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European Journal of Pharmacology 518 (2005) 83 – 89

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Phenolsulfonphthalein transport by potential-sensitive urate transport system

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Received 9 December 2004; received in revised form 10 May 2005; accepted 7 June 2005

Abstract

The purpose of this study was to elucidate the transporter-mediated secretion systems for phenolsulfonphthalein in brush-border membranes. In human and rat renal brush-border membranes, a potential-sensitive transport system has been shown to be involved in the efflux of organic anions. The uptake of phenolsulfonphthalein into rat renal brush-border membrane vesicles was stimulated by an inside-positive membrane potential. This potential-sensitive uptake of phenolsulfonphthalein was inhibited by probenecid, pyrazinoate and urate. p-Aminohippurate had no effect on the potential-sensitive uptake of phenolsulfonphthalein. Moreover, urate competitively inhibited the uptake of phenolsulfonphthalein. On the other hand, the uptake of phenolsulfonphthalein was slightly increased in the presence of an outward Cl^- gradient. These results suggest that phenolsulfonphthalein has high affinity for the potential-sensitive urate transport system but has low affinity for an anion exchanger.

Keywords: Urinary excretion; Phenolsulfonphthalein; p-Aminohippurate; Anion exchanger; Brush-border membrane

1. Introduction

Organic anions include a variety of drug metabolites and xenobiotics, many of which are harmful to the body. The kidney plays a central role in the elimination of anionic toxic compounds from the body. In humans and higher primates, urate is the end product of purine metabolism and is eliminated mainly by the kidney. Because of the complexity of the renal transport mechanism of urate, the transport mechanism for urate has not been fully elucidated (Sica and Schoolwerth, 2000). Recently, the long-hypothesized urate reabsorption transporter in the human kidney (URAT1/SLC22A13), an apical urate/anion exchanger regulating blood urate levels, has been identified (Enomoto et al., 2002). On the other hand, the transport mechanism for urate on the brush-border membranes of proximal tubules has been only partly elucidated. In human and rat renal brush-border membranes, a potential-sensitive transport system has been shown to be involved in the efflux of urate (Roch-Ramel et al., 1994). However, the urate efflux transporter in the human and rat kidney has not yet been elucidated at the molecular level.

p-Aminohippurate has been used as a model substrate to investigate renal handling of organic anions because of its high renal clearance and insusceptibility to metabolism (Moller and Sheikh, 1983). In humans as in rats, the efflux of p-aminohippurate from proximal cells to the tubular lumen also occurs through a potential-sensitive transport system (Hori et al., 1982; Ohoka et al., 1993). It is thought that the potential-sensitive p-aminohippurate transport system is different from the potential-sensitive urate transport system (Ohoka et al., 1993; Roch-Ramel et al., 1994).

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doi:10.1016/j.ejphar.2005.06.009

More than 80 years ago, Marshall and colleagues established the foundation for tubule secretory function using phenolsulfonphthalein (Marshall and Vickers, 1923). Subsequent studies using phenolsulfonphthalein have revealed that tubular secretion is a fundamental process in the elimination of urine by the kidney. Thus, phenolsulfonphthalein as well as p-aminohippurate have been used as representative substrates for renal organic anion transport systems. However, it has been suggested that the excretion mechanism of phenolsulfonphthalein is different from that of p-aminohippurate (Smith, 1951). The reason for this difference has not been determined. Excretion of organic anions in renal proximal tubules involves the uptake of organic anions across basolateral membranes into cells and exit into the lumen across brush-border membranes (Russel et al., 2002). Recently, we have found that rat organic anion transporter 1 and transporter 3 (rOAT1/Slc22a6 and rOAT3/Slc22a8) are involved in the renal uptake of phenolsulfonphthalein on the basolateral membrane of proximal tubules and that phenolsulfonphthalein is a high-affinity substrate for rOAT3 but is a relatively low-affinity substrate for rOAT1 (Itagaki et al., 2003). Since p-aminohippurate is a high-affinity substrate for rOAT1, our reported findings might be one of the reasons for the difference stated above. However, the transporters responsible for the transport of phenolsulfonphthalein in the brush-border membrane have not been identified.

