Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1778 (2008) 270–275
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbamem

Functional analysis of phenolsulfonphthalein transport system in Long–Evans Cinnamon rats

Shirou Itagaki [a], Makoto Chiba [a], Masaki Kobayashi [a], Mitsuru Sugawara [b], Michiya Kobayashi [b], Takeshi Hirano [a], Ken Iseki [a,*]

[a] *Laboratory of Clinical Pharmaceutics and Therapeutics, Division of Pharmasciences, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan*
[b] *Department of Pharmacy, Hokkaido University Hospital, Sapporo, Japan*

Received 12 April 2007; received in revised form 25 September 2007; accepted 27 September 2007
Available online 4 October 2007

Abstract

It has been reported that the transport function for organic anions on the kidney is maintained in multidrug resistance-associated protein 2 (Mrp2)-deficient rats. Different from Mrp2-deficient rats, Long–Evans Cinnamon (LEC) rats have impaired urinary excretion of Mrp2-substrate, phenolsulfonphthalein (PSP). PSP is transported by the potential-sensitive urate transport system in rat brush-border membranes. We analyzed the function of PSP transport system in LEC rats. Unlike Long–Evans Agouti (LEA) rats, the initial uptake of PSP and urate into the renal brush-border membrane vesicles of LEC rats were not significantly enhanced in the presence of positive intravesicular potential, suggesting that the potential-sensitive urate transport system is impaired in LEC rats. LEC rats should be useful for elucidating the potential-sensitive urate transport system in rats at the molecular level.

Keywords: LEC rats; Urinary excretion; Transporter; Organic anion; Urate

1. Introduction

A mutant strain of inbred Long–Evans rats that spontaneously develop acute hepatitis associated with jaundice, chronic hepatitis, and ultimately hepatocellular carcinoma, the Long–Evans Cinnamon (LEC) strain, has been established [1]. LEC rats suffer from fulminant hepatitis and severe jaundice at about 4 months of age [2]. Furthermore, liver cancer appears in long-surviving rats after recovery from jaundice [3,4]. Therefore, LEC rats provide a pertinent model for basic and clinical studies on hepatitis and liver cancer.

Multidrug resistance-associated protein 2 (Mrp2/Abcc2) has been shown to mediate the transport of endobiotics (e.g., bilirubin and bilirubin conjugates). Mutations in the Mrp2 gene have been identified as the pathogenetic basis of hereditary chronic conjugated hyperbilirubinemia [5]. The function of Mrp2 has been extensively studied by comparing the transport across the bile canalicular membrane in normal rats and that in Mrp2-deficient rats (e.g., Eisai hyperbilirubinemic rats (EHBR), transport deficient (TR^-) and Groningen Yellow (GY)) [6–8]. These animals have a defect in the hepatobiliary excretion of a broad range of organic anions, including bilirubin glucuronides and other multivalent organic anions. However, it has been reported that the transport function for organic anions on the kidney is maintained in these animals and that the contribution of Mrp2 to urinary excretion is minor [9,10].

Sulfobromophthalein (BSP) and phenolsulfonphthalein (PSP) are widely used clinically as drugs for testing liver function and renal function, respectively [11,12]. A previous study has revealed that LEC rats have not only impaired canalicular transport of BSP but also impaired urinary excretion of PSP [13]. However, urinary nitrogen and creatinine did not show serious renal failure [14]. These results suggest that the function of the renal secretion system for PSP in LEC rats is impaired.

* Corresponding author. Kita 12-jo, Nishi 6-chome, Kita-ku, Sapporo 060-0812, Japan. Tel./fax: +81 11 706 3770.
E-mail address: ken-i@pharm.hokudai.ac.jp (K. Iseki).

0005-2736/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2007.09.025

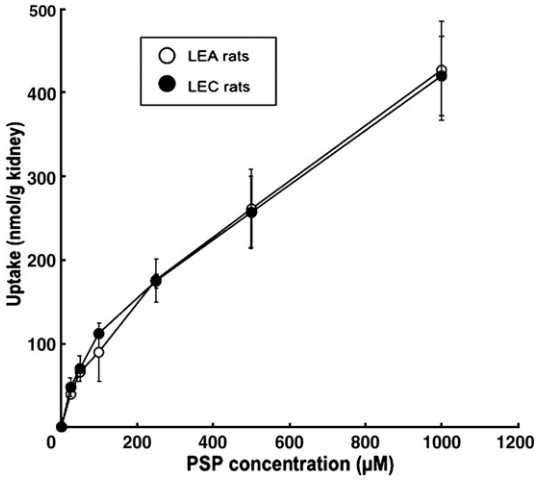

Fig. 1. Concentration dependence of the uptake of PSP by kidney slices. The uptake by kidney slices was measured at PSP concentrations of 25 μM to 1 mM over a period of 30 min. Each value represents the mean with SD of four determinations.

