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Major role of organic anion transporters in the uptake of phenolsulfonphthalein in the kidney

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Abstract

Phenolsulfonphthalein is used for testing renal function. However, its excretion mechanism has not been elucidated. The purpose of this study was therefore to elucidate the transporter-mediated excretion system for phenolsulfonphthalein. *p*-Aminohippuric acid, a substrate of rat organic anion transporter1 (rOat1), and cimetidine, a substrate of rOat3, reduced the urinary excretion of phenolsulfonphthalein. The uptake of phenolsulfonphthalein by kidney slices was found to consist of two components. The IC_{50} values of rOat1 substrates were higher than those of rOat3 substrates. In the presence of cimetidine, the Eadie–Hofstee plot gave a single straight line. The profile of the phenolsulfonphthalein uptake component in the presence of cimetidine was similar to that of the low-affinity component in the absence of cimetidine. We conclude that rOat1 and rOat3 are involved in the renal uptake of phenolsulfonphthalein and that phenolsulfonphthalein is a high-affinity substrate for rOat3 but is a relatively low-affinity substrate for rOat1.

Keywords: Basolateral membrane; Organic anion transporter 1; Organic anion transporter 3; Phenolsulfonphthalein; Renal uptake; Urinary excretion

1. Introduction

The kidney plays an important role in the urinary excretion of drugs and their metabolites via glomerular filtration and tubular secretion. The first step in tubular secretion is the uptake from blood through the basolateral membrane of epithelial cells in the proximal tubules. Transporter-mediated systems have been considered to play major roles in the tubular drug uptake. *p*-Aminohippuric acid is widely used as a model substrate to investigate renal handling of organic anions because of its high renal clearance and susceptibility to metabolism. Rat organic anion transporter 1 (rOat1/*Slc22a6*) has recently been isolated and identified as a basolateral *p*-aminohippuric acid transporter (Sweet et al., 1997). Phenolsulfonphthalein is widely used clinically as a drug for testing renal function because of its high renal clearance (Gault et al., 1967). However, it is thought that phenolsulfonphthalein and *p*-aminohippuric acid do not to share the same transporter system at the basolateral membrane of proximal tubular cells (Pritchard and Miller, 1992). An accumulation step across the basolateral membrane has been demonstrated. However, the transporters responsible for the renal uptake of phenolsulfonphthalein are not fully understood.

To date, another rOats, named rOat2 (*Slc22a7*) and rOat3 (*Slc22a8*), have been identified (Kusuhara et al., 1999; Sekine et al., 1998). However, rOat2 and rOat1/3 are transcripts from separate genes located on different chromosomes. rOat2 was first cloned as a novel liver transporter NLT (Simonson et al., 1994). rOat1 and rOat3 are the only organic anion transporters that are localized to the basolateral membrane of proximal tubules (Sekine et al., 2000; Tojo et al., 1999). rOat1 and rOat3 mediate the transport of many organic anions including endogenous metabolites, drugs and xenobiotics. It is thought to

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be the most important physiological process involved in the basolateral uptake of organic anions in renal epithelial cells (Sekine et al., 2000). It has been demonstrated that rOat1 mediates the transport of relatively small hydrophilic organic anions such as *p*-aminohippuric acid (Uwai et al., 1998). In contrast, the preferred substrates for rOat3 are larger and more hydrophobic compounds (Cha et al., 2001). There is a little information about the contribution of rOat1 and rOat3 to the total renal uptake of organic anions. The aim of the present study was to determine the validity of the hypothesis that phenolsulfonphthalein is cleared from peritubular blood by rat kidney organic anion transporters, particularly by rOat1 and rOat3, and to determine the contribution of rOat1 and rOat3 to the renal uptake of phenolsulfonphthalein. The results demonstrated that phenolsulfonphthalein is a high-affinity substrate for rOat3 but is a relatively low-affinity substrate for rOat1.

2. Materials and methods

2.1. Chemicals

Phenolsulfonphthalein, cimetidine, salicylate and *p*-aminohippuric acid were purchased from Wako (Osaka, Japan). Pravastatin was kindly donated from Sankyo (Tokyo, Japan). [^{3}H]*p*-Aminohippuric acid was purchased from NEN Life Science Products (Hoofddrop, The Netherlands). All other reagents were of the highest grade available and used without further purification.

