Full Paper

Human Organic Anion Transporter 4 Is a Renal Apical Organic Anion/Dicarboxylate Exchanger in the Proximal Tubules

Sophapun Ekaratanawong^{1,2}, Naohiko Anzai¹, Promsuk Jutabha¹, Hiroki Miyazaki¹, Rie Noshiro¹, Michio Takeda¹, Yoshikatsu Kanai¹, Samaisukh Sophasan³, and Hitoshi Endou^{1,*}

¹Department of Pharmacology and Toxicology, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan

²Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, Klong Luang Pathumthani 12121, Thailand

³Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

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Abstract. Human organic anion transporter OAT4 is expressed in the kidney and placenta and mediates high-affinity transport of estrone-3-sulfate (E₁S). Because a previous study demonstrated no *trans*-stimulatory effects by E₁S, the mode of organic anion transport via OAT4 remains still unclear. In the present study, we examined the driving force of OAT4 using mouse proximal tubular cells stably expressing OAT4 (S₂ OAT4). OAT4-mediated E₁S uptake was inhibited by glutarate (GA) (IC₅₀: 1.25 mM) and [¹⁴C]GA uptake via S₂ OAT4 was significantly *trans*-stimulated by unlabeled GA (5 mM) (*P*<0.001). [³H]E₁S uptake via S₂ OAT4 was significantly *trans*-stimulated by preloaded GA (*P*<0.001) and its [¹⁴C]GA efflux was significantly *trans*-stimulated by unlabeled E₁S in the medium (*P*<0.05). In addition, both the uptake and efflux of [¹⁴C]*p*-aminohippuric acid (PAH) and [¹⁴C]GA via S₂ OAT4 were significantly *trans*-stimulated by unlabeled GA or PAH. The immunoreactivities of OAT4 were observed in the apical membrane of proximal tubules along with those of basolateral organic anion/dicarboxylate exchangers such as hOAT1 and hOAT3 in the same tubular population. These results indicate that OAT4 is an apical organic anion/dicarboxylate exchanger and mainly functions as an apical pathway for the reabsorption of some organic anions in renal proximal tubules driven by an outwardly directed dicarboxylate gradient.

Keywords: organic anion, dicarboxylate, proximal tubule, organic anion transporter

Introduction

The kidney plays an important role in the elimination of harmful endogenous compounds and xenobiotics from the body. The proximal tubule is the primary site where numerous organic anions are taken up from the blood and excreted into the urine. (1, 2). The trans-cellular secretion of organic anion in the proximal tubule involves a two-step process: an uptake across the basolateral membrane of proximal tubular cells and an exit across the apical membrane into the tubular lumen. The former is energetically uphill and is accomplished by a tertiary active process via the organic anion/dicarboxy-

late exchanger that uses outwardly directed gradient of dicarboxylates such as α -ketoglutarate (3, 4). The latter is believed to be transporter-mediated, although this process is energetically downhill. In general, the efflux system for *p*-aminohippuric acid (PAH) (a prototypical substrate for the organic anion transport system) in the brush-border (apical) membrane has been investigated using brush-border membrane vesicles (BBMVs) (2). The existence of at least two transport systems was reported in human BBMVs, that is, a potential-driven facilitated diffusion and an organic anion/dicarboxylate exchange mechanism. To date, the mechanism of an apical organic anion exit pathway remains still uncertain.

Recently, several cDNAs encoding organic anion transporter have been successively identified in the

