Identification of a Novel Voltage-driven Organic Anion Transporter Present at Apical Membrane of Renal Proximal Tubule*

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A novel transport protein with the properties of voltage-driven organic anion transport was isolated from pig kidney cortex by expression cloning in Xenopus laevis oocytes. A cDNA library was constructed from size-fractionated poly(A)⁺ RNA and screened for p-aminohippurate (PAH) transport in high potassium medium. A 1856-base pair cDNA encoding a 467-amino acid peptide designated as OATV₁ (voltage-driven organic anion transporter 1) was isolated. The predicted amino acid sequence of OATV₁ exhibited 60–65% identity to those of human, rat, rabbit, and mouse sodium-dependent phosphate cotransporter type 1 (NPT1), although OATV₁ did not transport phosphate. The homology of this transporter to known members of the organic anion transporter family (OAT family) was about 25–30%. OATV₁-mediated PAH transport was affected by the changes in membrane potential. The transport was Na⁺-independent and enhanced at high concentrations of extracellular potassium and low concentrations of extracellular chloride. Under the voltage clamp condition, extracellularly applied PAH induced outward currents in oocytes expressing OATV₁. The current showed steep voltage dependence, consistent with the voltage-driven transport of PAH by OATV₁. The PAH transport was inhibited by various organic anions but not by organic cations, indicating the multispecific nature of OATV₁ for anionic compounds. This transport protein is localized at the apical membrane of renal proximal tubule, consistent with the proposed localization of a voltage-driven organic anion transporter. Therefore, it is proposed that OATV₁ plays an important role to excrete drugs, xenobiotics, and their metabolites driven by membrane voltage through the apical membrane of the tubular epithelial cells into the urine.

In kidney, the secretion of organic anions takes place in the renal proximal tubular epithelial cells via at least two steps. The first is the transport of organic anions from the peritubular plasma across the basolateral membrane into the proximal tubular cells. p-Aminohippurate (PAH) has been used as a prototypical substrate for the renal organic anion transport systems (1, 2). The transport of PAH across the basolateral membrane of proximal tubular cells against the electrochemical gradient (3) occurs in exchange for intracellular dicarboxylates such as α-ketoglutarate (4, 5). Two organic anion transporters, OAT1 (6–8) and OAT3 (9), have been proposed to be responsible for this step. The second step is the exit of organic anions across the apical membrane at the tubular epithelial cells into the urine. Although this process is energetically downhill for organic anions, it has been believed that this process is also mediated by specific transporters. The luminal efflux system for PAH has been investigated mostly using brush border membrane vesicles. In dogs (10–13) and rats (14), anion exchange mechanisms have been demonstrated for the luminal efflux systems. They mediate probenecid-sensitive electroneutral exchange of anionic compounds including both organic (e.g. PAH, urate, and lactate) and inorganic (e.g. Cl⁻, HCO₃⁻, OH⁻) anions. A distinct efflux system involving the voltage-driven transport was demonstrated in pig and rabbit (15–17). In bovine renal brush border membrane vesicles, a PAH/dicarboxylate exchange system was reported (18). PAH transport at the apical membrane in the OK kidney epithelial cell line was shown to be mediated by a voltage-driven transport system but not by an anion exchange system (19). In the physiological condition, a major component of the exit path of organic anions from renal proximal tubular cells has been proposed to be the facilitated diffusion along the electrochemical potential gradient (2, 23). Voltage-driven organic anion transport plays an important role for this step. However, the molecular nature and precise functional properties of these efflux systems are still unknown.

