Sug-

iyama et al., 2001). Uptake was initiated by adding the medium containing the radiolabeled compounds in the presence or absence of inhibitors after cells had been washed twice and preincubated with Krebs-Henseleit buffer (118 mM NaCl, 23.8 mM $NaHCO_3$, 4.83 mM KCl, 0.96 mM KH_2PO_4, 1.20 mM $MgSO_4$, 12.5 mM HEPES, 5 mM glucose, and 1.53 mM $CaCl_2$, pH 7.4). The uptake was terminated at designated times by aspirating the incubation buffer and adding ice-cold Krebs-Henseleit buffer. Cells were washed twice with ice-cold buffer and dissolved in 500 μl of 0.2 N NaOH. The aliquots neutralized with 2 N HCl were transferred to scintillation vials containing 2 ml of scintillation cocktail (Clearsol I; Nacalai Tesque Inc., Kyoto, Japan), and the radioactivity associated with the specimens was determined in a liquid scintillation counter. The remaining 50-μl aliquots of cell lysate were used to determine the protein concentration by the method of Lowry with bovine serum albumin as a standard.

Kinetic Analyses. Kinetic parameters were obtained using the following Michaelis-Menten equation: one saturable component,

$$v = \frac{V_{max} \times S}{K_m + S} \quad (1)$$

one saturable and one nonsaturable component,

$$v = \frac{V_{max} \times S}{K_m + S} + P_{dif} \times S \quad (2)$$

and two saturable components,

$$v = \frac{V_{max.1} \times S}{K_{m.1} + S} \times \frac{V_{max.2} \times S}{K_{m.2} \times S} \quad (3)$$

where v is the uptake velocity of the substrate (nanomoles per gram kidney per 15 min or picomoles per milligram of protein per minute), S is the substrate concentration of the medium (micromolar), K_m is the Michaelis constant (micromolar), V_{max} is the maximal uptake velocity (nanomoles per gram kidney per 15 min or picomoles per milligram of protein per minute), and P_{dif} is the nonsaturable uptake clearance. Fitting was performed by the nonlinear least-squares method using the MULTI program (Yamaoka et al., 1981). The input data were weighed as the reciprocals of the observed values, and the Damping Gauss Newton Method algorithm was used for fitting.

Results

Quantification of hOAT1 and hOAT3 mRNA Expression in Human Kidney Cortex. Figure 1A shows the relative mRNA expression levels of hOAT1 and hOAT3 in 42 human kidney cortical tissues. The expression levels of hOAT1 and hOAT3 mRNA, normalized by GAPDH, showed very large deviations: from 0.147 ($\times 10^{-2}$) to 31.1 ($\times 10^{-2}$) for hOAT1 and from 0.051 ($\times 10^{-2}$) to 40.6 ($\times 10^{-2}$) for hOAT3. The average of the relative expression of hOAT1 and hOAT3 was 8.97 ± 1.27 ($\times 10^{-2}$) and 15.0 ± 1.4 ($\times 10^{-2}$), respectively. A linear correlation between the relative expression of hOAT1 and hOAT3 was observed (Fig. 1B, $R^2 = 0.814$, $p < 0.001$).

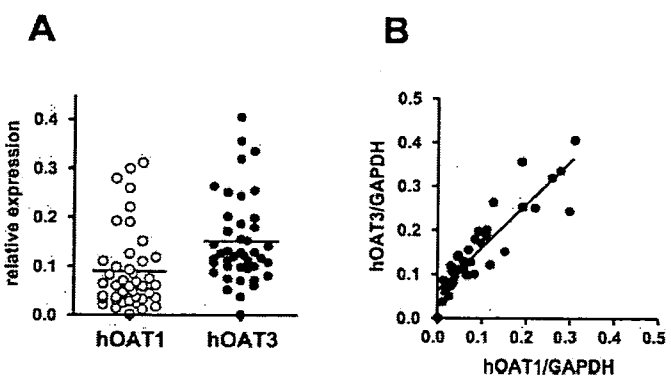

Fig. 1. Expression levels of hOAT1 and hOAT3 mRNA in human renal cortical tissues. A, relative expression levels of hOAT1 and hOAT3 mRNA in human kidney cortical tissues were measured by real-time PCR. Expression levels of hOAT1 and hOAT3 were normalized by that of GAPDH. B, correlation between the relative expression levels of hOAT1 and hOAT3 mRNA in human kidney cortical tissues. Solid line, linear regression line. Each point represents the results of one human kidney subject.