*Corresponding author. FAX: +81-422-79-1321
E-mail: endouh@kyorin-u.ac.jp

kidney, including organic anion transporter (OAT), organic anion transporting polypeptide (oatp), multidrug resistance-associated protein (MRP), and sodium-dependent inorganic phosphate transporter (NPT) families (3, 5, 6). Until now, it has been clarified that OAT1 and OAT3 are the PAH/dicarboxylate exchangers located at the basolateral membrane of proximal tubules (7–12). Rodent organic anion transporters, such as oatp1 and Oat-K1/K2, are located in the apical membrane of proximal tubules, but their homologues in humans have not yet been identified (3, 5, 6). Oatp5, a novel member of the oatp family in rodents, was cloned and shown to be expressed only in the kidney. However, its localization and functional characteristics have not yet been identified (13). In humans, MRP2 and NPT1, which are also localized in the apical membrane of proximal tubules (14–16), show PAH transport (K_m : 0.88–1.9 mM for MRP2; K_m : 2.66 mM for NPT1), although the relative contributions of both transporters to its apical exit are yet to be identified. Very recently, we have identified a novel voltage-driven organic anion transporter (OAT_v1) at the apical membrane of pig renal proximal tubules (17). OAT_v1 seems to be a long-hypothesized potential-driven facilitator in pig BBMVs (18). OAT_v1 exhibited the highest amino acid sequence identity to NPT1 (60–65%), although the functional properties of both transporters are different. Therefore, it needs to be elucidated whether OAT_v1 is an orthologue of human NPT1, but it is suggested that NPT1 is a good candidate for the apical potential-driven facilitator in human proximal tubules characterized in earlier experiments on BBMVs (2).

As previously reported, OAT4 is exclusively expressed in the human kidney and placenta, mediates the high-affinity transport of estrone-3-sulfate (E₁S) (K_m = 1.01 μ M) and dehydroepiandrosterone sulfate (DHEAS) (K_m = 0.63 μ M) (19), and is located in the apical membrane of renal proximal tubules (20). Although the apical membrane localization of OAT4 proposes its potential role in the organic anion secretion, its physiological importance in the kidney is still unclear due to the lack of information regarding its mode of transport. In the present study, we reinvestigated the driving force of OAT4. We report that OAT4 is not a facilitated transporter but an organic anion/dicarboxylate exchanger and mediate bidirectional organic anion transport. Furthermore, OAT4 is localized in the same population of hOAT1- and hOAT3-positive proximal tubular cells. These results indicate that OAT4 is a renal apical organic anion/dicarboxylate exchanger and mainly serves as a tubular reabsorptive pathway for some organic anions including sulfate conjugates driven by an outwardly directed gradient of dicarboxylates such as

α -ketoglutarate.

Materials and Methods

Materials

The materials used here were purchased from the following sources: [¹⁴C]PAH (1.90 GBq/mmol) and [³H]E₁S (1.61 TBq/mmol) were from PerkinElmer Life Science Products (Boston, MA, USA); [¹⁴C]glutarate (GA) (4.07 GBq/mmol) was from American Radio-labeled Chemicals, Inc. (St. Louis, MO, USA); GA was from Wako (Osaka). All other chemicals and reagents used were of analytical grade and obtained from commercial sources.

Cell culture and establishment of S₂ OAT4

The second segment of proximal tubules (S₂) cells, derived from transgenic mice harboring the temperature-sensitive simian virus 40 large T-antigen gene, were established as described previously (21). The establishments of S₂ OAT4 was reported previously (20, 22). These cells were grown in a humidified incubator at 33°C and under 5% CO₂ using RITC 80-7 medium containing 5% fetal bovine serum, 10 μ g/ml transferrin, 0.08 U/ml insulin, 10 ng/ml recombinant epidermal growth factor, and 400 μ g/ml geneticin. The cells were subcultured in a medium containing 0.05% trypsin-EDTA solution (137 mM NaCl, 5.4 mM KCl, 5.5 mM glucose, 4 mM NaHCO₃, 0.5 mM EDTA, and 5 mM HEPES, pH 7.2) and used for 25 to 35 passages.

Uptake study

Uptake experiments were performed as previously described (23). Organic anion transport in S₂ OAT4 was estimated by measuring the uptakes of [³H]E₁S, [¹⁴C]PAH, and [¹⁴C]GA. S₂ OAT4 or S₂ pcDNA 3.1(+) (1×10^5 cells) was plated in 24-well plates and cultured for two days. After the medium was removed, the cell monolayers were washed twice with Dulbecco's modified phosphate-buffered saline (D-PBS) (137 mM NaCl, 3 mM KCl, 8 mM Na₂HPO₄, 1 mM KH₂PO₄, 1 mM CaCl₂, and 0.5 mM MgCl₂, pH 7.4) supplemented with 5.5 mM D-glucose and preincubated for 10 min. Then the monolayer was incubated with 500 μ l of D-PBS containing 50 nM [³H]E₁S, 20 μ M [¹⁴C]PAH or 10 μ M [¹⁴C]GA for 2 min (5 min for GA) at 37°C. Cells were washed three times with the ice cold D-PBS and solubilized in 0.5 ml of 0.1 N sodium hydroxide. The amount of substrate accumulated within the cells was then determined by measuring radioactivity.