MRP2, an ABC (ATP-binding cassette) transporter, was suggested to be one of the exit paths of PAH in proximal tubular cells. Studies of membrane vesicles from MRP2-expressing HEK or SF9 cells have indicated that MRP2 transports PAH (20, 21) in an ATP-dependent manner. Recently, it was shown...
ties of NPT1, in particular its $K_{\text{Cl}}$ (22). Although NPT1 is a candidate of the exit path for PAH at intestine, and skeletal muscle were loaded onto 1.0% agarose/formamide gel, transferred to a nylon membrane, and then probed with $^{32}$P-labeled OAT1 cDNA fragment. b, Western blot analysis of OAT1 in pig kidney and brush border membrane (BBM) of the pig kidney cortex was performed using an anti-OAT1 antibody. The absorption test was performed by preincubation of the antibody with OAT1 peptides (100 µg/ml) (+). (Peptide (+)).

that the human sodium-dependent phosphate cotransporter type 1 (NPT1) present at the apical membrane of proximal tubules transports organic anions including PAH. The PAH transport by NPT1 expressed in HEK293 was $K_\text{Cl}$-sensitive (22). Although NPT1 is a candidate of the exit path for PAH at the apical membrane of proximal tubules, the reported properties of NPT1, in particular its $K^+$-dependence, is not consistent with those of the voltage-driven PAH transporter at the apical membrane. In the present study, to identify the transporter responsible for the exit of PAH through the apical membrane, we have performed expression cloning using pig kidney cortex poly(A)$^+$ RNA and identified a novel transporter present at the apical membrane of proximal tubules that mediates voltage-driven facilitated diffusion.

**EXPERIMENTAL PROCEDURES**

**Expression of Pig Kidney Cortex Poly(A)$^+$ RNA—**Northern blot and Western blot analyses of OAT1. a, 4 µg of poly(A)$^+$ RNA isolated from pig brain, lung, liver, kidney, small intestine, and skeletal muscle were loaded onto 1.0% agarose/formamide gel, transferred to a nylon membrane, and then probed with $^{32}$P-labeled OAT1 cDNA fragment. b, Western blot analysis of OAT1 in pig kidney and brush border membrane (BBM) of the pig kidney cortex was performed using an anti-OAT1 antibody. The absorption test was performed by preincubation of the antibody with OAT1 peptides (100 µg/ml) (+).

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**Functional Characterization—**Twenty-five nanograms of OAT1 cRNA synthesized in vitro using T7 RNA polymerase from the OAT1 cDNA in pSPORT1 linearized with SalI were injected into defolliculated Xenopus oocytes. Two or 3 days after injection the uptake of radiolabeled substrates was measured in various uptake solutions (differing in ion composition) to examine its dependence on sodium or chloride. In sodium-free solution, LiCl or choline-Cl were used to replace NaCl in the standard uptake solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl$_2$, 1 mM MgCl$_2$, 5 mM HEPES, pH 7.4) containing 20 µM $[^{14}]$CIPAH (1 µCi/ml).

To examine the effects of the extracellular concentration of $K^+$ and $Cl^-$ on the OAT1-mediated $[^{14}]$CIPAH uptake, the concentrations of $K^+$ or $Cl^-$ were varied over the range of 0 to 100 mM (0, 5, 10, 25, 50, and 100 mM). The concentrations of $K^+$ in the uptake solution were varied in Na$^+$-free solution in which [KCl] plus [chlorine-Cl] was equal to 100 mM. The concentration of $Cl^-$ was varied in the high potassium uptake solution in which [KCl] plus [K-glucuronate] was equal to 100 mM.

**Kinetic Study—**Because the uptake of substrates in high potassium uptake solution was linear longer than 2 h, the uptake was measured for 30 min for kinetic studies. The concentrations of PAH, urate, and estrone sulfate were varied from 10 µM to 10 mM, from 10 µM to 5 mM, and from 1 to 500 µM, respectively. OAT1-mediated substrate uptake...
was calculated as the difference between the values of uptake into cRNA-injected oocytes and those of control oocytes (without cRNA injection). The $K_m$ values were determined with the Eadie-Hofstee equation.