Uptake of [^{3}H]DHEAS by hOAT1- and hOAT3-Transfected HEK293 Cells. The uptake of [^{3}H]DHEAS by hOAT3-transfected cells was significantly greater than that by vector-transfected cells, which was saturable with K_m (micromolar) and V_{max} (picomoles per milligram of protein per minute) values of 12.9 ± 2.0 and 249 ± 29, respectively. The uptake of [^{3}H]DHEAS by hOAT1-transfected cells was similar to that by vector-transfected cells, whereas the uptake of [^{3}H]PAH by hOAT1- and vector-transfected cells was 38.8 ± 1.4 and 1.39 ± 0.17 μl/mg protein/min, respectively.

Interbatch Differences in the Uptake of PAH, 2,4-D, PCG, and DHEAS by Human Kidney Slices. The uptake of typical hOAT1 substrates (PAH and 2,4-D) and hOAT3 substrates (PCG and DHEAS) was examined in human kidney slices prepared from 42 subjects, and the saturable transport activities of PAH, 2,4-D, PCG, and DHEAS in human kidney slices are shown in Fig. 2. There were very large interbatch differences in their transport activities. The saturable uptake of PAH was also compared with that of PCG in each human kidney batch, and a linear correlation between PAH and PCG was observed ($R^2 = 0.715$, $p < 0.001$) (Fig. 3).

Transport Properties of the Uptake of PAH, 2,4-D, PCG, DHEAS, and ES by Human Kidney Slices. The uptake of PAH, 2,4-D, PCG, and DHEAS by human kidney slices was examined in the presence of KG in external buffer. Uptake of PAH was slightly stimulated in the presence of 10 to 30 μM KG (Fig. 4A) followed by an inhibition at greater extracellular KG concentration. KG slightly stimulated DHEAS uptake at 300 to 1000 μM, although the effect depended on the subjects (Fig. 4D). The uptake of 2,4-D and PCG was not stimulated by extracellular KG but rather inhibited at high KG concentrations. The concentration dependence of the uptake of PAH, 2,4-D, PCG, DHEAS, and ES by human kidney slices was examined (Fig. 5). The uptake of PAH, 2,4-D, PCG, and DHEAS was determined using three different human kidney batches and that of ES was determined using two different batches. Nonlinear regression analysis showed that the uptake of PAH, 2,4-D, PCG, and ES by human kidney slices consists of one saturable and one nonsaturable component, whereas that of DHEAS consists of two saturable components. The kinetic parameters are summarized in Table 1.

Effect of PAH and PCG on the Uptake of PAH, 2,4-D, PCG, DHEAS, and ES by Human Kidney Slices. The inhibitory effect of unlabeled PAH and PCG on the uptake of [^{3}H]PAH, [^{3}H]2,4-D, [^{14}C]PCG, and [^{3}H]DHEAS by human kidney slices was examined (Fig. 6). The uptake of [^{3}H]PAH and [^{3}H]2,4-D was more potently inhibited by unlabeled PAH than PCG (Fig. 6, A and B) and vice versa for the uptake of [^{14}C]PCG (Fig. 6, C and D). Although PCG was also a more potent inhibitor than PAH for the uptake of [^{3}H]DHEAS, the