The present study was carried out to characterize the functional properties of the renal transport system for PSP in LEC rats. In this study, we used Long–Evans Agouti (LEA) rats as a control.

2. Materials and methods

2.1. Chemicals

PSP was purchased from Wako Pure Chemical (Osaka, Japan). Probenecid was purchased from Sigma Chemical Co. (St. Louis, MO). [^{3}H]p-Aminohippurate (PAH) (4.90 Ci/mmol) was purchased from NEN Life Science Products (Hoofddrop, The Netherlands). [^{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 LEC and LEA rats, aged 6 to 9 weeks (200–250 g in weight), were obtained from the Center for Experimental Plants and Animals of Hokkaido University (Sapporo, Japan). The housing conditions were described previously [15]. 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" as adopted by the National Institutes of Health.

2.3. Study of uptake by kidney slices

Uptake studies were carried out as described in our previous report [16]. Slices of whole kidneys from 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 to 100 mg) were incubated in a 6-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 of PSP by kidney slices was measured at PSP concentrations of 25 μM to 1 mM over a period of 30 min at 37 °C. After incubation, 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 PSP in the supernatant was measured.

2.4. Preparation of brush-border membrane vesicles

Renal brush-border membrane vesicles (BBMV) were prepared from the rat kidney by the calcium precipitation method with some modification as described previously [17]. The purified membranes were suspended in a buffer containing 100 mM D-mannitol, 100 mM KCl and 20 mM HEPES/Tris (pH 7.4) or 100 mM D-mannitol, 100 mM Na–gluconate and 20 mM HEPES/Tris (pH 7.4). Alkaline phosphatase (a marker enzyme of the brush-border membrane) activity levels of the LEA and LEC rat brush-border membrane were 11.2±1.3-fold and 12.1±1.7-fold higher than those of the initial homogenates (mean with S.D. of 6 preparations), respectively. In contrast, Na^+–K^+ ATPase (a marker enzyme of the basolateral membrane) activity level of the LEA and LEC rat brush-border membrane were 1.3±0.2-fold and 1.1±0.2-fold higher than those of the initial homogenates (mean with S.D. of 6 preparations, $P<0.01$, significantly different from the ratio of alkaline phosphatase), respectively. These findings indicate that brush-border membranes were enriched at least 10-fold with respect to the basolateral membranes.

2.5. Study of uptake by brush-border membrane vesicles

The uptake of substrates into BBMV was determined by the rapid filtration technique described previously [18]. In a routine assay, 40 μl of membrane vesicles (0.4–0.6 mg protein) suspension was added to 200 μl of substrate