The aim of the present study was to determine the validity of the hypothesis that the efflux of phenolsulfonphthalein from proximal cells to the tubular lumen occurs through a potential-sensitive transport system and to determine the contribution of the potential-sensitive urate transport system and potential-sensitive p-aminohippurate transport system to the secretory process of phenolsulfonphthalein across proximal tubular cells.

2. Materials and methods

2.1. Chemicals

Phenolsulfonphthalein, pyrazinoate and p-aminohippurate were purchased from Wako Pure Chemical (Osaka, Japan). Probenecid and urate were purchased from Sigma Chemical Co. (St Louis, MO). [^{14}C] Urate (54 mCi/mmol) was purchased from Moravek Biochemicals, Inc. (Brea, CA). All other reagents were of the highest grade available and used without further purification.

2.2. Animals

Male Wistar rats, aged 7 to 8 weeks (300–350 g in weight), were obtained from NRC Haruna (Gunma, Japan). The housing conditions were described previously (Itoh et al., 2004). 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. Preparation of brush-border membrane vesicles

Renal brush-border membrane vesicles were prepared from the rat kidney by the calcium precipitation method with some modification as described previously (Evers et al., 1978). All steps were performed on ice or at 4 °C. Kidneys were excised from the rats under sodium pentobarbital anesthesia (40 mg/kg weight, i.p.). Kidney cortex slices (8 to 12 kidneys) were homogenized in 80 ml of ice-cold solution A (10 mM D-mannitol, 2 mM Tris/HCl, pH 7.1) with a Waring blender at 16,500 rpm for 8 min. $CaCl_2$ solution (0.5 M) was added to a final concentration of 10 mM, and the homogenate was allowed to stand for 15 min. The homogenate was centrifuged at 3000 $\times g$ for 12 min, and the supernatant was recentrifuged at 19,000 $\times g$ for 12 min. The resulting pellet was resuspended in 40 ml of ice-cold solution A and homogenized in a glass/Teflon Dounce-type homogenizer with 10 strokes. $CaCl_2$ solution (0.5 M) was added to a final concentration of 10 mM, and the homogenate was allowed to stand for 15 min. The homogenate was centrifuged at 3000 $\times g$ for 12 min, and the supernatant was recentrifuged at 27,000 $\times g$ for 30 min. The purified membranes were suspended in an experimental buffer containing 100 mM D-mannitol, 100 mM Na-gluconate and 20 mM HEPES/Tris (pH 7.4) or 100 mM D-mannitol, 100 mM KCl and 20 mM HEPES/Tris (pH 7.4) and were homogenized in a glass/Teflon Dounce-type homogenizer with 10 strokes. After a final centrifugation at 27,000 $\times g$ for 30 min, the brush-border membranes were suspended in an experimental buffer with or without valinomycin. Alkaline phosphatase (a marker enzyme of the brush-border membrane) activity level of the brush-border membrane was 12.4±1.7-fold higher than that of the initial homogenate (mean with S.D. of 6 preparations). In contrast, Na^+–K^+ ATPase (a marker enzyme of the basolateral membrane) activity level of the brush-border membrane was 1.1±0.4-fold higher than that of the initial homogenate (mean with S.D. of 6 preparations, $P<.01$, significantly different from the ratio of alkaline phosphatase). This means that brush-border membranes were enriched at least 10-fold with respect to the basolateral membranes.