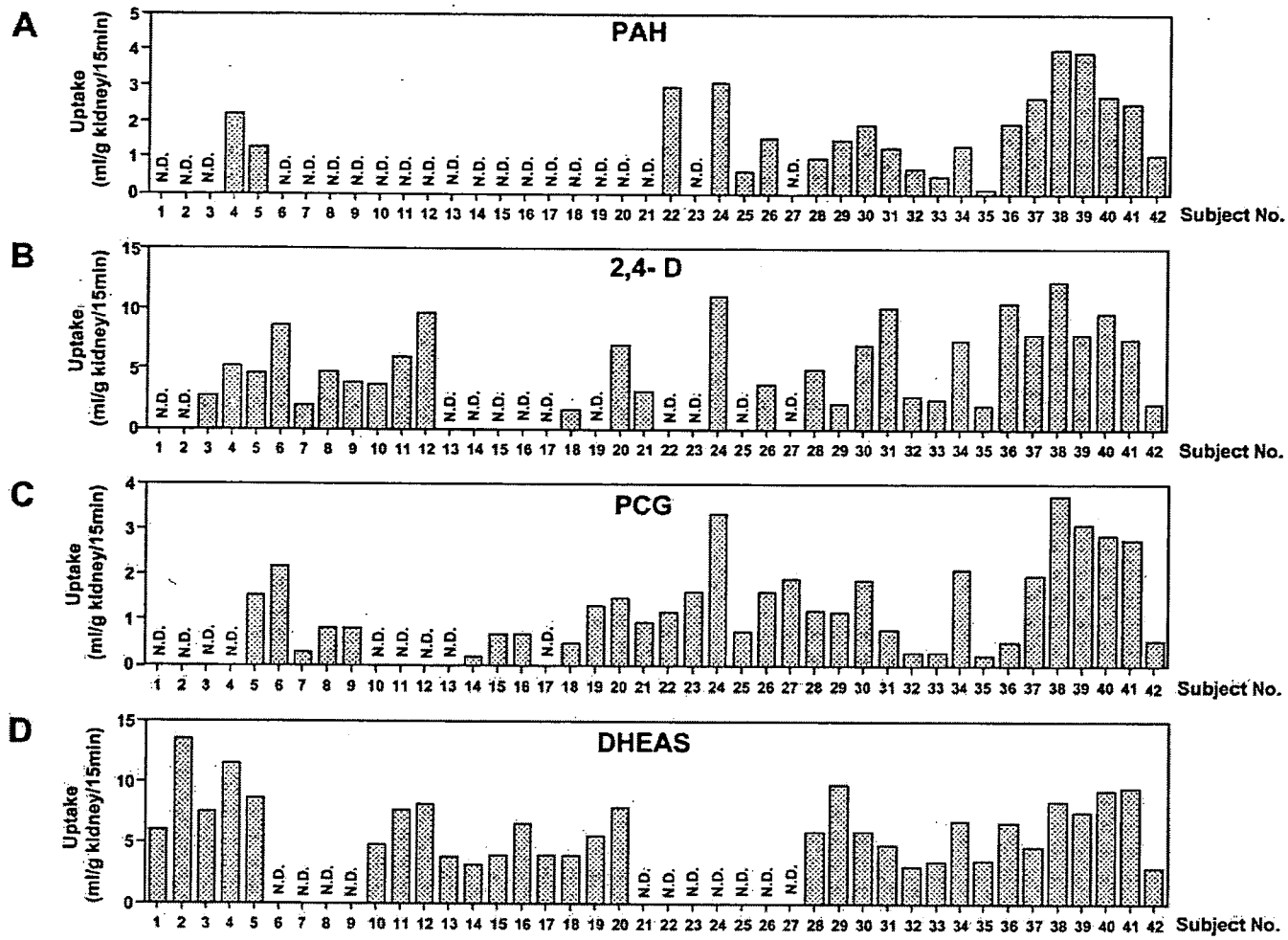

Fig. 2. Interbatch variability in the uptake of PAH, 2,4-D, PCG, and DHEAS by human kidney slices. Human kidney slices were prepared from intact renal cortical tissues donated from 42 nephrectomized patients with renal cell carcinoma, and the uptake of 0.1 μM [^{3}H]PAH (A), 0.1 μM [^{3}H]2,4-D (B), 1 μM [^{14}C]PCG (C), and 0.1 μM [^{3}H]DHEAS (D) was measured for 15 min at 37°C. The *y*-axis represents the saturable uptake clearance (milliliters per gram kidney per 15 min), which was obtained by subtracting the uptake clearance in the presence of excess amount of nonlabeled compounds (1 mM) from that under tracer conditions. All the data represent the mean (n = 2 slices). N.D., not determined due to a lack of sufficient tissue.

