

Characterization of the uptake of OAT1 and OAT3 substrates by human kidney slices

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Abbreviations :

OAT, organic anion transporter; PAH, *p*-aminohippurate; 2,4-D, 2,4-dichlorophenoxyacetate;

PCG, benzylpenicillin; DHEAS, dehydroepiandrosterone sulfate; ES, estrone sulfate;

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ABSTRACT

The activities of renal multispecific organic anion transporters (OAT1 and OAT3) have not been fully evaluated in human kidneys. In the present study, the uptake of some organic anions was characterized in kidney slices from human intact renal cortical tissues: hOAT1 and hOAT3 substrates (*p*-aminohippurate, (PAH) and 2,4-dichlorophenoxyacetate (2,4-D)) and hOAT3 substrates (benzylpenicillin (PCG), dehydroepiandrosterone sulfate (DHEAS) and estrone sulfate (ES)). In spite of large interbatch differences, hOAT1 and hOAT3 mRNA levels correlated well and there was a good correlation between the uptake of PAH and PCG by kidney slices. The uptake of organic anions by kidney slices was saturable with K_m values (μM) 31–48 for PAH, 0.73–4.9 for 2,4-D, 14–90 for PCG and 9.2–11 for ES. These parameters were comparable with those for hOAT1 and/or hOAT3. The uptake of DHEAS consists of two saturable components with K_m values (μM) of 2.2–3.9 and 1,300 and the K_m value of the high affinity component was close to that for hOAT3. Furthermore, PAH more potently inhibited the uptake of 2,4-D than that of PCG and DHEAS. PCG had a weaker effect on the uptake of PAH and 2,4-D than expected from its K_m value. Taken together, it is likely that the uptake of PAH and 2,4-D is due to OAT1, and the uptake of PCG and ES and part of DHEAS uptake are due to OAT3 in human kidney slices. Human kidney slices are useful tools for characterizing the renal uptake of drugs.

Introduction

The kidney plays an important role in the urinary excretion of endogenous wastes and xenobiotics, including drugs and their metabolites. Urinary excretion of drugs is mediated by glomerular filtration and tubular secretion, and selective and passive reabsorption. Various kinds of membrane transporters involved in the renal tubular secretion have been identified in the proximal tubules. In particular, organic anion transporter 1 (OAT1, *SLC22A6*) and OAT3 (*SLC22A8*) are exclusively expressed on the basolateral membrane of the proximal tubules (Tojo et al., 1999; Hosoyamada et al., 1999; Cha et al., 2001; Hasegawa et al., 2002; Motohashi et al., 2002), and play major roles in the uptake of a variety of organic anions on the basolateral membrane of kidney proximal tubule epithelial cells (Wright and Dantzler, 2004; Sweet, 2005; Sekine et al., 2006).

OAT1 and OAT3 are multispecific transporters accepting a variety of structurally unrelated compounds as substrates, including clinically important drugs, such as diuretics (Uwai et al., 2000; Hasannejad et al., 2004), β -lactam antibiotics (Jariyawat et al., 1999; Ueo et al., 2005), antiviral drugs (Cihlar et al., 1999; Wada et al., 2000), an anticancer drug (methotrexate) (Cha et al., 2001), nonsteroidal anti-inflammatory drugs (Apiwattanakul et al., 1999), HMG-CoA reductase inhibitors (Hasegawa et al., 2002; Takeda et al., 2004), and H_2 receptor antagonists (Tahara et al., 2005a). OAT3 exhibits a broader substrate specificity than OAT1, and accepts amphipathic and hydrophilic organic anions, and some organic cations although the transport activities of hydrophilic organic anions with a low molecular weight by OAT3 is 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), while 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; Sweet et al., 2002; Hasegawa et al., 2003; 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, while 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 although 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, while 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 prepared kidney slices from human kidney, and demonstrated the active accumulation of PAH and methotrexate, suggesting that human kidney slices also retains the activities of organic anion transporters (Fleck et al., 2000; Fleck et al., 2002). 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.