2.4. Uptake experiments

The uptake of phenolsulfonphthalein into brush-border membrane vesicles was determined by the rapid filtration technique described previously (Itagaki et al., 2005). In a routine assay, 40 μl of membrane vesicles (0.4–0.6 mg protein) suspension was added to 200 μl of incubation medium kept at 25 °C. The compositions of the media are described in the figure legend. At selected time intervals, the uptake was stopped by diluting the incubation medium with 5 ml of ice-cold 10 mM HEPES buffer (pH 7.4) containing 150 mM KCl. The mixture was immediately filtered through a Millipore filter (0.45 μm in pore size, 2.5 cm in diameter; HAWP). The filter was rinsed with 3 ml of the same buffer. Substrate trapped on the filter was extracted with 300 μl of water, and the concentration of substrate was determined. In experience where valinomycin was used, the ionophore dissolved in ethanol (150 μg/ml), or ethanol alone as a control, was added 30 min prior to the assay. The final concentration of ethanol was 0.1%. To assay the radiolabeled compounds, substrate trapped on the filter was extracted with 10 ml of ACSII (Amersham International, UK), and the radioactivity was determined.

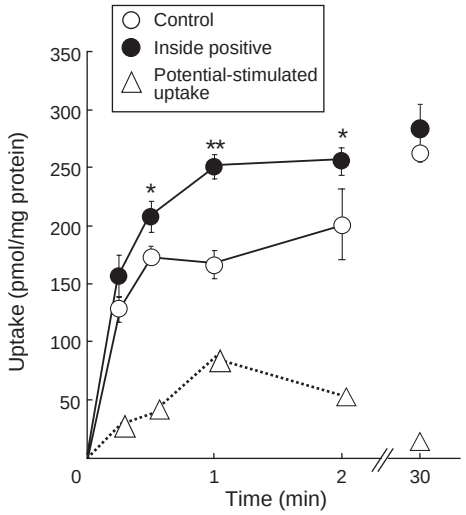

Fig. 1. Effect of positive intravesicular potential on the uptake of phenolsulfonphthalein by rat renal brush-border membrane vesicles. Membrane vesicles were suspended in 100 mM D-mannitol, 100 mM Na-gluconate and 20 mM HEPES/Tris (pH 7.4) and treated either with valinomycin (7 μg/mg protein) or ethanol (control). The drug solution contained 100 mM D-mannitol, 120 μM phenolsulfonphthalein, 100 mM K-gluconate and 20 mM HEPES/Tris (pH 7.4). Each point represents the mean with S.D. of 3 preparations. *$P<.05$, **$P<.01$, significantly different from the control.

2.5. Analytical procedures

Phenolsulfonphthalein concentration was determined using an HPLC system equipped with a Hitachi L-6000 pump and an L-4200H UV/VIS detector described previously (Itagaki et al., 2004a,b). 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. Radioactivity was determined using a liquid scintillation counter (Packard, 1600TR). Protein was measured by the method of Lowry et al. (1951) with bovine serum albumin as a standard. Statistical significance was evaluated using one-way analysis of variance (one-way ANOVA) or unpaired Student's *t*-test. A value of $P<0.05$ was considered significant. Kinetic parameters were obtained using the following equation:

$$v = V_{max}S/(K_m + S),$$

where v is the uptake rate of phenolsulfonphthalein (pmol/min/mg protein), 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/mg protein). Fitting was performed by the nonlinear least-squares method using Origin® (version 6.1J).

3. Results

3.1. Characteristics of potential-sensitive transport of phenolsulfonphthalein

In brush-border membranes of human and rat kidneys, organic anions such as urate and p-aminohippurate are transported by two different transport systems: a potential-sensitive transport system and an anion exchanger (Hori et al., 1982; Ohoka et al., 1993; Roch-Ramel et al., 1994). When an inside-positive membrane potential was generated by an inwardly directed K^+ gradient in the presence of the K^+ ionophore valinomycin, the uptake of phenolsulfonphthalein was significantly accelerated compared with that in the absence of valinomycin (Fig. 1). Potential-stimulated uptake was obtained as the difference between the uptake in the presence of positive intravesicular potential and that in the absence of positive intravesicular potential. Potential-stimulated uptake of phenolsulfonphthalein increased linearly over a period of 1 min. Phenolsulfonphthalein uptake at 1 min was used to determine concentration dependence and effects of various inhibitors. Fig. 2 shows the curve for the concentration dependence of the potential-stimulated uptake of phenolsulfonphthalein by brush-border membrane vesicles. The relationship between the concentration and the rate of uptake was nonlinear, providing evidence for saturability. The K_m and V_{max} values were determined by kinetic analysis to be 1.28 mM and 3.67 nmol/min/mg protein, respectively.