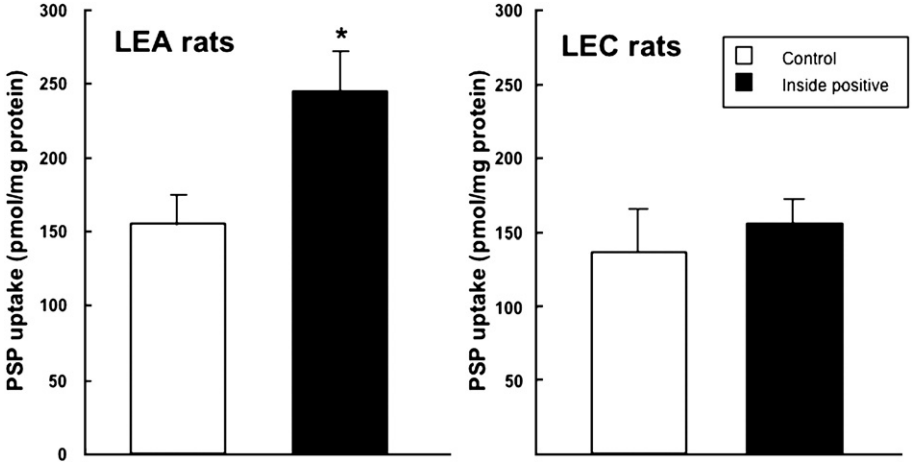

Fig. 2. Effect of a positive intravesicular potential on PSP uptake by LEA and LEC rat renal BBMV. Membrane vesicles were suspended in 100 mM D-mannitol, 100 mM Na-gluconate and 20 mM HEPES/Tris (pH 7.4), and treated either with valinomycin (7 μg/mg protein) or ethanol (control). The drug solution contained 100 mM D-mannitol, 120 μM PSP, 100 mM K-gluconate and 20 mM HEPES/Tris (pH 7.4). Uptake of PSP was determined after 1 min of incubation. Each point represents the mean with SD of three determinations *$P<0.05$, significantly different from the control.

Table 1
Effects of various compounds on the potential-stimulated substrate uptake

	Potential-stimulated uptake (% of Control)	
	PSP	Urate
Control	100	100
Probenecid	37.0±4.09*	27.5±15.2*
Urate	23.6±1.87*	–
PSP	–	38.5±4.03*

Incubation conditions were identical to those described in the legends to Figs. 2 and 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 3–6 preparations. The control values for potential-stimulated uptake of PSP and urate were 90.3±9.72 and 9.75±0.68 pmol/mg protein, respectively. *$P<0.05$, significantly different from the control.

mixture kept at 25 °C. The compositions of the media are described in the figure legends. After incubation for 1 min, 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 experiments 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.

2.6. Analytical procedures

PSP was determined using an HPLC system equipped with a Hitachi L-6000 pump and L-4200H UV/VIS detector described previously [19]. The column was a Hitachi ODS Gel #3053 (4 mm i.d.×250 mm). Column temperature and flow rate were 55 °C and 0.7 ml/min, respectively. In the assay for PSP, a mobile phase containing 20% acetonitrile and 50 mM H_3PO_4 with pH adjusted to 3.0 by NaOH was used. The wavelength of the detector for PSP was 432 nm. Radioactivity was determined using a liquid scintillation counter (Packard, 1600TR). Protein was measured by the method of Lowry et al. with bovine serum albumin as a standard [20]. Student's *t*-test was used for statistical analysis, and a value of $P<0.05$ was considered significant.

3. Results

3.1. Characteristics of the renal uptake system for PSP in LEC rats

Secretion 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 [21]. We recently reported that rat organic anion transporters 1 and 3 (rOat1/Slc22a6 and rOat3/Slc22a8) are involved in the renal uptake of PSP and that PSP is a high-affinity substrate for rOat3 but is a relatively low-affinity substrate for rOat1 [16]. The first experiment was performed to evaluate the renal uptake of PSP in LEC rats. The uptake of PSP by slices of kidneys from LEA and LEC rats was examined. The uptake of PSP by kidney slices at 30 min was used to examine the concentration-dependence. The uptake behavior of PSP was almost the same in each rat (Fig. 1).

3.2. Potential-sensitive transport system in LEC rats

Next we evaluated the exit system of PSP in LEC rats. The uptake of PSP into the renal BBMV of LEA and LEC rats was examined. We recently reported that PSP has high affinity for the potential-sensitive urate transport system [22]. When an inside-positive membrane potential was created by applying an inwardly directed K^+ gradient in the presence of the K^+ ionophore valinomycin, the uptake of PSP into the renal BBMV of LEA rats was significantly accelerated compared that in the absence of valinomycin (Fig. 2), and PSP uptake stimulated by the inside-positive membrane potential was significantly inhibited by probenecid, an organic anion transporter inhibitor (Table 1). On the other hand, the uptake of PSP into the renal BBMV of LEC rats was not significantly enhanced by positive intravesicular potential (Fig. 2). Next, the effect of positive intravesicular potential on the uptake of urate into the renal BBMV of LEA and LEC rats was investigated. As well as the uptake of PSP, the uptake of urate into the renal BBMV of LEA rats was significantly accelerated by positive intravesicular potential, but that of LEC