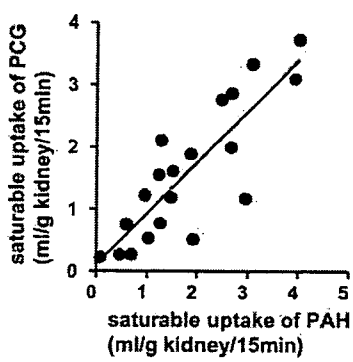

Fig. 3. Correlation of saturable uptake of PAH and PCG by human kidney slices The uptake of [^{3}H]PAH (0.1 μM) and [^{14}C]PCG (1 μM) was measured for 15 min at 37°C. The saturable uptake clearance (milliliters per gram kidney per 15 min) was obtained by subtracting the uptake clearance in the presence of 1000 μM substrate concentrations from that under tracer conditions. This plot was taken from the results of subjects 5, 22, 24 to 26, and 28 to 42 (see Fig. 2). Each point represents the results of one human kidney subject.

inhibition curves of [^{3}H]DHEAS uptake by PCG and PAH were shifted to the right in comparison with that of PCG (Fig. 6, C and D).

Discussion

Human-derived materials, such as cryopreserved hepatocytes, canalicular membrane vesicles, and microsomes, have been widely used in drug development to predict the pharmacokinetic properties of potential drug candidates (metabolism and membrane transport), and they also serve as essential tools to evaluate drug-drug interactions in addition to in vitro-in vivo scaling. Kidney slices from experimental animals have been widely used to characterize the renal uptake of organic anions through the basolateral membrane. Fleck et al. (2000) demonstrated that human kidney slices retain organic anion transport activity. The present study focused on the use of human kidney slices to characterize the uptake of hOAT1 and hOAT3 substrates. For that purpose, PAH, 2,4-D, PCG, DHEAS, and ES were selected for probing hOAT1 and hOAT3 activities. PAH and 2,4-D are common substrates of hOAT1 and hOAT3, but the transport activities by hOAT1 were markedly greater than those by hOAT3 (Tahara et al., 2005b), and PCG, DHEAS, and ES are specific substrates of hOAT3 (Tahara et al., 2005b; this study).

The mRNA expression of hOAT3 in human kidney slices was 1.7-fold higher than that of hOAT1 on average, which is