Inhibition study

To evaluate the inhibitory effect of GA on E₁S uptake

mediated by OAT4, the cells were incubated in a medium containing [^3H]E₁S (50 nM) for 2 min in the absence or presence of various concentrations of GA at 37°C.

Examination of trans-stimulatory effect on the organic anion transport via OAT4

In order to clarify the driving force and its transport direction, we examined the *trans*-stimulatory effect on OAT4-mediated organic anion transport. For the uptake of radiolabeled substrates, the cells were prepared as described above, and the cells were preloaded with 0.5 ml of D-PBS with or without unlabeled substrates (E₁S, PAH, or GA; 5 mM each) at 37°C for 15 min before the uptake experiments. For the efflux of radiolabeled substrates, S₂ OAT4 and mock cells were seeded in 6-well tissue culture plates at a density of 4×10^5 cells/well. The cells were preloaded with 1 ml of D-PBS containing [^3H]E₁S (50 nM), [^{14}C]PAH (20 μM), or [^{14}C]GA (10 μM) at 37°C for 15 min, respectively. Then the cells were incubated in a medium with or without unlabeled substrates (GA and E₁S, PAH). Their radioactivities in the medium and that remaining in the cells were determined after 1-min (E₁S and PAH) or 5-min (GA) incubation.

Immunohistochemical analysis

The antibodies against hOAT1, hOAT3, and OAT4 used in this study have been shown to be specific for each protein, as previously described (11, 20, 24). We used human single-tissue slides (Biochain) for light microscopic immunohistochemical analysis using the streptavidin-biotin-HRP complex technique (LSAB kit; DAKO, Carpinteria, CA, USA). Sections were deparaffinized, rehydrated, and incubated with 3% H₂O₂ for 10 min to abrogate endogenous peroxidase activity. After rinsing in 0.05 M Tris-buffered saline containing 0.1% Tween 20 (TBST), the sections were treated with 10 $\mu\text{g}/\text{ml}$ primary rabbit polyclonal antibodies (4°C overnight). Then the sections were incubated with the secondary antibody, biotinylated goat polyclonal antibody against rabbit immunoglobulin (DAKO), diluted 1:400, for 30 min with HRP-labeled streptavidin. This step was followed by incubation with diaminobenzidine and hydrogen peroxide. The sections were counterstained with hematoxylin and examined by light microscopy.

Statistical analyses

Data are expressed as the mean $\pm$ S.E.M. Statistical differences were determined by Student's *t*-test. The reproducibility of the results in the present study was confirmed using two or three separate experiments. The

results from the representative experiments are shown in the figures.

Results

In our previous study, we reported that OAT4-mediated E₁S uptake is sodium-independent and not inhibited by 500 μM GA (a non-metabolized dicarboxylate) nor *trans*-stimulated by unlabeled E₁S (0.2 and 2 μM) (19). However, very recently, Sweet et al. and Bakhiya et al. showed that OAT3, which is considered to be a facilitated-diffusion carrier, is an organic anion/dicarboxylate exchanger similar to OAT1 (10, 12). Therefore, to test the interaction of GA with OAT4, we carried out *cis*-inhibition of OAT4-mediated E₁S uptake by GA at first.