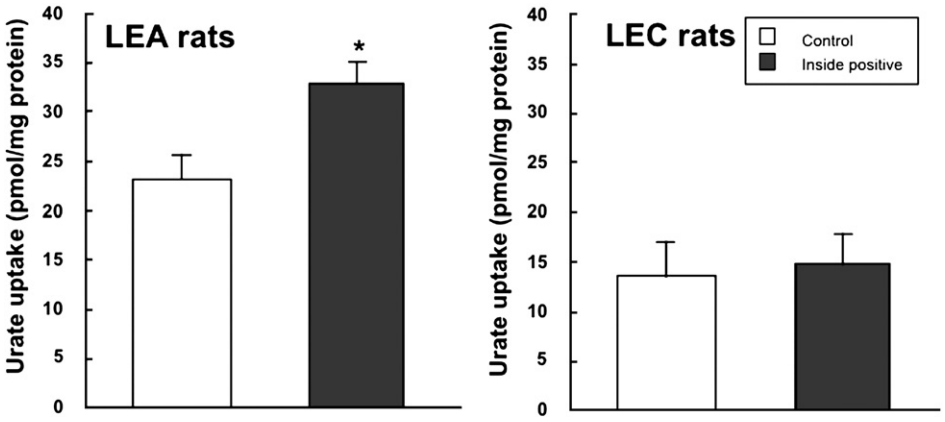

Fig. 3. Effect of a positive intravesicular potential on urate uptake by LEA and LEC rat renal BBMV. Membrane vesicles were suspended in 100 mM D-mannitol, 100 mM Na-gluconate and 20 mM HEPES/Tris (pH 7.4), and treated either with valinomycin (7 μg/mg protein) or ethanol (control). The drug solution contained 100 mM D-mannitol, 48 μM [^{14}C]urate, 100 mM K-gluconate and 20 mM HEPES/Tris (pH 7.4). Uptake of urate was determined after 1 min of incubation. Each point represents the mean with SD of three determinations *$P<0.05$, significantly different from the control.

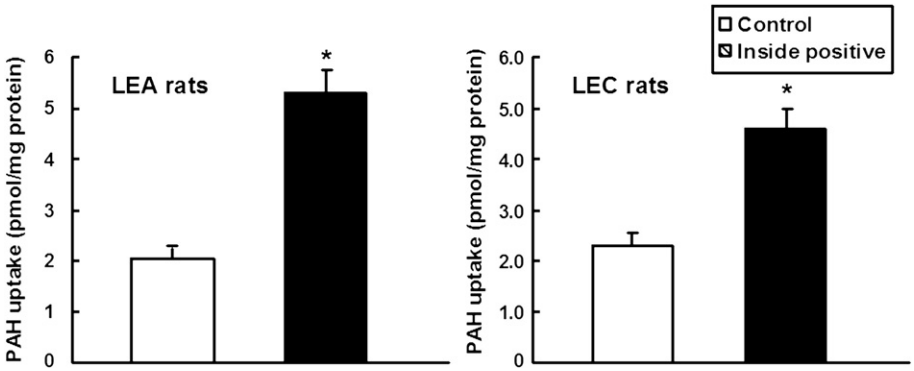

Fig. 4. Effect of a positive intravesicular potential on PAH uptake by LEC rat renal BBMV. Membrane vesicles were suspended in 100 mM D-mannitol, 100 mM Na-gluconate and 20 mM HEPES/Tris (pH 7.4), and treated either with valinomycin (7 μg/mg protein) or ethanol (control). The drug solution contained 100 mM D-mannitol, 2.4 μM [^{3}H] PAH, 100 mM K-gluconate and 20 mM HEPES/Tris (pH 7.4). Uptake of PAH was determined after 1 min of incubation. Each point represents the mean with SD of three determinations. *$P<0.05$, significantly different from the control.

rats was not significantly increased in the presence of positive intravesicular potential (Fig. 3). Moreover, urate and PSP uptake into the renal BBMV of LEA rats stimulated by the inside-positive membrane potential was significantly inhibited by PSP and urate, respectively (Table 1). On the other hand, probenecid, urate and PSP had no effect on the initial uptakes of substrates into the renal BBMV of LEC rats (data not shown).