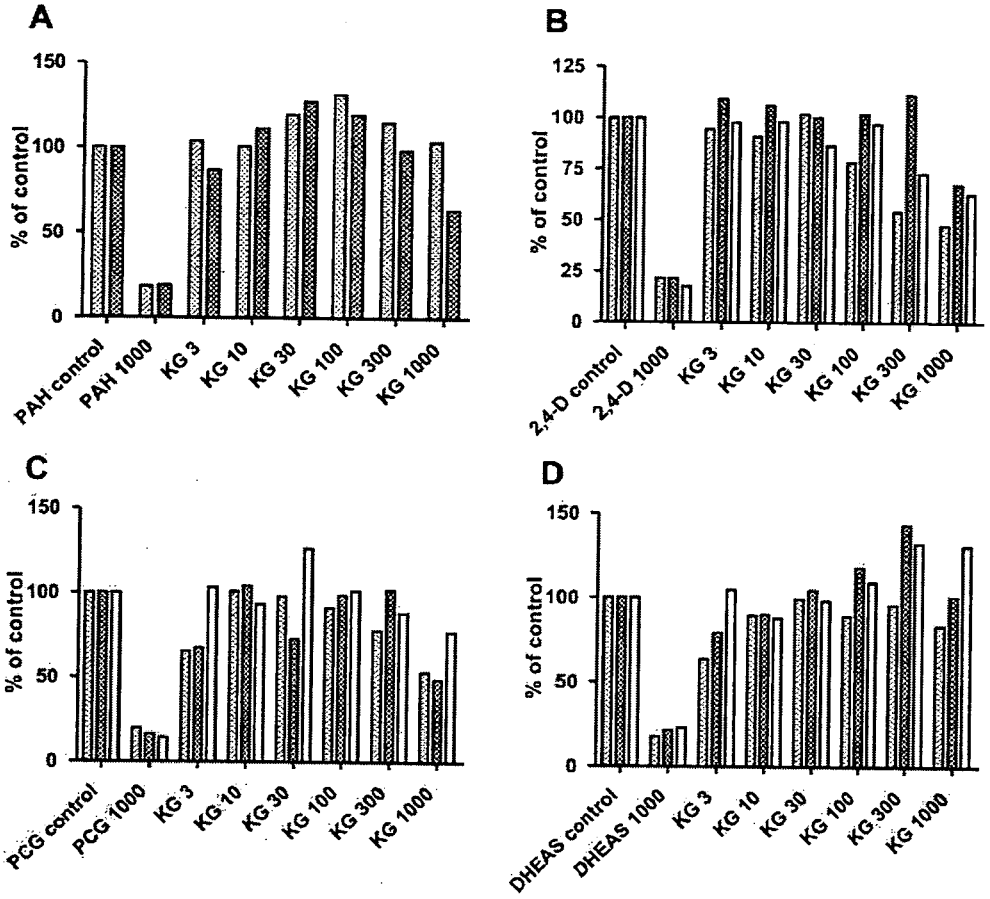

Fig. 4. Effect of α-ketoglutarate on the uptake of PAH, 2,4-D, PCG, and DHEAS by human kidney slices. The uptake of 0.1 μM [^{3}H]PAH (A), 0.1 μM [^{3}H]2,4-D (B), 1 μM [^{14}C]PCG (C), and 0.1 μM [^{3}H]DHEAS (D) was measured for 15 min at 37°C in the presence or absence of α-ketoglutarate (0–1 mM). All experiments were repeated in two or three subjects (two slices per subject), and the uptake of PAH was determined in subjects 22 and 24, that of 2,4-D in subjects 6, 11, and 12, that of PCG in subjects 6, 20, and 24, and that of DHEAS in subjects 6, 16, and 20. Each column represents an individual subject. The values are shown as a percentage of the uptake in the absence of any unlabeled compounds (n = 2 slices).

consistent with the previous determination (Motohashi et al., 2002). There was a very large interbatch difference in the mRNA expression of hOAT1 and hOAT3 (Fig. 1A) and the uptake of test compounds in human kidney slices (Fig. 2). However, the mRNA expression of hOAT1 and hOAT3 was closely correlated (Fig. 1B), and the saturable uptake of PAH and PCG by human kidney slices also correlated well (Fig. 3). The interbatch difference is probably due to the large difference in the expression of OAT1 and OAT3, but the close correlations suggests that the functional contribution of hOAT1 and hOAT3 to the net uptake is maintained irrespective of the batch of slices used (Figs. 2 and 3). Because the normal part of the kidney was obtained from surgically nephrectomized patients with renal cell carcinoma for preparation of slices, it is possible that renal cell carcinoma and chemotherapy indirectly affect the expression of OATs. Indeed, Fleck et al. (1997) reported that the uptake of PAH by slices from intact kidney was influenced by the tumor stage and size, although kidney slices were prepared from intact cortical tissues (Fleck et al., 1997). Furthermore, during the process of nephrectomy, kidney tissue is kept under warm ischemic conditions. Considering that ischemic reperfusion injury affects the mRNA and/or protein expression levels of ATP-binding cassette transporters in the kidney (Huls et al., 2006), it is also possible that the warm ischemic conditions during nephrectomy affect the expression of hOAT1 and/or hOAT3.

The basolateral uptake of organic anions in renal proximal tubule cells is indirectly coupled to the Na^+ gradient through Na^+-dicarboxylate cotransport and organic anion/dicarboxylate exchange (Bakhiya et al., 2003; Sweet et al., 2003). In the kidney slices used in this study, the effect of extracellular KG was compound-dependent (Fig. 4), and KG only slightly stimulated the uptake of PAH and DHEAS (Fig. 4). There are two possibilities to account for this discrepancy. One is the difference in the incubation time. In the present study, the incubation time was shorter than in the previous study to characterize the initial uptake process (15 versus 60 min) (Sweet et al., 2003). It is also possible that the kidney slice may retain sufficient activity to allow the intracellular concentration of KG to drive OAT1 and OAT3. However, further studies are required to draw more definite conclusions.

Nonlinear regression analyses suggest that the uptake of test compounds, except DHEAS, by human kidney slices consists of one saturable and one nonsaturable component, whereas that of DHEAS consists of two saturable components, and the high-affinity component accounts for the major part (56–76%) (Fig. 5). The kinetic parameters for the uptake of organic anions by human kidney slices are summarized in Table 1. The K_m value of PAH determined in human kidney slices was similar to the K_m of hOAT1 (20 μM; Tahara et al., 2005b) and K_i of hOAT3 (100 μM; Deguchi et al., 2004), whereas the K_m values of 2,4-D and those of PCG and ES were similar to that of hOAT1 (5.8 μM; Tahara et al., 2005b) and hOAT3 (52 and 9.5 μM; Tahara et al., 2005b), respectively. For DHEAS, the K_m value for hOAT3 (13 μM, this study) is close to that of the high-affinity component rather than the low-affinity component.