As shown in Fig. 1A, E₁S uptake by OAT4 was largely inhibited by GA in millimolar concentrations (IC₅₀: 1.25 mM). This low-affinity seems to indicate the lack of inhibitory effect by 500 μM GA on OAT4-mediated E₁S transport in our previous work (19). Next, to test whether OAT4 transports GA, we performed a GA uptake study as well as the *trans*-stimulation experiment (Fig. 1B). For a 5-min incubation, the [^{14}C]GA uptake via OAT4 was twofold higher than that by mock cells ($P < 0.01$). In addition, [^{14}C]GA uptake via OAT4 is *trans*-stimulated by preloaded GA (5 mM) ($P < 0.001$), not by 1 mM GA. Then, to determine whether OAT4 is an organic anion/dicarboxylate exchanger, we tested the *trans*-stimulatory effect on the uptake and efflux of radiolabeled organic anion substrates via OAT4.

First, we used E₁S, an endogenous sulfate conjugate of steroid that is known to be a prototypical substrate for OAT4. The uptake of [^3H]E₁S was significantly *trans*-stimulated by preloaded GA (5 mM) (Fig. 2A), and the efflux of preloaded [^{14}C]GA was significantly stimulated with unlabelled E₁S in the medium (5 mM) (Fig. 2B). On the other hand, the uptake of [^{14}C]GA was not *trans*-stimulated by preloaded E₁S and the efflux of preloaded [^3H]E₁S was not stimulated with unlabelled GA in the medium (5 mM) (data not shown).

Then we used PAH, the prototypical xenobiotic substrate for renal organic anion transport. The uptake of [^{14}C]PAH was significantly *trans*-stimulated by preloaded GA (Fig. 3A) and the efflux of preloaded [^{14}C]GA was significantly stimulated with unlabelled PAH (5 mM) (Fig. 3B). In contrast to E₁S, the uptake of [^{14}C]GA was significantly *trans*-stimulated by preloaded PAH (Fig. 4A) and the efflux of preloaded [^{14}C]PAH was significantly stimulated with unlabelled GA in the medium (5 mM) (Fig. 4B). Similar *trans*-stimulatory effects were also observed in S₂ hOAT1 and S₂ hOAT3

Fig. 1. Interaction of glutarate (GA) with OAT4. A: Inhibitory effect of GA on OAT4-mediated uptake of [^3H]E $_1$ S. S $_2$ OAT4 cells were incubated in a solution containing 50 nM [^3H]E $_1$ S at 37°C for 2 min in the absence or presence of various concentrations of GA at 37°C. B: OAT4-mediated [^{14}C]GA uptake. S $_2$ OAT4 cells and mock cells were preloaded with 0.5 ml of D-PBS with 0 mM (open column), 1 mM (hatched column), or 5 mM (solid column) of unlabeled GA at 37°C for 15 min before uptake experiments. Then they were incubated in a solution containing 10 μM [^{14}C]GA at 37°C for 5 min. Each value represents the mean $\pm$ S.E.M. of four determinations from one typical experiment of the two or three separate ones. ** P <0.01, *** P <0.001.

cells with 1 mM unlabeled compounds (data not shown).

To determine whether the apical organic anion/dicarboxylate exchanger (OAT4) is co-expressed along with basolateral organic anion/dicarboxylate exchanger (hOAT1 and hOAT3) in the same proximal tubules, we performed immunohistochemical analysis using serial sections of a human kidney (Fig. 5). The IgG fractions of rabbit polyclonal antibodies against the carboxyl termini of hOAT1, hOAT3, and OAT4 were used. The specificity of these antibodies was already reported previously (11, 20, 24). Light microscopy of 2- μm -thick paraffinized serial sections demonstrated that there is specific immunostaining of all three

Fig. 2. Trans-stimulatory experiments regarding OAT4-mediated [^3H]E $_1$ S uptake. A: Trans-stimulatory effect on the uptake of [^3H]E $_1$ S by preloaded GA (5 mM). S $_2$ OAT4 cells and mock cells were preloaded with D-PBS in the absence (open column) or the presence of unlabeled GA (dotted column) at 37°C for 15 min before uptake experiments. The uptake rates of [^3H]E $_1$ S by mock and S $_2$ OAT4 cells for 2 min were measured. B: Trans-stimulatory effect on the efflux of preloaded [^{14}C]GA by extracellular E $_1$ S (5 mM). S $_2$ OAT4 cells and mock cells were preloaded with D-PBS in a solution containing 10 μM [^{14}C]GA at 37°C for 15 min before efflux experiments. The efflux rates of [^{14}C]GA by mock and S $_2$ OAT4 cells for 5 min were measured with (solid column) or without outside unlabeled E $_1$ S (open column). Each value represents the mean $\pm$ S.E.M. of three or four determinations from one typical experiment of the three separate ones. * P <0.05, *** P <0.001.