In rats, in addition to the potential-sensitive urate transport system, the potential-sensitive PAH transport system has been thought to be present in the brush-border membrane [23,24]. We have shown that PSP has low affinity for the potential-sensitive PAH transport system [22]. The function of the potential-sensitive PAH transport system in LEC rats was evaluated. The uptake of PAH into the renal BBMV of LEA and LEC rats was examined. In the presence of positive intravesicular potential, PAH uptake was significantly accelerated compared with that in the absence of positive intravesicular potential (Fig. 4). Moreover, D-glucose uptake into the renal BBMV of LEA and LEC rats was stimulated by the inside-positive membrane potential (3.2±0.5-fold and 2.9±0.4-fold higher than those in the absence of positive intravesicular potential, respectively).

4. Discussion

The LEC rat is a mutant strain established at the Center for Experimental Plants and Animals of Hokkaido University [1]. LEC rats, like Mrp2-deficient rats, exhibit hyperbilirubinemia [2]. However, different from Mrp2-deficient animals, LEC rats have not only impaired canalicular transport of BSP but also impaired urinary excretion of PSP [13]. Thus, we focused on the urinary excretion of PSP in LEC rats. We previously reported that PSP was transported by some transport systems, rOat3, rOat1 and a potential-sensitive urate transport system and that the contribution of organic anion exchanger to the excretion of PSP is minor [16,22]. Thus, it is possible that dysfunction of one or some of these transport systems for PSP is responsible for the reduction of PSP excretion in LEC rats. The purpose of this study was to clarify the cause of the impairment of the renal transport system for PSP in LEC rats.

In the first part of this study, we investigated the function of basolateral organic anion transporters in LEC rats. No significant difference was found between the uptake of PSP by slices of kidneys from LEA rats and that by slices of kidneys from LEC rats. These results suggest that the functions of organic anion transporters in the renal basolateral membrane are normal in LEC rats.

In the next part of this study, we investigated the function of brush-border organic anion transporters in LEC rats. We recently reported that the efflux of PSP from proximal cells to the tubular lumen occurs through a potential-sensitive urate transport system [22]. In the presence of an inwardly directed K^+ gradient, the initial uptakes of PSP and urate into the renal BBMV of LEC rats were not significantly enhanced compared with that in the absence of valinomycin. Moreover, the uptake of urate into the renal BBMV of LEA rats stimulated by the inside-positive membrane potential was significantly inhibited by PSP. Taking these findings into consideration, we conclude that potential-sensitive urate transport system in LEC rats is impaired. In rat renal brush-border membranes, it is thought that the potential-sensitive PSP/urate transport system is different from the potential-sensitive PAH transport system [23,24]. Finally, we investigated the function of this transport system in LEC rats. In the presence of a positive intravesicular potential, the uptake of PAH into the renal BBMV of LEC rats was significantly enhanced. This find indicates that the potential-sensitive PAH transport system in LEC rats is normal. Although the aim of this study is to investigate the efflux pathway, we performed uptake studies using brush-border membrane vesicles [23,24]. Since transporters are not symmetric, different results can be obtained depending on the direction of the fluxes that are measured. Further studies are needed to elucidate the function of this transporter more precisely.

The renal excretion of urate is the result of rather complex mechanisms [25]. Due to the complexity of the mechanism of renal transport of urate, it is difficult to predict *in vivo* urate disposition from *in vitro* data [26]. The major models of renal urate handling include filtration, reabsorption, secretion, and postsecretory reabsorption. Recently, the long-hypothesized urate

transporter in the human kidney (URAT1/SLC22A13), an apical urate–anion exchanger regulating blood urate levels, was identified [27]. 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 [28]. However, the transporter that is responsible for the secretion of urate in rats has not yet been elucidated at the molecular level. LEC rats should be useful for elucidating the potential-sensitive urate transport system in rats at the molecular level.

To date, a novel potential-sensitive transporter (OATv1) has been identified at the brush-border membranes of pig renal proximal tubules [29]. In addition to OATv1, 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 including urate [30]. We have suggested that the rat homologue of mouse RST plays a major role in the renal secretion of PSP [22]. It is possible that this transport system in LEC rats is impaired. Moreover, urate transporter/channel (UAT) have been proposed to be involved in renal urate transport [31]. Further studies are needed to elucidate the function of these transport systems in LEC rats.

In summary, the functions of basolateral organic anion transporters in the renal proximal tubules of LEC rats are normal; however, a functional disorder of the potential-sensitive urate transport system is responsible for the marked reduction in urinary excretion of PSP in LEC rats.