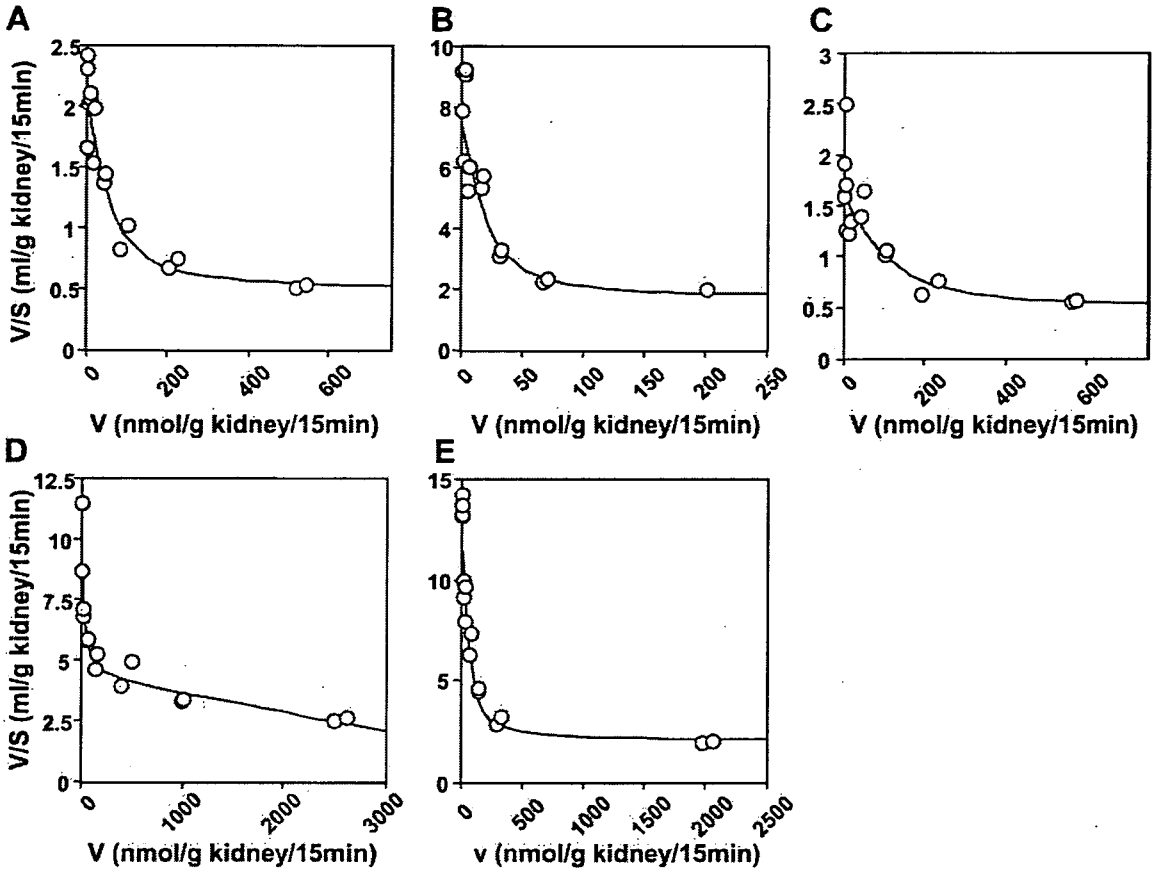

Fig. 5. Concentration dependence of the uptake of PAH, 2,4-D, PCG, DHEAS, and ES by human kidney slices. The concentration dependence of the uptake of [^{3}H]PAH (A), [^{3}H]2,4-D (B), [^{14}C]PCG (C), [^{3}H]DHEAS (D), and [^{3}H]ES (E) is shown as an Eadie-Hofstee plot. The uptake was measured for 15 min at 37°C. The concentration dependence of PAH, 2,4-D, PCG, and DHEAS was determined using three different human kidney batches and that of ES was determined using two different batches. A to E, data for human kidney slices prepared from subjects 30, 20, 20, 3, and 37, respectively. Each point represents the result from one slice. Solid lines represent the fitted lines obtained by nonlinear regression analysis.