transporters in proximal tubular cells. At a high magnification, OAT4 localized in the apical membrane of proximal tubules (Fig. 5: E and F) along with hOAT1 (Fig. 5: A and B) and hOAT3 (Fig. 5: C and D) proteins expressed in the basolateral membrane of the same tubular cells.

Discussion

OAT4 was isolated from the human kidney cDNA library. When expressed in *Xenopus laevis* oocytes, OAT4 mediated the uptake of various substrates including E $_1$ S, DHEAS, and PAH (19). The recent finding that OAT4 is localized at the apical membrane of proximal tubules suggested its role as an exit pathway

Fig. 3. *Trans*-stimulatory experiments regarding OAT4-mediated $[^{14}\text{C}]\text{PAH}$ uptake. A: *trans*-stimulatory effect on the uptake of $[^{14}\text{C}]\text{PAH}$ by preloaded GA (5 mM). S_2 OAT4 cells and mock cells were preloaded with D-PBS in the absence (open column) or the presence of unlabeled GA (hatched column) at 37°C for 15 min before uptake experiments. The uptake rates of $[^{14}\text{C}]\text{PAH}$ by mock and S_2 OAT4 cells for 2 min were measured. B: *Trans*-stimulatory effect on the efflux of preloaded $[^{14}\text{C}]\text{GA}$ by extracellular PAH (5 mM). S_2 OAT4 cells and mock cells were preloaded with D-PBS in a solution containing $10\ \mu\text{M}$ $[^{14}\text{C}]\text{GA}$ at 37°C for 15 min before efflux experiments. The efflux rates of $[^{14}\text{C}]\text{GA}$ by mock and S_2 OAT4 cells for 5 min were measured with (solid column) or without outside unlabeled PAH (open column). Each value represents the mean $\pm$ S.E.M. of three or four determinations from one typical experiment of the three separate ones. * $P < 0.05$, *** $P < 0.001$.

Fig. 4. *Trans*-stimulatory experiments regarding OAT4-mediated $[^{14}\text{C}]\text{PAH}$ efflux. A: *trans*-stimulatory effect on the efflux of preloaded $[^{14}\text{C}]\text{PAH}$ by extracellular GA (5 mM). S_2 OAT4 cells and mock cells were preloaded with D-PBS in a solution containing $20\ \mu\text{M}$ $[^{14}\text{C}]\text{PAH}$ at 37°C for 15 min before efflux experiments. The efflux rates of $[^{14}\text{C}]\text{PAH}$ by mock and S_2 OAT4 cells for 1 min were measured with (hatched column) or without outside unlabeled GA (open column). B: *Trans*-stimulatory effect on the uptake of $[^{14}\text{C}]\text{GA}$ by preloaded PAH (5 mM). S_2 OAT4 cells and mock cells were preloaded with D-PBS in the absence (open column) or the presence of unlabeled PAH (solid column) at 37°C for 15 min before uptake experiments. The uptake rates of $[^{14}\text{C}]\text{GA}$ by mock and S_2 OAT4 cells for 5 min were measured. Each value represents the mean $\pm$ S.E.M. of four determinations from one typical experiment of the three separate ones. * $P < 0.05$, *** $P < 0.001$.

for organic anion (20). In the present study, we examined the properties of OAT4 as an apical organic anion/dicarboxylate exchanger.