Fig. 3 shows that the uptake of phenolsulfonphthalein into brush-border membrane vesicles stimulated by the inside-positive membrane potential was significantly inhibited by potential-sensitive urate transport system inhibitors, probenecid, urate and pyrazinoate (Roch-Ramel et al., 1994). On the other hand, p-aminohippurate had no effect on the potential-stimulated uptake of phenolsulfonphthalein. Moreover, in the absence of an inside-positive potential, these compounds did not affect the uptake of phenolsulfonphthalein into brush-border membrane vesicles (data not shown).

Results of Dixon plot analysis of phenolsulfonphthalein uptake in the presence of urate and a positive intravesicular potential are shown in Fig. 4. Urate was found to inhibit the uptake of phenolsulfonphthalein competitively. The regression line obtained from the replot of slopes of the Dixon plot almost coincided with the origin (Fig. 4, inset), indicating that urate transport is mediated by a common potential-sensitive transport system with phenolsulfonphthalein. The apparent K_i value calculated from Dixon plots for urate was 0.45 mM.

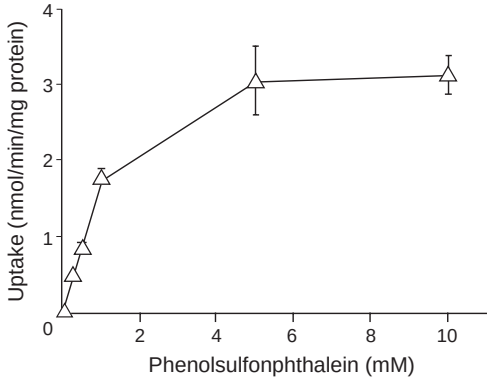

Fig. 2. Concentration dependence of potential-stimulated phenolsulfonphthalein uptake. Incubation conditions were identical to those described in the legend to Fig. 1. Potential-stimulated uptake was obtained as the difference between the uptake in the presence of positive intravesicular potential and that in the absence of positive intravesicular potential. Each point represents the mean with S.D. of 3 preparations.

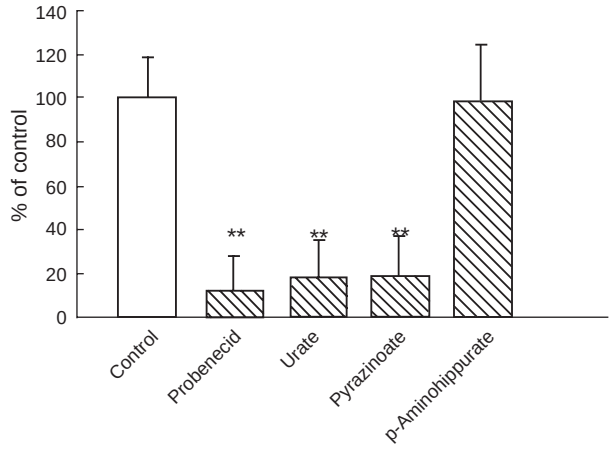

Fig. 3. Effects of various compounds on the potential-stimulated uptake of phenolsulfonphthalein. Membrane vesicles were suspended in 100 mM D-mannitol, 100 mM Na-gluconate and 20 mM HEPES/Tris (pH 7.4) and treated either with valinomycin (7 μg/mg protein) or ethanol (control). The drug solution contained 100 mM D-mannitol, 120 μM phenolsulfonphthalein, 100 mM K-gluconate and 20 mM HEPES/Tris (pH 7.4) in the presence of various compounds. The final concentration of each inhibitor was 1 mM. Potential-stimulated uptake was obtained as the difference between the uptake in the presence of positive intravesicular potential and that in the absence of positive intravesicular potential. The control value for potential-stimulated uptake of phenolsulfonphthalein was 105.7±19.0 pmol/mg protein. Each column represents the mean with S.D. of 3 preparations. $^{**}P>.01$, significantly different from the control.