Acknowledgment

This work was supported in part by NOASTEC.

References

[1] M. Sasaki, M.C. Yoshida, K. Kagami, H. Kobayashi, K. Dempo, M. Mori, Spontaneous hepatitis in an inbred strain of Long–Evans rats, Rat News Letter 14 (1985) 4–6.
[2] M.C. Yoshida, R. Masuda, M. Sasaki, N. Takeichi, H. Kobayashi, K. Dempo, M. Mori, New mutation causing hereditary hepatitis in the laboratory rat, J. Heredity 78 (1987) 361–365.
[3] R. Masuda, M.C. Yoshida, M. Sasaki, K. Dempo, M. Mori, High susceptibility to hepatocellular carcinoma development in LEC rats with hereditary hepatitis, Jpn. J. Cancer Res. 79 (1988) 828–835.
[4] M. Oyamada, K. Dempo, Y. Fujimoto, H. Takahashi, M.I. Satoh, M. Mori, R. Masuda, M.C. Yoshida, K. Satoh, K. Sato, Spontaneous occurrence of placental glutathione S-transferase positive foci in the livers of LEC rats, Jpn. J. Cancer Res. 79 (1988) 5–8.
[5] C.C. Paulusma, R.P.J. Oude Elferink, The canalicular multispecific organic anion transporter and conjugated hyperbilirubinemia in rat and man, J. Mol. Med. 75 (1997) 420–428.
[6] A. Ayrton, P. Morgan, Role of transport proteins in drug absorption, distribution and excretion, Xenobiotica 31 (2001) 469–497.
[7] P.L.M. Jansen, W.H.M. Peters, W.H. Lamers, Hereditary chronic conjugated hyperbilirubinemia in mutant rats caused by defective hepatic anion transport, Hepatology 5 (1985) 573–579.
[8] F. Kuipers, M. Enserink, R. Havinga, A.B. van der Steen, M.J. Hardonk, J. Fevery, R.J. Vonk, Separate transport systems for biliary secretion of sulfated and unsulfated bile acids in the rat, J. Clin. Invest. 81 (1988) 1593–1599.
[9] A. Morikawa, Y. Goto, H. Suzuki, T. Hirohashi, Y. Sugiyama, Biliary excretion of 17beta-estradiol 17beta-D-glucuronide is predominantly mediated by cMOAT/MRP2, Pharm. Res. 17 (2000) 546–552.
[10] S.A. Terlouw, R. Masereeuw, P.H. van den Broek, S. Notenboom, F.G.M. Russel, Role of multidrug resistance protein 2 (MRP2) in glutathione–bimane efflux from Caco-2 and rat renal proximal tubule cells, Br. J. Pharmacol. 134 (2001) 931–938.
[11] S.E. Bradley, F.J. Ingelfinger, G.P. Bradley, J.J. Curry, The estimation of hepatic blood flow in man, J. Clin. Invest. 24 (1945) 890–897.
[12] M.H. Gault, B. Koch, J.B. Dossetor, Phenolsulfonphthalein (PSP) in assessment of renal function, J. Am. Med. Assoc. 200 (1967) 871–873.
[13] S. Itagaki, M. Sugawara, M. Kobayashi, K. Miyazaki, T. Hirano, K. Iseki, Comparison of the disposition behavior of organic anions in an animal model for Wilson's disease (Long–Evans Cinnamon rats) with that in normal Long–Evans Agouti rats, Drug Metab. Pharmacokinet. 19 (2004) 150–154.
[14] K. Iseki, M. Kobayashi, A. Ohba, K. Miyazaki, Y. Li, Y. Togashi, N. Takeichi, Comparison of disposition behavior and de-coppering effect of triethylenetetramine in animal model for Wilson's disease (Long–Evans Cinnamon rat) with normal Wistar rat, Biopharm. Drug Dispos. 13 (1992) 273–283.
[15] S. Itagaki, M. Chiba, S. Shimamoto, M. Sugawara, M. Kobayashi, K. Miyazaki, T. Hirano, K. Iseki, Characterization of secretory intestinal transport of phenolsulfonphthalein, Drug Metab. Pharmacokinet. 20 (2005) 72–78.