2.2. Animals

Male Wistar rats, aged 6–7 weeks (300–350 g in weight), were obtained from NRC Haruna (Gunma, Japan). The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the Guide for the Care and Use of Laboratory Animals.

2.3. In vivo study

Three to six male Wistar rats were used in all experiments. The rats were anesthetized with sodium pentobarbital (40 mg/kg weight, i.p.). Phenolsulfonphthalein (2.2 μmol/kg) and an inhibitory drug solution were injected through the femoral vein. Blood was collected at 1, 15, 30, 45 and 60 min after injections. Plasma was prepared by centrifugation ($850 \times g$ for 15 min) of blood samples. Methanol, corresponding to a double volume of plasma, was added to each plasma specimen. After centrifugation ($15{,}000 \times g$ for 15 min) of the mixture, the concentration of phenolsulfonphthalein in the supernatant was measured. The whole contents of the bladder were withdrawn with a syringe at 60 min after injections.

2.4. Preparation of basolateral membrane vesicles

Renal basolateral membrane vesicles were prepared by self-orienting Percoll-gradient centrifugation (Inui et al., 1981) with some modification. Kidneys were excised from the rats under sodium pentobarbital anesthesia (40 mg/kg weight, i.p.). Kidney cortex slices (8–12 kidneys) were homogenized in ice-cold solution A (300 mM sucrose, 5 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride and 12 mM Tris/HCl, pH 7.4) with a waring blender at 16,500 rpm for 4 min. The homogenate was rapidly centrifuged at $1500 \times g$ for 15 min, and the supernatant was recentrifuged at $20{,}500 \times g$ for 20 min. The fluffy upper-layer pellet was collected and resuspended in 30 ml of the buffer with 8% of Percoll and homogenized using a glass Teflon homogenizer with 10 strokes. The crude membrane suspension was centrifuged at $50{,}000 \times g$ for 60 min. The third fraction from the bottom (approx. 8 ml) was withdrawn and diluted to 32 ml with the buffer. After centrifugation of this fraction at $48{,}000 \times g$ for 30 min, the pellet on the Percoll solid was collected and resuspended in 20 ml of solution B (100 mM D-mannitol, 100 mM KCl and 20 mM HEPES/Tris, pH 7.5) containing 5 mM EGTA and 10 mM $MgCl_2$. The basolateral membrane was precipitated by centrifugation at $3400 \times g$ for 15 min. Finally, the pellet was suspended in solution B and recentrifuged at $27{,}000 \times g$ for 30 min and the basolateral membrane pellet was resuspended in solution B. Na^+–K^+ ATPase (a marker enzyme of basolateral membrane) activity of the basolateral membrane was routinely more than 15-fold higher than that of the initial homogenate. In contrast, alkaline phosphatase (a marker enzyme of brush border membrane) activity of basolateral membrane was routinely as same as that of the initial homogenate. This means that basolateral membranes were enriched at least 15-fold with respect to the brush border membranes.

2.5. Uptake experiments

The uptake of substrates into basolateral membrane vesicles was measured by the rapid filtration technique described previously (Kobayashi et al., 1992). In a routine assay, 20 μl of membrane vesicles (0.2–0.3 mg protein) suspension was added to 100 μl of incubation medium kept at 25 °C. The drug solution contained 100 mM D-mannitol, 0.5 μM glutaric acid, 2 μM *p*-aminohippuric acid or 100 μM phenolsulfonphthalein, and 100 mM KCl or NaCl. At selected time intervals, the uptake was stopped by diluting the incubation medium with 3 ml of ice-cold 10 mM HEPES buffer (pH 7.5) containing 150 mM KCl. The mixture was immediately filtered through a Millipore filter (0.45 μm, 2.5 cm diameter; HAWP). The filter was rinsed with 3 ml of the same buffer. Substrate trapped on the filter was extracted with 500 μl of water and the concentration of substrate was determined.