3.2. Characteristics of potential-sensitive transport of urate

In order to clarify whether urate shares a transporter with phenolsulfonphthalein, the opposite inhibitory effect of phenolsulfonphthalein on the uptake of urate was investigated. In the presence of positive intravesicular potential, the uptake of urate was significantly accelerated compared with that in the absence of positive intravesicular potential (Fig. 5A). Potential-stimulated uptake of urate increased linearly over a period of 1 min. Urate uptake at 1 min was used to determine the effect of phenolsulfonphthalein. Fig. 5B shows that the uptake of urate into brush-border membrane vesicles stimulated by the inside-positive membrane potential was significantly inhibited by phenolsulfonphthalein.

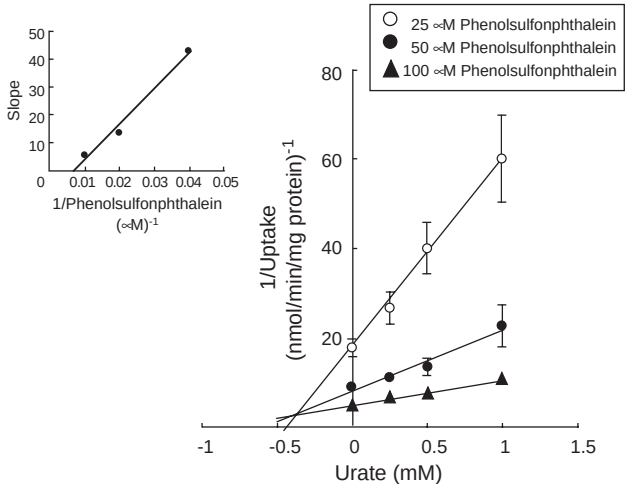

Fig. 4. Dixon plot of potential-stimulated phenolsulfonphthalein uptake into rat renal brush-border membrane vesicles in the presence of urate. Uptake of 25, 50 and 100 μM phenolsulfonphthalein was measured for 1 min in the presence of a positive intravesicular potential with urate. Incubation conditions were identical to those described in the legend to Fig. 3. Potential-stimulated uptake was obtained as the difference between the uptake in the presence of positive intravesicular potential and that in the absence of positive intravesicular potential. Each point represents the mean with S.D. of 3 preparations. The apparent K_i value was determined to be 0.45 mM by linear regression analysis from the Dixon plot. The insets shows a replot of the slopes of Dixon plot.

3.3. Phenolsulfonphthalein transport by an anion exchanger

Not only human URAT1 but also the anion exchanger of rat brush-border membranes uses Cl^- as a substrate (Enomoto et al., 2002; Ohoka et al., 1993). The effect of an outwardly directed Cl^- gradient on phenolsulfonphthalein uptake was investigated.