Methods

Materials

[³H]PAH (4.1 Ci/mmol), [³H]DHEAS (60 Ci/mmol) and [³H]ES (43.1 Ci/mmol) were purchased from Perkin-Elmer Life Science (Boston, MA). [¹⁴C]PCG (59 mCi/mmol) and [³H]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; Hasegawa et al., 2003). Kidney slices (300 μ m 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 CaCl₂, 1.2 mM MgSO₄ and 10mM NaH₂PO₄/Na₂HPO₄ 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 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 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 9mers 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 manufacturer's instructions. Nucleotide sequences of the primers for hOAT1 and hOAT3 and GAPDH are listed below :

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

GAPDH, reverse: 5-GAAGATGGTGATGGGATTTC-3

GAPDH was used as a house-keeping 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 % CO₂ 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 (Sugiyama 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 NaHCO₃, 4.83 mM KCl, 0.96 mM KH₂PO₄, 1.20 mM MgSO₄, 12.5 mM HEPES, 5 mM glucose and 1.53 mM CaCl₂, 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

0.2 N NaOH. The aliquots neutralized with 2 N HCl were transferred to scintillation vials containing 2 ml 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 μ l 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,

$$v = \frac{V_{\max} \times S}{K_m + S}$$

one saturable and one nonsaturable components,

$$v = \frac{V_{\max} \times S}{K_m + S} + P_{\text{dif}} \times S$$

two saturable components,

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

where, v is the uptake velocity of the substrate (nmol/g kidney/15min or pmol/mg protein/min), S is the substrate concentration of the medium (μ M), K_m is the Michaelis constant (μ M), V_{\max} is the maximum uptake velocity (nmol/g kidney/15min or pmol/mg protein/min), 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

Fig. 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 $0.147 (x 10^{-2})$ to $31.1 (x 10^{-2})$ for hOAT1, and from $0.051 (x 10^{-2})$ to $40.6 (x 10^{-2})$ for hOAT3. The average of the relative expression of hOAT1 and hOAT3 was $8.97 \pm 1.27 (x 10^{-2})$ and $15.0 \pm 1.4 (x 10^{-2})$, respectively. A linear correlation between the relative expression of hOAT1 and hOAT3 was observed (Fig. 1B, $R^2 = 0.814$, $p < 0.001$).

Uptake of [^3H]DHEAS by hOAT1-, hOAT3-transfected HEK293 cells.

The uptake of [^3H]DHEAS by hOAT3-transfected cells was significantly greater than that by vector-transfected cells, which was saturable with K_m (μM) and V_{\max} (pmol/mg protein/min) values of 12.9 ± 2.0 and 249 ± 29 , respectively. The uptake of [^3H]DHEAS by hOAT1-transfected cells was similar to that by vector-transfected cells, while the uptake of [^3H]PAH by hOAT1- and vector-transfected cells was 38.8 ± 1.4 , 1.39 ± 0.17 $\mu\text{l/mg protein/min}$, 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 300-1000 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. Non-linear regression analysis showed that the uptake of PAH, 2,4-D, PCG and ES by human kidney slices consists of one saturable and one non-saturable component, while 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 [^3H]PAH, [^3H]2,4-D, [^{14}C]PCG and [^3H]DHEAS by human kidney slices was examined (Fig. 6). The uptake of [^3H]PAH and [^3H]2,4-D was more potently inhibited by unlabeled PAH than PCG (Fig. 6A and 6B) and, vice versa, for the uptake of [^{14}C]PCG (Fig. 6C and 6D). Although PCG was also a more potent inhibitor than PAH for the uptake of [^3H]DHEAS, the inhibition curves of [^3H]DHEAS uptake by PCG and PAH were shifted to the right in comparison with that of PCG (Fig. 6C and 6D).

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. demonstrated that human kidney slices retain organic anion transport activity (Fleck et al., 2000). 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 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 were 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 likely 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 (Fig 2 and 3). Because 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 affect 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 min *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 conclusion.