2.6. Uptake by kidney slices

Uptake studies were carried out as described in a previous report (Urakami et al., 1999). Slices of whole kidneys from Wistar rats were put in ice-cold oxygenated incubation buffer. The incubation buffer consisted 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. Slices (80–100 mg) were incubated in a six-well plate with 3 ml of oxygenated incubation buffer in each well after they had been preincubated with incubation buffer for 5 min. The uptake study of 30 μM phenolsulfonphthalein was carried out at 37 °C. After incubating for an appropriate time, each slice was immediately removed from the incubation buffer, washed with ice-cold saline, weighed and homogenized in 0.5 ml saline and the same volume of methanol. After centrifugation (15,000 × *g* for 15 min) of the mixture, the concentration of phenolsulfonphthalein in the supernatant was measured. When the uptake of phenolsulfonphthalein consists of two components, kinetic parameters were obtained using the following equation:

$$v = V_{max1} \cdot S/(K_{m1} + S) + V_{max2} \cdot S/(K_{m2} + S) + K_d \cdot S$$

where v is the uptake rate of phenolsulfonphthalein (pmol/min/g kidney), S is the phenolsulfonphthalein concentration in the medium (μM), K_m is the Michaelis–Menten constant (μM) and V_{max} is the maximum uptake rate (pmol/min/g kidney). K_d is the rate constant of nonsaturated permeation (nl/min/g kidney). K_{m1} and V_{max1} represent the high-affinity component parameter. K_{m2} and V_{max2} represent the low-affinity component parameter. When the Eadie–Hofstee plot gives a single straight line kinetic parameters were obtained using the following equation:

$$v = V_{max} \cdot S/(K_m + S) + K_d \cdot S$$

2.7. Analytical procedures

Substrates were determined using an HPLC system equipped with a Hitachi L-6000 pump and L-4200H UV/VIS detector. The column was a Hitachi ODS Gel #3053 (4 mm i.d. × 250 mm). A mobile phase containing 20% acetonitrile and 50 mM H_3PO_4 with pH adjusted to 3.0 by NaOH was used. Column temperature and flow rate were 55 °C and 0.7 ml/min, respectively. Wavelength for detection of phenolsulfonphthalein was 432 nm. [^{3}H]*p*-Aminohippuric acid was measured by liquid scintillation counting. Protein was measured by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

2.8. Data analysis

The area under the plasma concentration–time curve (AUC) was estimated by the trapezoidal rule using plasma data obtained from 0 to 60 min. The clearance values of urine (CLurine) were determined by dividing the amounts of phenolsulfonphthalein excreted into urine by the AUC from 0 to 60 min. Analysis of variance (ANOVA) and unpaired Student's *t*-test were used for the statistical analysis, and a value of $P < 0.05$ was considered significant. The log–concentration inhibition curves were analyzed, and the concentration at 50% inhibition (IC_{50}) of phenolsulfonphthalein uptake was determined. IC_{50} values were obtained by fitting the data to the nonlinear least-squares regression analysis program MULTI.

3. Results

3.1. Inhibitory effects of various drugs on urinary excretion of phenolsulfonphthalein

To characterize the uptake process for phenolsulfonphthalein by the kidney in vivo, the inhibitory effects of *p*-aminohippuric acid, a relatively specific substrate of rOat1, and cimetidine, a specific substrate of rOat3, on the urinary excretion of phenolsulfonphthalein were determined (Hasegawa et al., 2002; Nagata et al., 2002). The amount of urinary excretion of phenolsulfonphthalein over a period of 1 hr after intravenous injection in Wistar rats is shown in Fig. 1. *p*-Aminohippuric acid and cimetidine reduced the urinary excretion of phenolsulfonphthalein in a dose-dependent manner. The plasma concentration was determined to calculate CLurine (Fig. 2). The administration of *p*-aminohippuric acid and cimetidine reduced the CLurine value of phenolsulfonphthalein.