[16] S. Itagaki, M. Sugawara, M. Kobayashi, S. Nishimura, M. Fujimoto, K. Miyazaki, K. Iseki, Major role of organic anion transporters in the uptake of phenolsulfonphthalein in the kidney, Eur. J. Pharmacol. 475 (2003) 85–92.
[17] M. Kobayashi, Y. Saito, S. Itagaki, T. Hirano, K. Iseki, Nateglinide uptake by a ceftibuten transporter in the rat kidney brush-border membrane, Biochim. Biophys. Acta 1715 (2005) 19–24.
[18] S. Itagaki, Y. Saito, S. Kubo, Y. Otsuka, Y. Yamamoto, M. Kobayashi, T. Hirano, K. Iseki, H^+-dependent transport mechanism of nateglinide in the brush-border membrane of the rat intestine, J. Pharmacol. Exp. Ther. 312 (2005) 77–82.
[19] S. Itagaki, M. Sugawara, M. Kobayashi, K. Miyazaki, K. Iseki, Mechanism of active secretion of phenolsulfonphthalein in the liver via MRP2 (abcc2), an organic anion transporter, Drug Metab. Pharmacokinet. 18 (2003) 238–244.
[20] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[21] F.G.M. Russel, R. Masereeuw, R.A.M.H. van Aubel, Molecular aspects of renal anionic drug transport, Annu. Rev. Physiol. 64 (2002) 563–594.
[22] S. Itagaki, S. Shimamoto, M. Sugawara, M. Kobayashi, K. Miyazaki, T. Hirano, K. Iseki, Phenolsulfonphthalein transport by potential-sensitive urate transport system, Eur. J. Pharmacol. 518 (2005) 83–89.
[23] K. Ohoka, M. Takano, T. Okano, S. Maeda, K. Inui, R. Hori, p-Aminohippurate transport in rat renal brush-border membranes: a potential-sensitive transport system and an anion exchanger, Biol. Pharm. Bull. 16 (1993) 395–401.
[24] F. Roch-Ramel, D. Werner, B. Guisan, Urate transport in brush-border membrane of human kidney, Am. J. Physiol. 266 (1994) F797–F805.
[25] F. Roch-Ramel, B. Guisan, Renal transport of urate in humans, News Physiol. Sci. 14 (1999) 80–84.
[26] D.A. Sica, A.C. Schoolwerth, Renal handling of organic anions and cations: excretion of uric acid, in: B.M. Brenner, F.C. Rector (Eds.), The Kidney, sixth ed., Saunders, Philadelphia, PA, 2000, pp. 680–700.
[27] A. Enomoto, H. Kimura, A. Chairoungdua, Y. Shigeta, P. Jutabha, S.H. Cha, M. Takeda, M. Hosoyamada, T. Sekine, T. Igarashi, H. Matsuo, Y. Kikuchi, T. Oda, K. Ichida, T. Hosoya, K. Shimokata, T. Niwa, Y. Kanai, H. Endou, Molecular identification of a renal urate anion exchanger that regulates blood urate levels, Nature 417 (2002) 447–452.
[28] M. Takano, K. Hirozane, M. Okamura, A. Takayama, J. Nagai, R. Hori, p-Aminohippurate transport in apical and basolateral membranes of the OK kidney epithelial cells, J. Pharmacol. Exp. Ther. 269 (1994) 970–975.

[29] P. Jutabha, Y. Kanai, M. Hosoyamada, A. Chairoungdua, D.K. Kim, Y. Iribe, E. Babu, J.Y. Kim, N. Anzai, V. Chatsudthipong, H. Endou, Identification of a novel voltage-driven organic anion transporter present at apical membrane of renal proximal tubule, J. Biol. Chem. 278 (2003) 27930–27938.

[30] T. Imaoka, H. Kusuhara, S. Adachi-Akahane, M. Hasegawa, N. Morita, H. Endou, Y. Sugiyama, The renal-specific transporter mediates facilitative transport of organic anions at the brush border membrane of mouse renal tubules, J. Am. Soc. Nephrol. 15 (2004) 2012–2022.

[31] E. Leal-Pinto, W. Tao, J. Rappaport, M. Richardson, B.A. Knorr, R.G. Abramson, Molecular cloning and functional reconstitution of a urate transporter/channel, J. Biol. Chem. 272 (1997) 617–625.