Non-linear regression analyses suggest that the uptake of test compounds, except DHEAS, by human kidney slices consists of one saturable and one non-saturable component,

while 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 K_m of hOAT1 (20 μ M, Tahara et al, 2005b) and K_i of hOAT3 (100 μ M, Deguchi et al., 2004), while the K_m value of 2,4-D, and those of PCG and ES were similar to that of hOAT1 (5.8 μ M Tahara et al, 2005b), and hOAT3 (52 and 9.5 μ M, Tahara et al, 2005b), respectively. For DHEAS, the K_m value for hOAT3 (13 μ M, this study) is close to that of the high affinity component rather than the low affinity component.

To estimate the contribution of OAT1 and OAT3, an inhibition study was carried out. Hasegawa et al previously demonstrated different inhibition potencies of PAH and PCG for rOat1 and rOat3-mediated uptake (Hasegawa et al., 2002). 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 μ M), whereas PCG exhibits a 30-fold difference (52 *versus* 1700 μ M) (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 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 likely smaller than hOAT1.

As in the case of PCG uptake, PCG more potently inhibited the uptake of DHEAS than PAH (Fig. 6C and 6D). However, the inhibition curves shifted to the right in comparison with that of PCG uptake (Fig. 6D). Since 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 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 likely 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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Footnotes.

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Legends for Figures

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. The solid line represents the linear regression line. Each point represents the results of one human kidney subject.

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 [^3H]PAH (A), 0.1 μM [^3H]2,4- D (B), 1 μM [^{14}C]PCG (C) and 0.1 μM [^3H]DHEAS was measured for 15 min at 37 °C. The Y axis represents the saturable uptake clearance (ml/g kidney/15 min), which was obtained by subtracting the uptake clearance in the presence of excess amount of non-labeled compounds (1mM) 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 [^3H]PAH (0.1 μM) and [^{14}C]PCG (1 μM) was measured for 15 min at 37 °C. The saturable uptake clearance (ml/g kidney/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 – 26, 28 – 42 (see Fig. 2). Each point

represents the results of one human kidney subject.

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 μ M [3 H]PAH (A), 0.1 μ M [3 H]2,4-D (B), 1 μ M [14 C]PCG (C) and 0.1 μ M [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 2 or 3 subjects (2 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 individual subject. The values are shown as a percentage of the uptake in the absence of any unlabeled compounds (N = 2 slices).

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. Figs. 5A, B, C, D and E represent the 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.

Fig. 6 Inhibitory effect of PAH and PCG on the uptake of [³H]PAH, [³H]2,4-D, [¹⁴C]PCG and [³H]DHEAS by human kidney slices. The uptake of [³H]PAH (0.1 μM, closed circles), [³H]2,4-D (0.1 μM, open circles), [¹⁴C]PCG (1 μM, closed squares) and [³H]DHEAS (0.1 μM, open squares) was determined in the presence and absence of unlabeled PAH (Fig. 6A and C) and PCG (Fig. 6B 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 3 subjects (2 slices per subject), and each point represents the mean ± S.E. (N = 6).

Table.1 Kinetic parameters of the uptake of organic anions by human kidney slices.

Substrate /inhibitor	Human kidney slices			
	Subject No.	K_m μM	V_{max} $nmol/g\ kidney/15\ min$	P_{dif} $nmol/g\ kidney/15\ min$
PAH	30	39.5 \pm 1.0	62.9 \pm 15.4	0.438 \pm 0.048
	31	31.1 \pm 18.9	25.4 \pm 14.9	0.412 \pm 0.060
	36	47.8 \pm 26.4	50.1 \pm 27.5	0.436 \pm 0.079
2,4-D	20	3.94 \pm 1.04	23.0 \pm 5.5	1.65 \pm 0.13
	24	0.727 \pm 0.449	7.03 \pm 2.91	1.41 \pm 0.23
	30	4.85 \pm 1.39	25.6 \pm 6.2	1.09 \pm 0.11
PCG	20	89.8 \pm 44.0	103 \pm 53	0.461 \pm 0.096
	24	42.9 \pm 10.0	126 \pm 27	0.439 \pm 0.064
	30	13.9 \pm 4.6	22.9 \pm 6.4	0.455 \pm 0.041
DHEAS	3	3.57 \pm 1.67 (1340 \pm 260)	19.2 \pm 8.9 (5750 \pm 760)	-
	4	2.19 \pm 0.91 (1270 \pm 580)	20.9 \pm 8.2 (3810 \pm 1240)	-
	5	3.91 \pm 1.38 (1260 \pm 490)	26.6 \pm 10.3 (4800 \pm 1340)	-
ES	37	9.18 \pm 2.01	91.4 \pm 17.4	2.01 \pm 0.17
	39	10.7 \pm 2.2	148 \pm 26	2.31 \pm 0.21