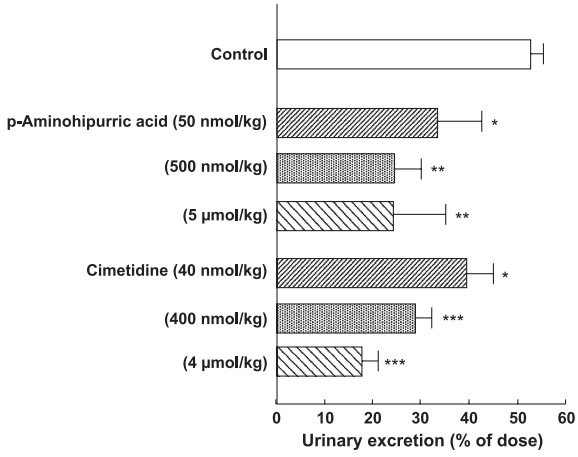

Fig. 1. Effects of various doses of *p*-aminohippuric acid and cimetidine on urinary excretion of phenolsulfonphthalein in Wistar rats. Phenolsulfonphthalein (2.2 μmol/kg) was injected through the femoral vein with or without *p*-aminohippuric acid and cimetidine. The ratio of the excreted amount of phenolsulfonphthalein over a period of 60 min in a urine sample to the percent of the injected amount was determined. Each value represents the mean with S.D. of four determinations. $^{**}P < 0.01$, $^{***}P < 0.01$, significantly different from control.

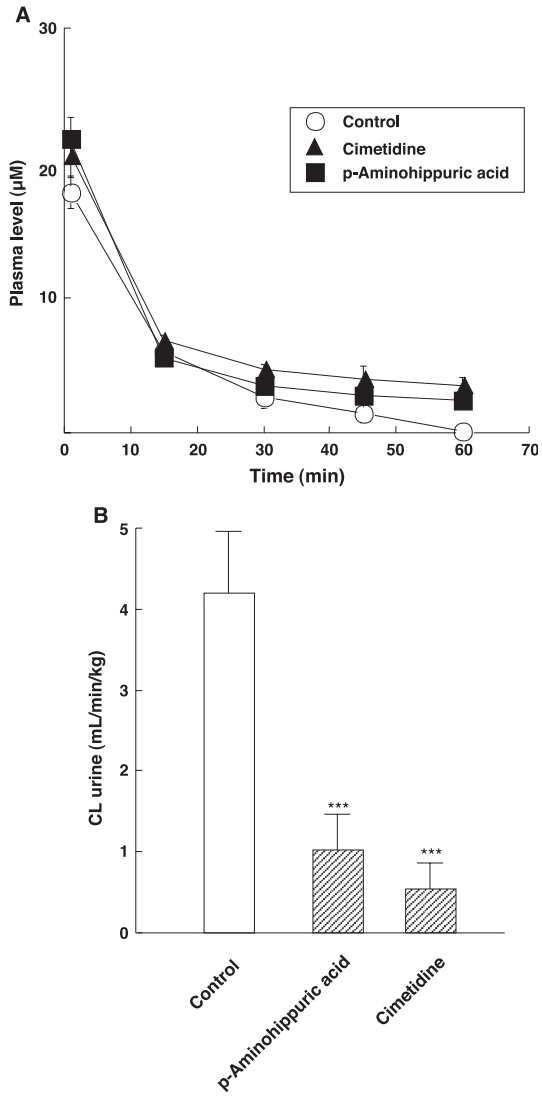

Fig. 2. Plasma concentrations and urinary excretion clearances of phenolsulfonphthalein in Wistar rats. The time profiles of plasma concentrations (A) and CLurine values (B) were determined after i.v. injection of phenolsulfonphthalein in the presence or absence of *p*-aminohippuric acid (5 μmol/kg) and cimetidine (4 μmol/kg). Each value represents the mean with S.D. of three to four determinations. ***$P<0.001$, significantly different from control.

3.2. Function of rOat1 in the basolateral membrane of Wistar rats

rOat1 and rOat3 have been shown to function as a dicarboxylate-coupled anion exchanger (Shimada et al., 1987; Sweet et al., 2003). In basolateral membrane vesicles, an inward Na^+ gradient stimulates glutaric acid uptake and this intravesicular glutaric acid is exchanged with extravesicular *p*-aminohippuric acid (Sekine et al., 1997; Shimada et al., 1987). To confirm that rOat1 and rOat3 mediate uptake of phenolsulfonphthalein by exchange with intravesicular glutaric acid, the effect of inward Na^+ and glutarate gradient on phenolsulfonphthalein transport was assessed. As shown in Fig. 3, the initial uptake of *p*-aminohippuric acid and phenolsulfonphthalein into basolateral membrane vesicles was stimulated by the inward Na^+ and glutarate gradients. In the presence of an inward Na^+ gradient, but absence of glutarate, the uptake of phenolsulfonphthalein was as same as that in the absence of Na^+ gradient (data not shown).