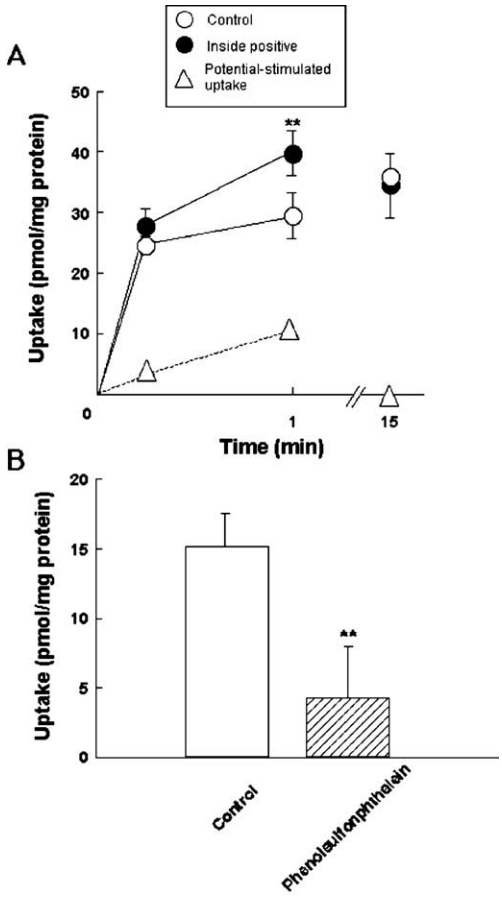

Fig. 5. (A) Effect of positive intravesicular potential on the uptake of urate by rat renal brush-border membrane vesicles. Membrane vesicles were suspended in 100 mM D-mannitol, 100 mM Na-gluconate and 20 mM HEPES/Tris (pH 7.4) and treated either with valinomycin (7 μg/mg protein) or ethanol (control). The drug solution contained 100 mM D-mannitol, 48 μM urate, 100 mM K-gluconate and 20 mM HEPES/Tris (pH 7.4). Each point represents the mean with S.D. of 3 preparations. $^{*}P<.05$, $^{**}P<.01$, significantly different from the control. (B) Effect of phenolsulfonphthalein on the potential-stimulated uptake of urate. The final concentration of phenolsulfonphthalein was 1 mM. Potential-stimulated uptake was obtained as the difference between the uptake in the presence of positive intravesicular potential and that in the absence of positive intravesicular potential. Each column represents the mean with S.D. of 3 preparations. $^{**}P<.01$, significantly different from the control.

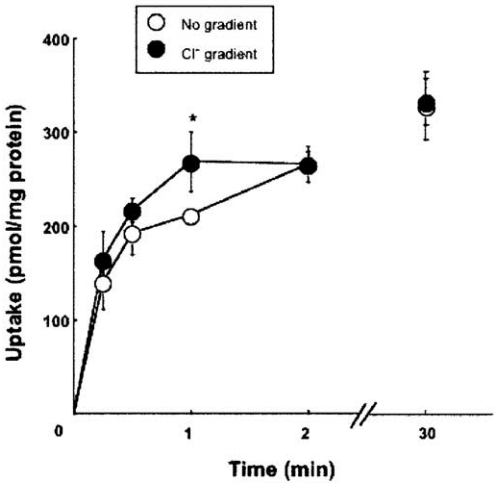

Fig. 6. Effect of an outwardly directed Cl^- gradient on the uptake of phenolsulfonphthalein by rat renal brush-border membrane vesicles. Membrane vesicles were suspended in 100 mM D-mannitol, 100 mM KCl and 20 mM HEPES/Tris (pH 7.4) in the presence of valinomycin (7 μg/mg protein) and FCCP (10 μg/mg protein). The drug solution contained 100 mM D-mannitol, 120 μM phenolsulfonphthalein, 100 mM KCl (no gradient) or 100 mM K-gluconate (chloride gradient) and 20 mM HEPES/Tris (pH 7.4). Each point represents the mean with S.D. of 3 preparations. $*P<.05$, significantly different from the control.

Because the diffusion of Cl^- along its gradient may induce an electropositive intravesicular space and alkalinization of the intravesicular space may result from the activity of a Cl^-/OH^- exchanger, two protocols were adopted for the experiments for which the results are shown in Fig. 6 (Roch-Ramel et al., 1994). The proton ionophore p-trifluoromethoxyphenylhydrazone (FCCP) was used to maintain a pH equilibrium across the membranes. Membrane potential was clamped to zero by using valinomycin. The uptake of phenolsulfonphthalein into brush-border membrane vesicles in the presence of an outwardly directed Cl^- gradient was compared with the uptake measured at equal Cl^- concentrations on both sides of the membrane. The results of a representative experiment are shown in Fig. 6. The uptake of phenolsulfonphthalein into brush-border membrane vesicles was slightly increased in the presence of an outwardly directed Cl^- gradient. The concentration dependence of the Cl^-/phenolsulfonphthalein exchange was examined. The Cl^-/phenolsulfonphthalein exchange was not saturated up to 10 mM (data not shown).