Abbreviations are: PAH, *p*-aminohippurate; 2,4-D, 2,4-dichlorophenoxyacetate; PCG, benzylpenicillin; DHEAS, dehydroepiandrosterone sulfate; ES, estrone-3-sulfate.

Kinetic parameters were determined by nonlinear regression analysis using data shown in Fig. 5.

The parameters in parenthesis represent that of low affinity components. Each value represents the mean \pm S.D.

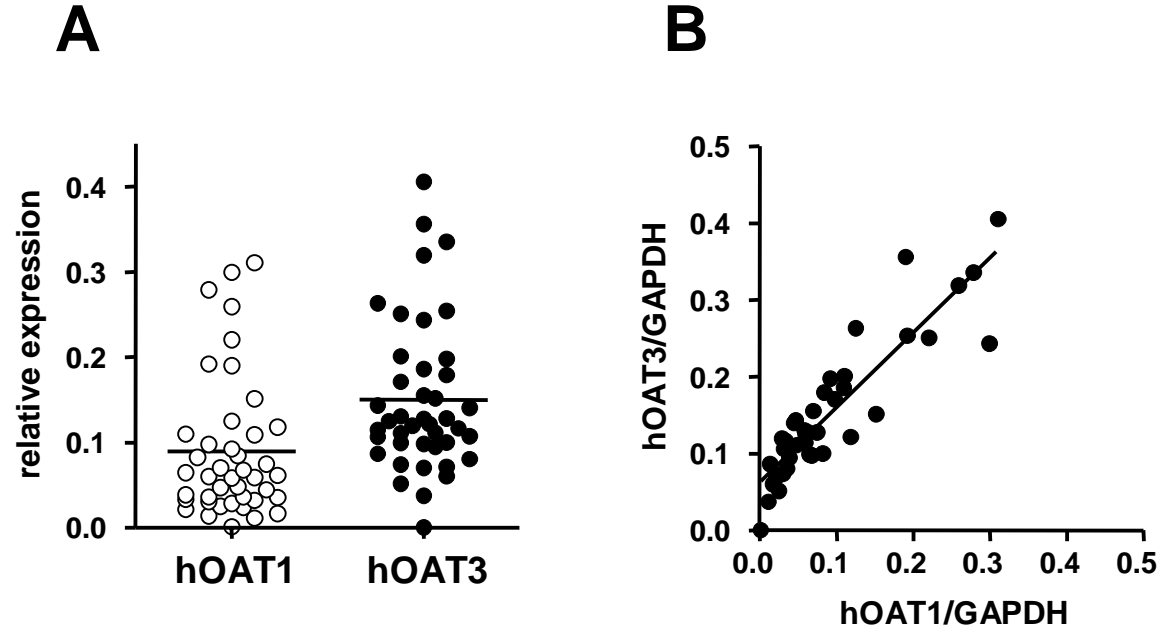


Fig. 1

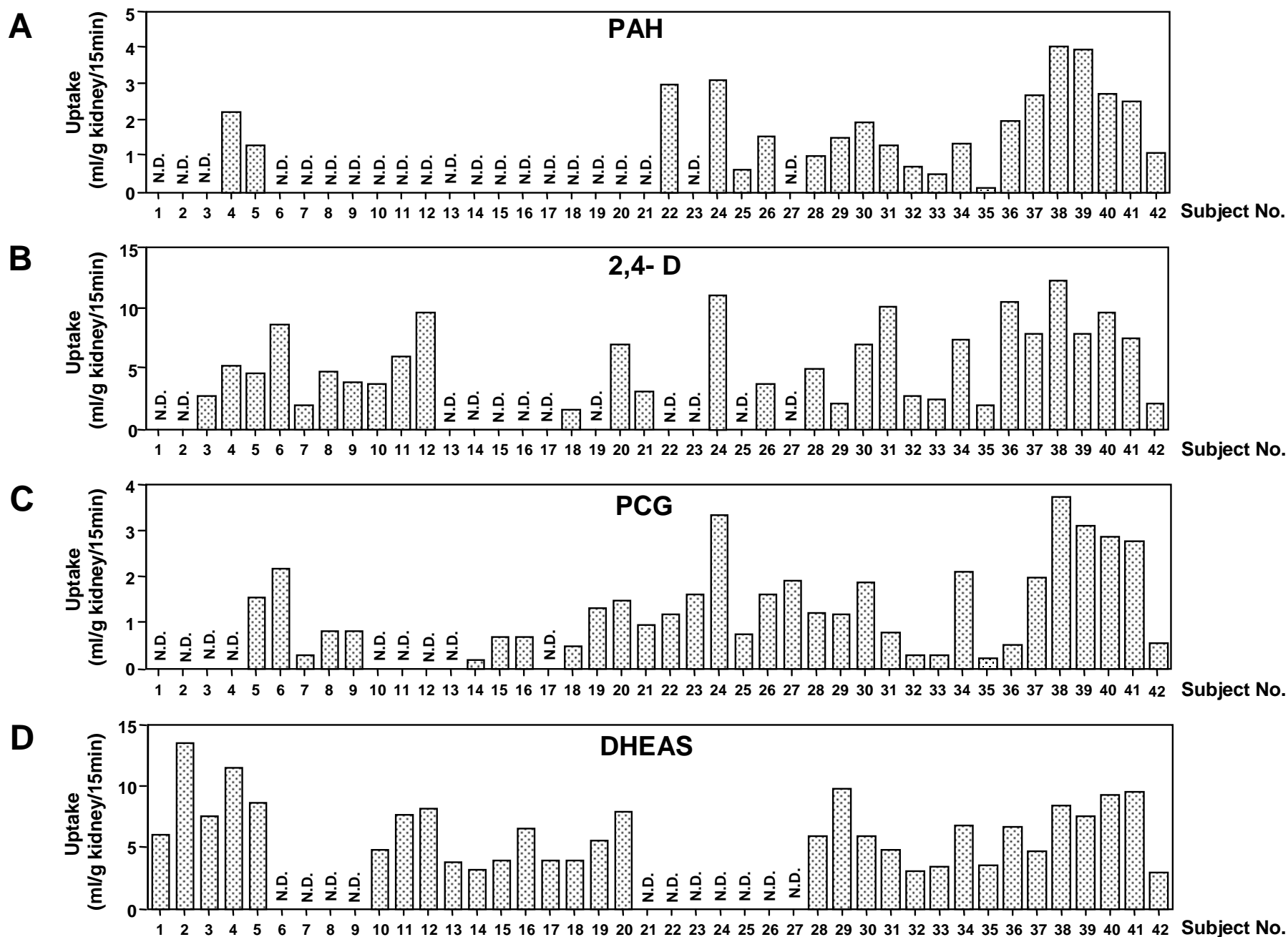