3.3. Uptake of phenolsulfonphthalein by kidney slices

The time course of the uptake of phenolsulfonphthalein by kidney slices is shown in Fig. 4A. The uptake of phenolsulfonphthalein increased linearly over a period of 30 min. Phenolsulfonphthalein uptake at 30 min was used to examine the concentration-dependence and the effects of various inhibitors. The uptake of phenolsulfonphthalein was saturated at a higher phenolsulfonphthalein concentration (Fig. 4B). Kinetic analysis revealed that the uptake of phenolsulfonphthalein consists of two components (Table 1).

3.4. Effects of rOat1 and rOat3 substrates on the uptake of phenolsulfonphthalein by kidney slices

Fig. 5 shows the effects of *p*-aminohippuric acid and salicylate, high-affinity substrates for rOat1, and cimetidine

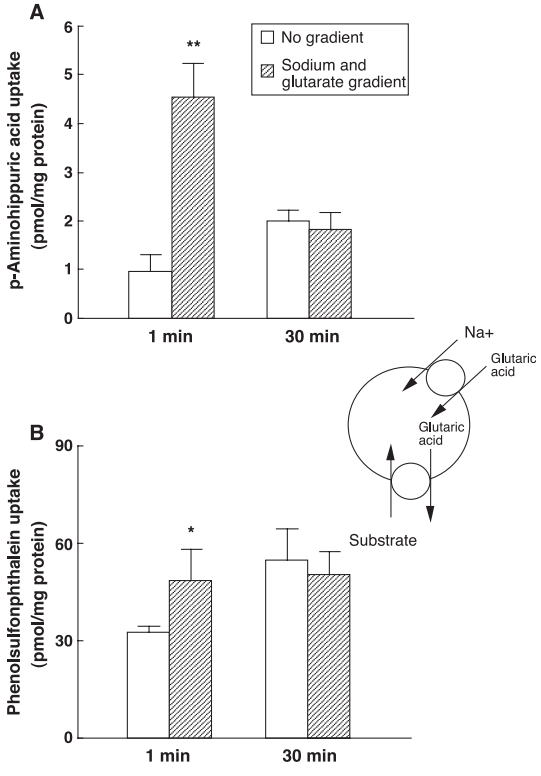

Fig. 3. Effects of inward sodium and glutarate gradients on the uptake of *p*-aminohippuric acid (A) and phenolsulfonphthalein (B) by Wistar rat renal basolateral membrane vesicles. Membrane vesicles were suspended in solution B. The drug solution contained 100 mM D-mannitol, 0.5 μM glutaric acid, 2 μM *p*-aminohippuric acid or 100 μM phenolsulfonphthalein, and 100 mM KCl or NaCl. Each column represents the mean with S.D. of three preparations. *$P<0.05$, **$P<0.01$, significantly different from control.