4. Discussion

The kidney plays an important role in the urinary excretion of drugs and xenobiotics via glomerular filtration and tubular excretion. Cumulative studies have revealed the importance of transporters in tubular excretion (Miyazaki et al., 2004). Vectorial transport across renal tubules is achieved by an interplay between the uptake and efflux transport at the basolateral membrane and brush-border membrane, respectively (Russel et al., 2002). It has been reported that the potential-sensitive transport system plays an important role in the efflux of organic anions across brush-border membranes in rats, because the intracellular compartment has a more negative electrical potential than that of the luminal fluid compartment in proximal tubules (Takano et al., 1994). In humans and rats, it is thought that there are two different potential-sensitive transport systems in the renal brush-border membranes. One is a potential-sensitive urate transport system and the other is a potential-sensitive p-aminohippurate transport system (Ohoka et al., 1993; Roch-Ramel et al., 1994).

Phenolsulfonphthalein is widely used clinically as a drug for testing renal function because of its high renal clearance (Gault et al., 1967). However, it has been suggested that phenolsulfonphthalein and p-aminohippurate do not to share the same transporter in the kidney (Smith, 1951). Recently, we have found that phenolsulfonphthalein is a high-affinity substrate for rOAT3 (Itagaki et al., 2003), whereas p-aminohippurate is a high-affinity substrate for rOAT1. However, the role of the potential-sensitive transport systems in renal handling of phenolsulfonphthalein has not been elucidated. In this study, therefore, we attempted to clarify the nature of the potential-sensitive transport of phenolsulfonphthalein using rat renal brush-border membrane vesicles.

The uptake of phenolsulfonphthalein into brush-border membrane vesicles was stimulated by an inside-positive membrane potential created by an inward K^+ gradient in the presence of valinomycin. Hence, the magnitude of the membrane potential should be an important determinant of phenolsulfonphthalein transport across rat renal brush-border membranes. The potential-stimulated phenolsulfonphthalein uptake was inhibited by probenecid, urate and pyrazinoate. On the other hand, p-aminohippurate had no effect on the potential-stimulated uptake of phenolsulfonphthalein into brush-border membrane vesicles. It has been reported that urate inhibited p-aminohippurate uptake by the anion exchanger more strongly than potential-stimulated uptake (Ohoka et al., 1993). These findings suggest that phenolsulfonphthalein has high affinity for the potential-sensitive urate transport system but has low affinity for the potential-sensitive p-aminohippurate transport system. Furthermore, the results of Dixon plot analysis indicated that phenolsulfonphthalein transport occurs via the potential-sensitive urate transport system.

In the present study, the opposite inhibitory effect of phenolsulfonphthalein on urate uptake was investigated. Phenolsulfonphthalein significantly inhibited the uptake of urate. Taking all of the results presented in this paper into consideration, we conclude that urate and phenolsulfonphthalein share the same transport system at brush-border membranes of proximal tubular cells.

In some species, urate can be transported by an anion exchanger in renal brush-border membranes (Enomoto et al., 2002; Kahn and Aronson, 1983; Roch-Ramel et al., 1994). The exchanger is assumed to exist only in urate-reabsorbing species such as dogs, humans and rats (Enomoto et al., 2002; Kahn and Aronson, 1983; Ohoka et al., 1993; Roch-Ramel et al., 1994). It has been suggested

that this anion exchanger plays a major role in urate reabsorption (Pritchard and Miller, 1993). Phenolsulfonphthalein transport by the anion exchanger was also examined in the present study using rat renal brush-border membranes. In the presence of an outward Cl^- gradient, the uptake of phenolsulfonphthalein into brush-border membrane vesicles was only slightly increased. Moreover, the Cl^-/phenolsulfonphthalein exchange was not saturated up to 10 mM. These results suggest that phenolsulfonphthalein is a low-affinity substrate for the anion exchanger. It has been reported that only a small amount of phenolsulfonphthalein is reabsorbed in the proximal tubule (Gerdes et al., 1978). Our findings are identical to the results presented in that report.