Fig. 2

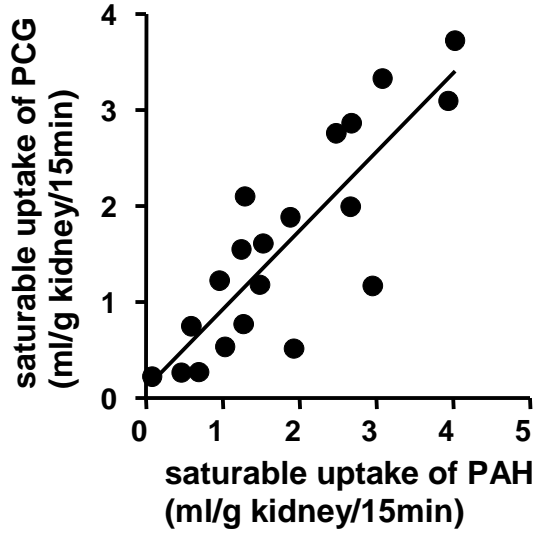


Fig. 3

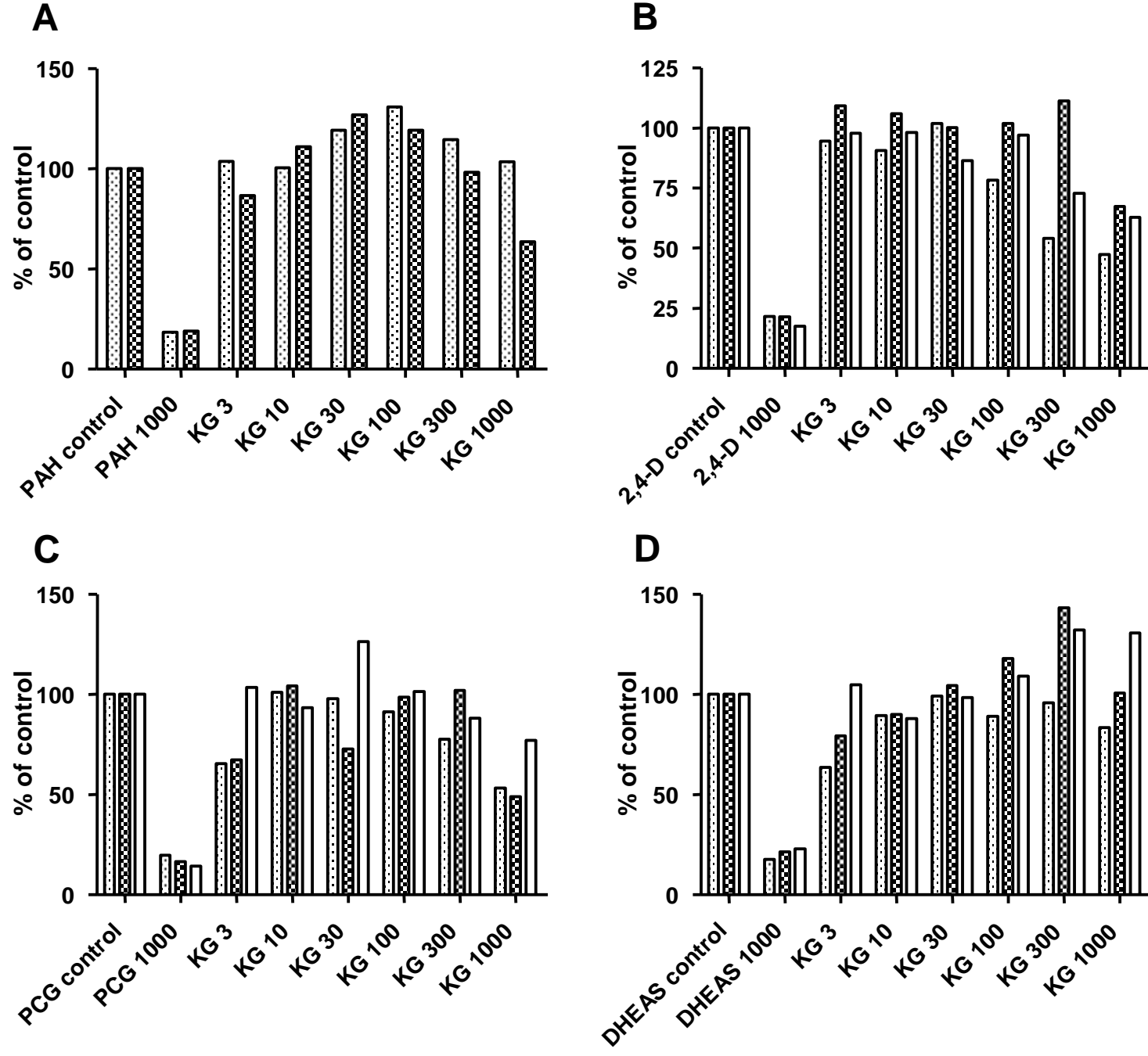
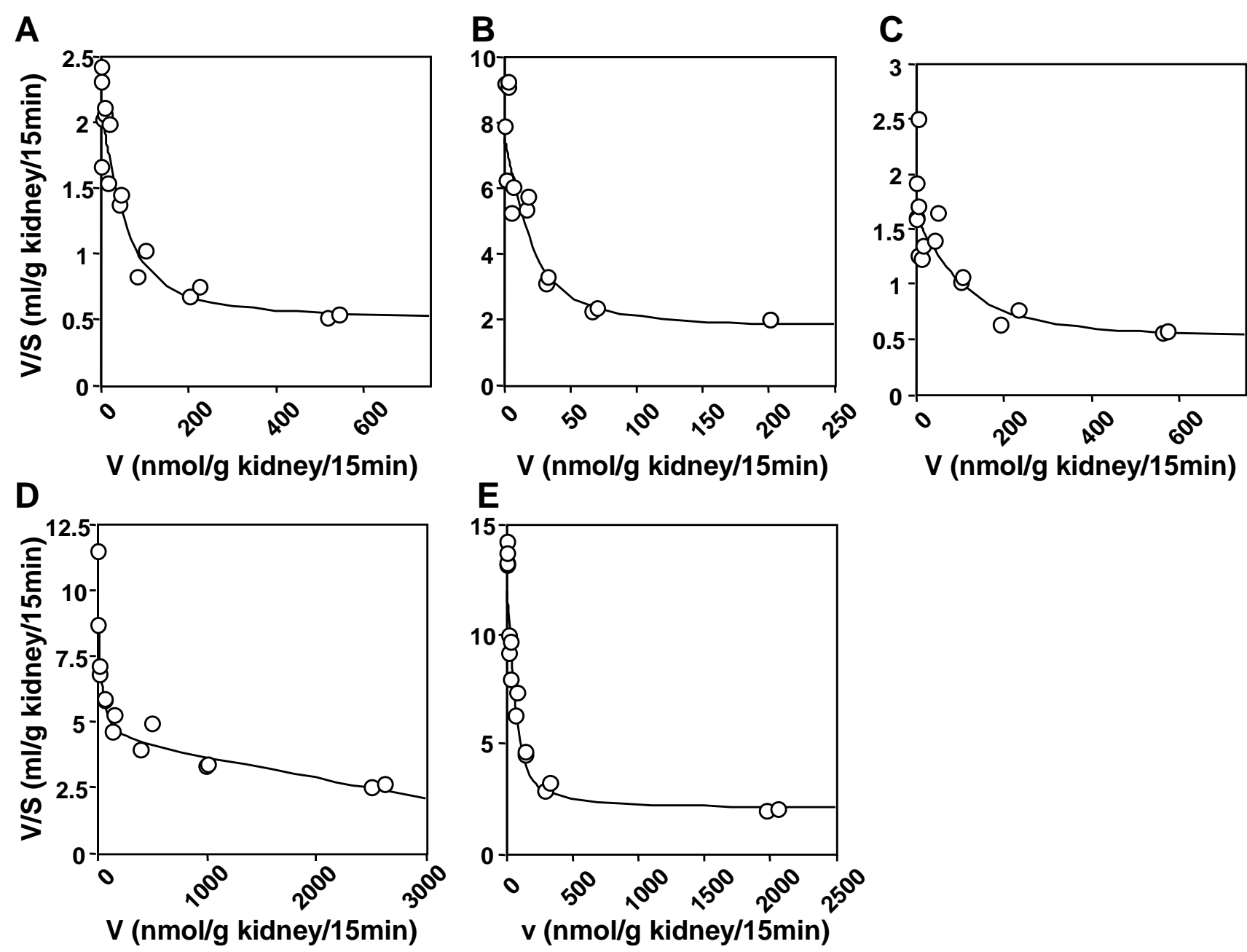