This is the first report regarding the transport direction and the driving force of OAT4. Our data derived from stable OAT4-expressing cell lines from the S_2 segment of the mouse renal proximal tubule (S_2 OAT4) demonstrated that OAT4 functions as an organic anion/dicarboxylate exchanger. We used GA as a model compound for dicarboxylates in this study because GA is a non-metabolized dicarboxylate and exhibited the similar inhibitory potency on OAT4-mediated E_1S transport to α -ketoglutarate, physiologically the most abundant endogenous dicarboxylate in the proximal tubular cells (data not shown). The uptakes of $[^{14}\text{C}]\text{GA}$,

$[^3\text{H}]\text{E}_1\text{S}$, and $[^{14}\text{C}]\text{PAH}$ via S_2 OAT4 were significantly increased by the preincubation of unlabeled GA (Figs. 2A and 3A), and the $[^{14}\text{C}]\text{GA}$ efflux was also significantly increased by the incubation with unlabeled E_1S or PAH (Figs. 2B and 3B). These results indicate that intracellular GA not only binds to OAT4 and *trans*-stimulates its organic anion uptake but also is translocated to the outside. Furthermore, the findings that $[^{14}\text{C}]\text{GA}$ uptake via S_2 OAT4 was significantly increased by the preincubation of unlabeled PAH (Fig. 4B) and $[^{14}\text{C}]\text{PAH}$ efflux was significantly increased by the incubation with unlabeled PAH (Fig. 4A) manifested the bidirectional transport characteristic of OAT4. These results raise the possibility that OAT4 is the organic anion/dicarboxylate exchange mechanism

Fig. 5. Immunohistochemical analysis of human kidney. Two-micrometer serial sections were incubated with a polyclonal antibody against hOAT1, hOAT3, and OAT4. The apical membrane of proximal tubules was stained by OAT4 (E and F) and the same tubules showed basolateral membrane stained by both hOAT1 (A and B) and hOAT3 (C and D). (A, C, and E, 100 $\times$; B, D, and F, 400 $\times$). Scale bars = 100 μ m for A, C, and E; 20 μ m for B, D, and F.

previously found in BBMVs from human kidney (25).

As demonstrated in Fig. 5, now it became apparent that organic anion/dicarboxylate exchangers exist at both the apical (OAT4) and basolateral (OAT1 and OAT3) membrane of the same proximal tubular cells. As Schmitt and Burekhardt discussed in the case of bovine BBMVs (26), it seems unlikely that two exchangers of the same type existing in both sides of membranes lead to the vectorial transport of organic anions. At the basolateral side of proximal tubules, it is widely accepted that the outwardly directed gradient of α -ketoglutarate, the most abundant dicarboxylate within proximal tubular cells, drives organic anion uptake. In contrast, little is known about the coupling between the dicarboxylate gradient and the anion exchange system, but taking the existence of the outwardly directed dicarboxylate gradient in the tubular cells into account, OAT4 seems to contribute to the tubular reabsorption of organic anions. One possible role of OAT4 is an apical backflux pathway (27) for some organic anions coupling to the apical organic anion efflux transporters such as MRP2, NPT1, and possibly, the human homologue of OATv1 (3, 5, 6, 17), as well as to the apical low affinity Na^+ /dicarboxylate cotransporter NaDC-1 (28). Because the transport mode of these transporters is normally unidirectional, the stimu-

lation or the inhibition of bidirectional OAT4 transport function may influence the net transepithelial secretion

Fig. 6. Proposed model of transepithelial transport pathway for organic anions in human kidney. Organic anions including xenobiotics (e.g., PAH) enter into the proximal tubular cells via basolateral OAT1 and OAT3, and they are secreted into urine via apical MRP2 and NPT1, and in some cases, via OAT4. In contrast, endogenous organic anions such as E_1S and DHEAS reabsorbed via OAT4 may be effluxed via basolateral OAT3 or an unidentified transporter(s). Some organic anions may be reabsorbed from tubular lumen via OAT4 and may function as a backflux pathway. Abbreviations, OA: organic anion, DCs: dicarboxylates.

rate for organic anions. Recently, we have identified the intracellular interacting protein that regulates the OAT4-mediated transport function by the yeast two-hybrid assay (H. Miyazaki et al., manuscript in preparation). The functional coupling among apical transporters via intracellular protein(s) needs to be further clarified. Moreover, the substrate specificity of OAT4, which favors sulfate conjugates, is rather narrower than that of MRP2, NPT1, and OAT_v1. Therefore, another possibility is that OAT4 may contribute to avoid losing endogenous sulfated conjugates of steroid such as E₁S and DHEAS, which are the reservoir of steroids in the blood circulation (29, 30). These steroids are known to be tubular reabsorbed (31, 32). In this case, OAT3 may be a basolateral efflux pathway for sulfate conjugates of steroids. A proposed model of the transepithelial transport pathway for organic anions in human kidney is shown in Fig. 6.