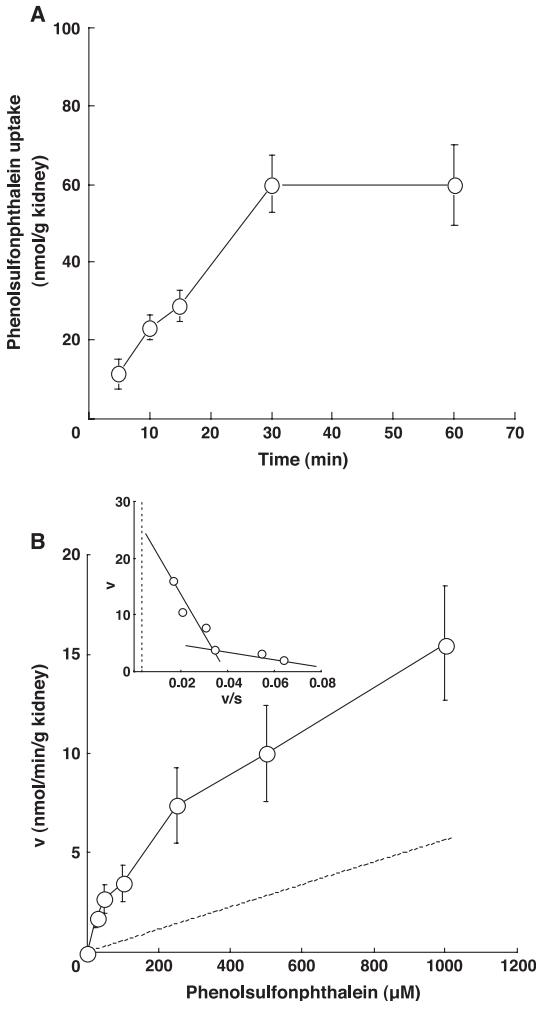

Fig. 4. Time courses (A) and concentration dependence (B) of the uptake of phenolsulfonphthalein by kidney slices. The uptake by kidney slices was measured at phenolsulfonphthalein concentrations of 25 μM to 1 mM over a period of 30 min. The solid and broken lines represent the fitted line and the uptake corresponding to the nonsaturable component, respectively. Each value represents the mean with S.D. of seven determinations.

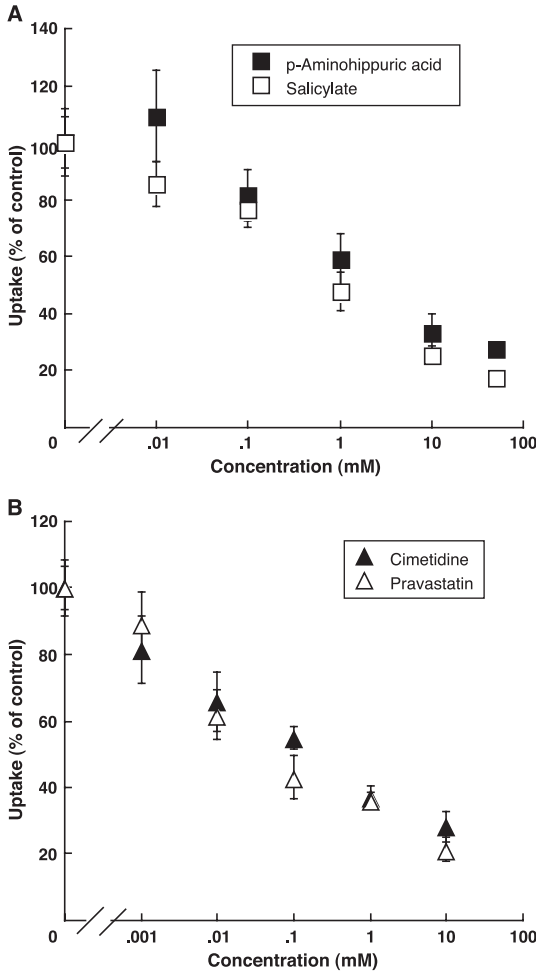

Fig. 5. Inhibitory effects of *p*-aminohippuric acid, salicylate, cimetidine and pravastatin on the uptake of phenolsulfonphthalein by kidney slices. The uptake of phenolsulfonphthalein (30 μM) by kidney slices was determined in the presence and absence of inhibitors at the designed concentrations. Each value is expressed as a percentage of the uptake in the absence of inhibitors. Each value represents the mean with S.D. of four determinations.

and pravastatin, rOat3-specific substrates, on the uptake of phenolsulfonphthalein by kidney slices (Russel et al., 2002). rOat3 substrates were more potent inhibitors of the uptake of phenolsulfonphthalein than are rOat1 substrates. The IC_{50} values of these compounds were obtained and they are summarized in Table 2. The IC_{50} values of rOat1 substrates were at least 10-times higher than those of rOat3 substrates.

3.5. Kinetics of phenolsulfonphthalein uptake in the presence of cimetidine

We examined the effect of cimetidine on the concentration dependence of the uptake of phenolsulfonphthalein by

Table 1
K_m and V_{max} values for the uptake of phenolsulfonphthalein by kidney slices

	No inhibitors		+Cimetidine
	High-affinity component	Low-affinity component	
K_m (μM)	33.1	1011	967
V_{max} (nmol/min/g kidney)	2.51	19.8	23.3

Data shown in Figs. 4B and 6 were used to determine the K_m and V_{max} values for the uptake of phenolsulfonphthalein by kidney slices. The K_m and V_{max} values were determined by nonlinear regression analysis.