TABLE 1

Kinetic parameters of the uptake of organic anions by human kidney slices

Kinetic parameters were determined by nonlinear regression analysis using data shown in Fig. 5. The parameters within parentheses represent that of low-affinity components. Each value represents the mean ± S.D.

Substrate	Human Kidney Slices			
	Subject Nos.	K_m	V_{max}	P_{dif}
		μM	*nmol/g kidney/15 min*	
PAH	30	39.5 ± 1.0	62.9 ± 15.4	0.438 ± 0.048
	31	31.1 ± 18.9	25.4 ± 14.9	0.412 ± 0.060
	36	47.8 ± 26.4	50.1 ± 27.5	0.436 ± 0.079
2,4-D	20	3.94 ± 1.04	23.0 ± 5.5	1.65 ± 0.13
	24	0.727 ± 0.449	7.03 ± 2.91	1.41 ± 0.23
	30	4.85 ± 1.39	25.6 ± 6.2	1.09 ± 0.11
PCG	20	89.8 ± 44.0	103 ± 53	0.461 ± 0.096
	24	42.9 ± 10.0	126 ± 27	0.439 ± 0.064
	30	13.9 ± 4.6	22.9 ± 6.4	0.455 ± 0.041
DHEAS	3	3.57 ± 1.67 (1340 ± 260	19.2 ± 8.9 (5750 ± 760)	
	4	2.19 ± 0.91 (1270 ± 580)	20.9 ± 8.2 (3810 ± 1240)	
	5	3.91 ± 1.38 (1260 ± 490)	26.6 ± 10.3 (4800) ± 1340)	
ES	37	9.18 ± 2.01	91.4 ± 17.4	2.01 ± 0.17
	39	10.7 ± 2.2	148 ± 26	2.31 ± 0.21

To estimate the contribution of OAT1 and OAT3, an inhibition study was carried out. Hasegawa et al. (2002) previously demonstrated different inhibition potencies of PAH and PCG for rOat1- and rOat3-mediated uptake. According to the published data, the K_m of PAH for hOAT1 is only 5-fold different from the K_i value for hOAT3 (20 versus 100 μM), whereas PCG exhibits a 30-fold difference (52 versus 1700 μM) (Deguchi et al., 2004; Tahara et al., 2005b). As shown in Fig. 6, PAH and PCG showed different potencies for the uptake of PAH and 2,4-D and that of PCG and DHEAS in