In humans and higher primates, urate is the end product of purine metabolism and is eliminated mainly by the kidney. The renal excretion of urate is the result of rather complex mechanisms (Sica and Schoolwerth, 2000). The major models of renal urate handling include filtration, reabsorption, secretion, and postsecretory reabsorption. Although urate is freely filtered, a large amount of filtered urate is reabsorbed. The renal urate reabsorption mechanism has been studied. It has been clarified that an urate/anion exchanger, such as URAT1, plays a principal role in the regulation of urate transport. However, the role of secretion of urate remains unclear. Because of the complexity of the renal transport mechanism of urate, it is difficult to predict in vivo urate disposition from in vitro data. Unlike urate, only 6% of phenolsulfonphthalein is filtered. Phenolsulfonphthalein should be useful for clarifying the renal secretion mechanism of urate.

Hyperuricemia is mainly caused by impaired urinary excretion of urate. Although the renal excretion of urate is the result of rather complex mechanisms, it is difficult to distinguish impaired urinary excretion of urate caused by increased urate reabsorption from that caused by decreased urate secretion. It is known that hyperuricemia contributes to the development of cardiovascular diseases, including hypertension and ischemic heart diseases (Waring et al., 2000). Recently, Iwai et al. (2004) investigated the contribution of inactivating mutations in *SLC22A12* to hyperuricemia and showed that inactive *SLC22A12* is not a major gene for hyperuricemia. However, the contribution of the urate secretory system to hyperuricemia has been only partly elucidated. Since phenolsulfonphthalein is a low-affinity substrate for the anion exchanger, phenolsulfonphthalein should be useful for assessing the function of the renal urate secretory system.

Recently, a novel potential-sensitive transporter (OATv1) has been identified at the brush-border membranes of pig renal proximal tubules (Jutabha et al., 2003). In addition to OATv1, Imaoka et al. (2004) showed that a mouse renal-specific transporter (RST) is a classical facilitative transporter at the brush-border membranes of renal proximal tubules, which has been hypothesized to be involved in the efflux of organic anions, and that the substrate specificity of mouse RST overlaps with that of basolateral organic anion transporters. The K_m values of urate for OATv1 and mouse RST were shown to be >5 and 1.2 mM, respectively (Hosoyamada et al., 2004; Jutabha et al., 2003). In this study, the estimated K_i value of urate for phenolsulfonphthalein uptake (0.45 mM) was not greatly different from the K_m value of urate for mouse RST. In humans and rats, the potential-sensitive p-aminohippurate transport system is thought to be different from the potential-sensitive urate transport system, whereas OATv1 mediated the transport of p-aminohippurate and urate (Jutabha et al., 2003). Unlike OATv1, although RST1 mediates the transport of p-aminohippurate, p-aminohippurate did not have any inhibitory effect on urate uptake by mouse RST (Hosoyamada et al., 2004; Imaoka et al., 2004). It has been suggested that mouse RST has multiple substrate recognition sites for p-aminohippurate and urate (Imaoka et al., 2004). In contrast to humans, rats and mice, pigs exhibit minimal urate reabsorption. Taking all of the facts into consideration, it is possible that the rat homologue of mouse RST plays a major role in the renal secretion of phenolsulfonphthalein. However, this transport system has not been elucidated at the molecular level. Further studies are needed to assess the importance of the rat homologue of mouse RST in the renal secretion of organic anions in the kidney.

In summary, the results of this study suggest that phenolsulfonphthalein has high affinity for the potential-sensitive urate transport system but has low affinity for the potential-sensitive p-aminohippurate transport system and anion exchanger. Since phenolsulfonphthalein is a low-affinity substrate for the anion exchanger, phenolsulfonphthalein should be useful for assessing the function of the renal urate secretory system.

Acknowledgement

This work was supported in part by NOASTEC.

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