Table 2
IC_{50} values of phenolsulfonphthalein uptake by kidney slices

	IC_{50} (μM)
p-Aminohippuric acid	575
Salicylate	614
Cimetidine	55.5
Pravastatin	13.4

Data shown in Fig. 5 were used to determine the IC_{50} values for the uptake of phenolsulfonphthalein by kidney slices. The IC_{50} values were determined by nonlinear regression analysis.

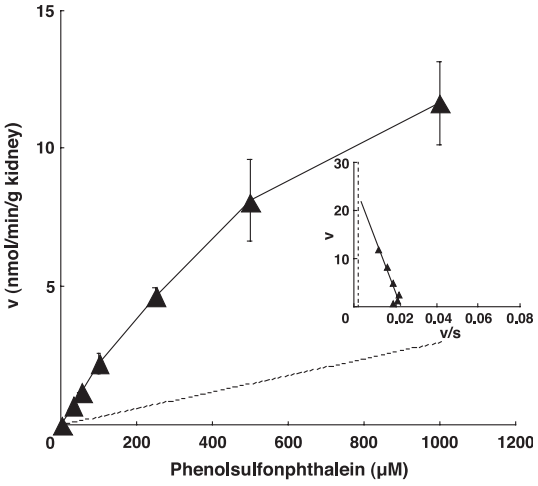

Fig. 6. Effect of cimetidine on the concentration dependence of the uptake of phenolsulfonphthalein by kidney slices. The uptake by kidney slices was measured at phenolsulfonphthalein concentrations of 25 μM to 1 mM over a period of 30 min in the presence of 10 mM cimetidine. The solid and broken lines represent the fitted line and the uptake corresponding to the nonsaturable component, respectively. Each value represents the mean with S.D. of four determinations.

kidney slices. Kinetic analysis by the Eadie–Hofstee plot gave a single straight line (Fig. 6). The profile of the phenolsulfonphthalein uptake component in the presence of cimetidine was similar to that of the low-affinity component but not that of the high-affinity component in the absence of cimetidine (Table 1).

4. Discussion

Phenolsulfonphthalein is normally excreted by the kidneys in humans (Gault et al., 1967). Thus, phenolsulfonphthalein has been used widely to assess renal function. However, the renal uptake mechanism has not been clarified yet. rOat1 and rOat3 mediate the initial active step in the process of organic anion secretion across the proximal tubule (Sekine et al., 2000; Tojo et al., 1999). The aim of the present study was to determine whether phenolsulfonphthalein is a substrate for rOat1 and rOat3, which are localized to the basolateral membrane of rat proximal tubule cells.

In the first part of this study, the urinary excretion of phenolsulfonphthalein after intravenous injections of *p*-aminohippuric acid and cimetidine was determined. *p*-Aminohippuric acid, a typical substrate of rOat1 (Hasegawa et al., 2002), and cimetidine, a specific substrate of rOat3 (Nagata et al., 2002), inhibited phenolsulfonphthalein excretion and remarkably reduced the CLurine value.

rOat1 and rOat3 are known to function as an dicarboxylate-coupled anion exchanger (Sekine et al., 1997; Shimada et al., 1987; Sweet et al., 1997, 2003). The glutalate gradient across the basolateral membrane is large and provides a powerful driving force for organic anion uptake via rOat1 and rOat3. Indeed, the magnitude of this driving force is sufficiently large to account for efficient organic anion secretion, often mediating substrate clearance in a single pass through the kidney. The initial uptake of *p*-aminohippuric acid and phenolsulfonphthalein into basolateral membrane vesicles was stimulated by inward Na^+ and glutarate gradients, suggesting that both drugs are recognized by rOat1 or rOat3. It has been reported that a Na^+ gradient alone is not sufficient for *p*-aminohippuric acid uptake into rat renal basolateral membrane vesicles (Shimada et al., 1987). In the presence of an inward Na^+ gradient, but absence of glutarate, the uptake of phenolsulfonphthalein was as same as that in the absence of Na^+ gradient.