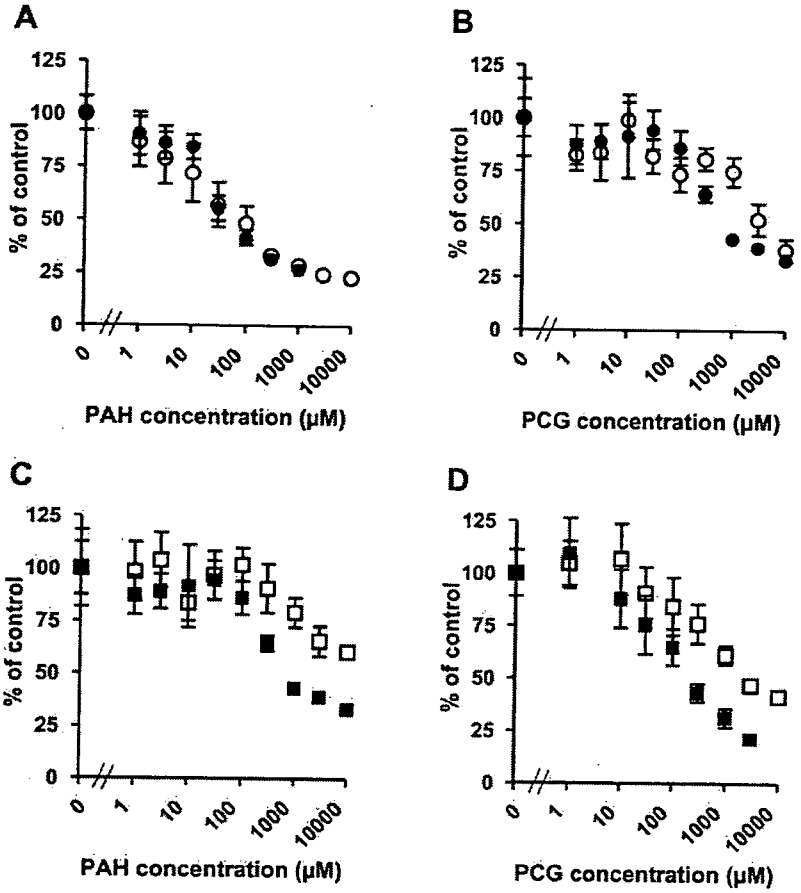

Fig. 6. Inhibitory effect of PAH and PCG on the uptake of [^{3}H]PAH, [^{3}H]2,4-D, [^{14}C]PCG, and [^{3}H]DHEAS by human kidney slices. The uptake of [^{3}H]PAH (0.1 μM, closed circles), [^{3}H]2,4-D (0.1 μM, open circles), [^{14}C]PCG (1 μM, closed squares), and [^{3}H]DHEAS (0.1 μM, open squares) was determined in the presence and absence of unlabeled PAH (A and C) and PCG (B and D) for 15 min at 37°C. The values are shown as a percentage of the uptake in the absence of any unlabeled compounds. All experiments were repeated in three subjects (two slices per subject), and each point represents the mean ± S.E. (n = 6).

human kidney slices. PAH inhibited 2,4-D uptake with an IC_{50} value similar to its own K_m value, whereas it inhibited the uptake of PCG and DHEAS, but with lower potencies. On the contrary, PCG had only a weak effect on the uptake of PAH and 2,4-D by the kidney slices, and, particularly, at a concentration similar to its K_m value, it had no effect. These mutual inhibition studies kinetically suggest that PAH shares the same transporter (hOAT1) with 2,4-D, but not with PCG. PAH and 2,4-D are also substrates of hOAT3 with low transport activities (Tahara et al., 2005b), and its contribution to PAH and 2,4-D uptake by human kidney slices is probably smaller than hOAT1. As in the case of PCG uptake, PCG more potently inhibited the uptake of DHEAS than PAH (Fig. 6, C and D). However, the inhibition curves shifted to the right in comparison with that of PCG uptake (Fig. 6D). Because the uptake DHEAS consists of two saturable components, PCG and PAH may have different inhibition potencies to these two components.

Taken together, it is likely that the uptake of PAH and 2,4-D in human kidney slices is due to hOAT1 and that of PCG and ES, and part of DHEAS uptake is due to hOAT3. There was a 6.3-fold difference in the intrinsic transport activities (V_{max}/K_m) of PAH and 2,4-D in hOAT1-expressing HEK293 cells (Tahara et al., 2005b), which was close to the difference observed in the human kidney (6-fold) (Table 1). This holds true for PCG, ES, and DHEAS. Assuming that the high-affinity component of DHEAS uptake is solely explained by hOAT3, the intrinsic transport activities of ES and DHEAS were 5.2- and 7.5-fold greater than that of PCG in human kidney, and the corresponding values were 4.7 and 7.7 in hOAT3-expressing HEK293 cells, respectively. The transport activities relative to PAH and PCG transport are probably preserved between human kidney slices and the cDNA transfectants, which allows the application of relative activity factor method in which the transport activities of test compounds in the kidney/liver were predicted from the intrinsic parameter of test compound in cDNA transfectants multiplied by the scaling factor obtained using reference compounds (Hasegawa et al., 2003; Hirano et al., 2004).

The present study shows that human kidney slices maintain the transport activities due to hOAT1 and hOAT3 and thus enable us to determine the contribution of hOAT1 and hOAT3 ex vivo. This experimental system will help in the characterization of the renal uptake of novel drugs and the quantitative evaluation of likelihood of drug-drug interactions caused by inhibition of the renal uptake transporters. However, the possible impact of disease state and patient drug treatments on OAT function in the available source tissues is unknown, and caution must be used when extrapolating such data to quantitative evaluation of the normal human response.

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