Although OAT4 is expected to drive the uptake of organic anions into the cells, it is still uncertain whether OAT4 functions as an exit pathway for organic anions because it is not evident whether dicarboxylate concentrations in the cytoplasm are homogenous throughout tubular cells. The fact that the dicarboxylate is the counterion of OAT4 suggests that the directions of organic anion transport via OAT4 depend on the local concentration gradient of dicarboxylates across the plasma membrane, but there is no precise report regarding the efficiency or the contribution of the apical dicarboxylate gradient. In addition, in some pathological conditions, OAT4 may function as an apical exit pathway for organic anions because of its insensitivity to intracellular ATP content, Na⁺ gradient, and membrane depolarization. For example, a mycotoxin, Ochratoxin A, produces alterations in renal function by inhibiting mitochondrial oxidation (33). Due to the mitochondrial damage, ATP production decreases, depolarization of the membrane potential occurs, and the Krebs-cycle reaction may be inhibited. In such a case, the bidirectional transport characteristic of OAT4 may contribute to the xenobiotics secretion into the urine in exchange for extracellular dicarboxylates regardless of ATP, Na⁺, and membrane potential.

In this study, we observed no *trans*-stimulatory effect on E₁S efflux by outside GA and on GA uptake by preloaded E₁S from S₂ OAT4. A similar observation was reported by Bakhiya et al. in hOAT3-expressing oocytes (12). They explained that due to the high lipophilicity of E₁S, binding to intracellular components upon oocyte injection reduces the free submembraneous E₁S concentration available for efflux. This may not be true because, as we have reported recently, approximately 25% of injected E₁S is effluxed by the voltage-driven organic

anion transporter OAT_v1 (17). Therefore, we propose another possibility that these findings relate to the difference of intracellular and extracellular side substrate binding sites. That is, OAT4 can recognize PAH from both intracellular and extracellular sides, but it can recognize E₁S only from the extracellular side. Further studies are necessary to clarify this problem.

The present findings with OAT4 raise the possibility that dicarboxylate exchange is a common mechanism for this family of transporters. It is well established that OAT1 is a classical PAH/dicarboxylate exchanger. In addition, two recent reports support our idea. First, Sweet et al. and Bakhiya et al. reported that OAT3 functions as an organic anion/dicarboxylate exchanger (10, 12). Second, we reported the cloning of a novel member of the OAT family, urate/anion exchanger 1 (URAT1) (34). URAT1 is expressed at the apical membrane of proximal tubules and it is thought to be the long-hypothesized urate/anion exchanger in the human kidney; however, the transport of PAH has not been observed. OAT4 exhibited the highest amino acid sequence identity to URAT1 and higher amino acid sequence identities to OAT1 and OAT3 among the OAT family. Particularly, all these clones (OAT4, OAT1, OAT3, and URAT1) have long intracellular loops (about 70 amino acids) that are thought to be a structural characteristic of antiporter (35). The fact that not only OAT1 and OAT3 but also URAT1 has an exchange mode seems to be appropriate for the OAT4 functions as an exchanger. Therefore it is interesting to know the common inherent structural traits responsible for such similar exchange mechanisms developed among different transporters. Further studies are needed to elucidate this possibility.

In conclusion, we first demonstrated that OAT4 is the bidirectional organic anion/dicarboxylate exchanger. OAT4 may function as an apical reabsorptive pathway of some organic anions in the proximal tubules under the physiological condition, but it may also drive the tubular secretion of organic anions in some conditions. Although the relative contribution of OAT4 in renal organic anion handling needs to be clarified, this study provides new insights into the functional basis of the transepithelial transport pathway of organic anions, including xenobiotics and endogenous compounds, in the human kidney.

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