Fig. 4

Fig. 5



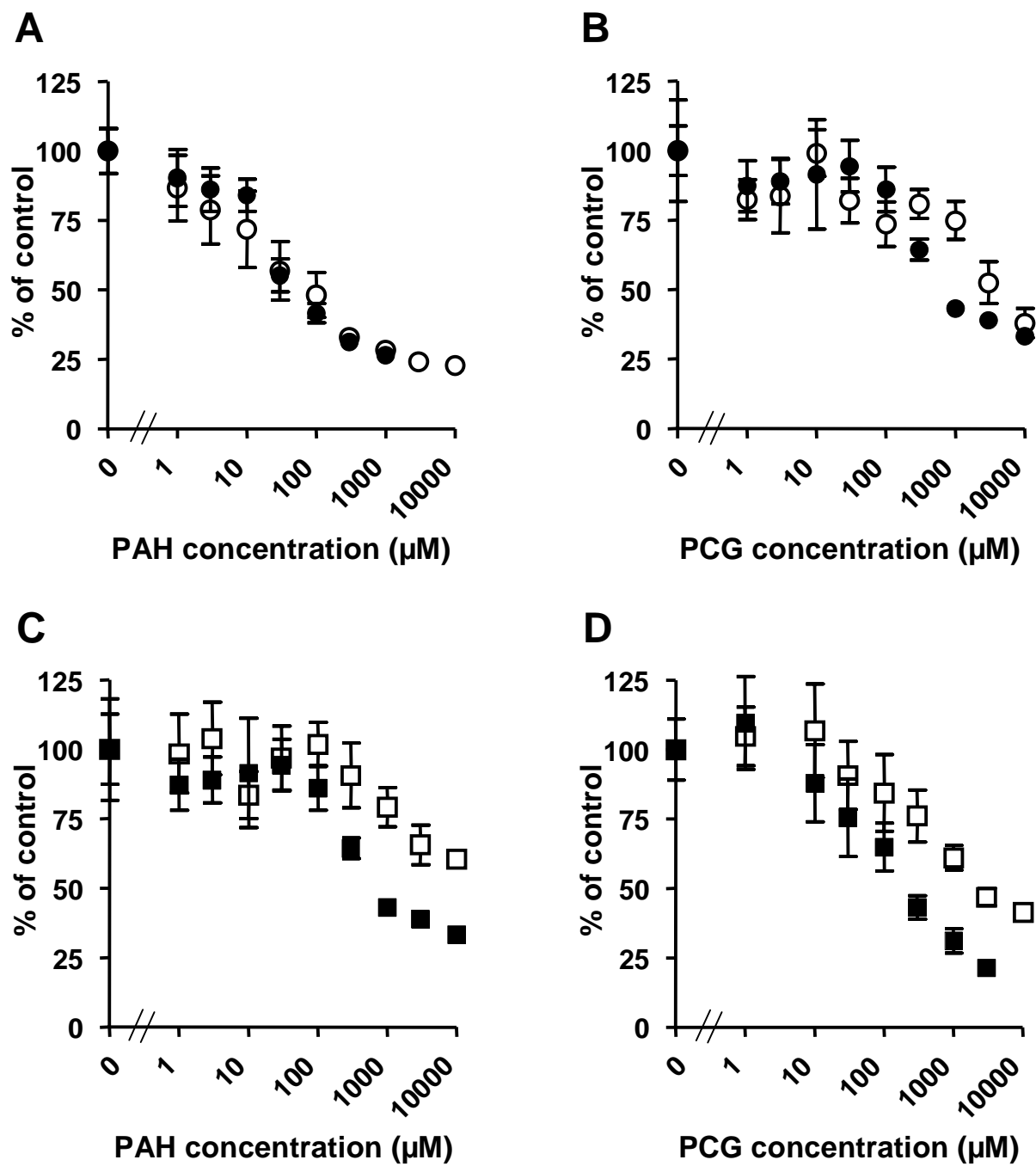


Fig. 6