The results of these in vivo studies and basolateral membrane vesicle studies suggest that the renal uptake of phenolsulfonphthalein is accounted for by rOat1 and rOat3. To confirm this hypothesis, we carried out a study on inhibition of uptake of phenolsulfonphthalein by kidney slices. We studied the contribution of rOat1 and rOat3 to the renal uptake of phenolsulfonphthalein by examining the inhibitory effects of their substrates on phenolsulfonphthalein uptake. The profile of phenolsulfonphthalein uptake indicated that the uptake of phenolsulfonphthalein consists of two components, suggesting that two transporters are involved in the basolateral uptake of phenolsulfonphthalein in renal epithelial cells. As summarized in Table 2, the results from the inhibition study suggest that, kinetically, there is a difference in the degrees of inhibition (Russel et al., 2002). rOat3 substrates are more potent inhibitors of the uptake of phenolsulfonphthalein by kidney slices, and the IC_{50} values were at least 10-fold smaller than those of rOat1 substrates. Uwai et al. (1998) demonstrated that hydrophilic dicarboxylates with a backbone of five or more carbons, but not those with a backbone of only three or four carbons, are able to inhibit Oat1-mediated *p*-aminohippuric acid transport. In contrast, the preferred substrates for Oat3 are larger and more hydrophobic compounds such as estrone sulfate (Cha et al., 2001). These findings suggest that rOat1 is responsible for the low-affinity component and that rOat3 is responsible for the high-affinity component. This was confirmed by examining the inhibitory effect of cimetidine on the concentration dependence for the uptake of phenolsulfonphthalein. Only the low-affinity component of phenolsulfonphthalein uptake remained in the presence of cimetidine. Taking of the all results presented in this paper into consideration, we conclude that phenolsulfonphthalein is a high-affinity substrate for rOat3 but is a relatively low-affinity substrate for rOat1. It has been reported that inhibition of organic anion transport consistently reduced *p*-aminohippuric acid accumulation in kidney slices to a larger extent than that of phenolsulfonphthalein (Sheikh, 1972). This finding supports our conclusion.

The rat organic anion transporting polypeptide 1 (rOatp1/*Slc21a1*) is thought to play a major role in the hepatic uptake of amphipathic organic anions (Meier et al., 1997). It

has been reported that substrates of rOat1 include hydrophilic and small molecules and do not overlap with those of the rOatp family. In contrast, many of the substrates of rOat3 are also substrates of rOatps (Eckhardt et al., 1999; Hasegawa et al., 2002; Kusuhara et al., 1999; Sugiyama et al., 2001). This overlap in substrates between rOatps and rOat3 but not rOat1 suggests that the substrates of rOat1 are mainly distributed in the kidney, whereas those of rOat3 are distributed not only in the kidney but also in the liver, because the substrates of rOat3 are recognized also by rOatps. Phenolsulfonphthalein is normally excreted largely by the kidneys in humans, but significant amounts have been found in the bile of some other species, including chickens, dogfish and rats (Adamson and Guarino, 1972; Hart and Schanker, 1966; Sperber, 1954). rOat3 is expressed in the rat kidney and liver (Kusuhara et al., 1999), while human OAT3 (hOAT3/*SLC22A8*) is predominantly expressed in the kidney (Cha et al., 2001). rOatp1, rOatp2 (*Slc21a5*) and rOatp4 (*Slc21a10*) have been demonstrated to be localized on the sinusoidal membrane of rat hepatocytes (Kusuhara and Sugiyama, 2002). A human counterpart of these rat transporters, hOATP-A (*SLC21A3*), has been isolated (Bossuyt et al., 1996). However, the expression level of hOATP-A in the liver is very low (Abe et al., 1999). Recently, hOATP-C (LST-1/*SLC21A6*) has been isolated from the liver (Abe et al., 1999; Hsiang et al., 1999). The amino acid sequence of hOATP-C exhibits a relatively low homology with that of hOATP-A. In addition to hOATP-C, hOATP-B (*SLC21A9*) and hOATP8 (LST-2/*SLC21A8*) have been demonstrated to be localized on the sinusoidal membrane of human hepatocytes (Kusuhara and Sugiyama, 2002). These observations may contribute to elucidation of the difference between biliary excretion of phenolsulfonphthalein in rats and humans. Further quantitative studies are required to confirm this hypothesis by examining the uptake of common substrates of rOat3 and rOatps by the liver and kidney.

In summary, we have demonstrated that rOat1 and rOat3 are involved in the renal uptake of phenolsulfonphthalein on the basolateral membrane of the proximal tubules. We have also found that phenolsulfonphthalein is a high-affinity substrate for rOat3 but is a relatively low-affinity substrate for rOat1.

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