To measure the $K_v$ values for the transport, oocytes expressing OATV1 were incubated for 30 min in high potassium uptake solution with various concentrations of $[^{14}C]$PAH with or without addition of inhibitor. The $K_v$ values were determined by double-reciprocal plot analysis in which 1/uptake rate of $[^{14}C]$PAH was plotted against 1/[14C]PAH concentration. The $K_v$ values were calculated from the following equation when competitive inhibition was observed: $K_v = (K_v[I])/(K_v - K_v[I])$, where $K_v$, $K_v[I]$, and $I$ are $K_v$ of PAH with inhibitor, $K_v[I]$, of PAH without inhibitor, and concentration of inhibitor, respectively (31).

Efflux Measurement—Fifty nanoliters of $[^{14}C]$PAH (2.5 nCi, -1 mM), $[^{14}C]$urate (2.5 nCi, -1 mM), or $[^{3}H]$estrone sulfate (25 nCi, -20 mM) was injected into oocytes with a fine-tipped glass micropette as described elsewhere (32, 33). The individual oocytes were incubated in ice-cold standard uptake solution (98 mM NaCl, 2 mM KCl, 1.8 mM CaCl$_2$, 1 mM MgCl$_2$, 5 mM HEPES, pH 7.4) for 5 min and then transferred to the standard uptake solution kept at room temperature and incubated at room temperature for 2, 5, 15, 30, 60, 90, 120, or 150 min. Then, the incubation solution was collected to determine the efflux of substrates from the oocytes into bath solution at the end of the incubation period. The radioactivity remaining in the oocytes was also measured. The efflux value was expressed as % radioactivity calculated from the radioactivity in the bathing solution × 100% (the radioactivity in oocyte plus the radioactivity in bathing solution). The dependence of $[^{14}C]$PAH efflux on inorganic ions was determined by comparing the $[^{14}C]$PAH uptake in the standard uptake solution and that in the solutions in which NaCl was replaced by KCl, sodium gluconate, potassium gluconate, LiCl, or choline-Cl as mentioned above. The pH dependence of $[^{14}C]$PAH efflux was measured in the standard uptake solution with varied pH to 5.5, 6.5, 7.4, and 8.5. The efflux was measured for 15 min over which the linear efflux value was obtained. To examine the trans-stimulation of the efflux of PAH, $[^{14}C]$PAH-injected oocytes were incubated in the standard uptake solution with or without non-radioiabeled PAH (0.01, 0.1, 1, 5, or 10 mM) for 15 min at room temperature.

For the uptake and efflux measurements in the present study, 8–10 oocytes were used for each data point. The values are expressed as mean ± S.E. The reproducibility of the results was confirmed by three separate experiments with different batches of oocytes. The results from representative experiments are shown in figures.

Electrophysiological Measurements—The electrical currents induced by 5 mM PAH in both control and OATV1-cRNA-injected X. laevis oocytes were recorded during voltage clamp at -60 to +40 mV in the standard uptake solution (24, 34, 35). At each step (20 mV interval) of voltage clamp, the baseline electrical current was first determined and then 5 mM PAH was added to the bath medium for 2 min. The solution was then changed back to the standard uptake solution and the record was continued until the electrical current was returned to the baseline level.

Northern Analysis—Poly(A)$^+$ RNA (4 μg/plate) isolated from pig tissues was electrophoresed on 1% agarose, 22.5% formaldehyde gel and transferred to nitrocellulose filter (Schleicher & Schuell). The filter was hybridized with 500-bp OATV1 cDNA labeled with $[^{32}P]$dCTP at 42 °C overnight and washed finally in 0.1× SSC, 0.1% SDS at 65 °C (25).

Anti-peptide Antibody—A rabbit polyclonal antibody against a keyhole limpet hemocyanin-conjugated synthetic peptide, EVQDWAKER-EQNTYL, corresponding to 14 amino acid residues near the carboxyl terminus (454–467 of the amino acid sequence) of OATV1 was generated and affinity purified as described elsewhere (36, 37).

Western Blot Analysis—Kidney epithelial membrales were prepared as described previously (38) and subjected to SDS-polyacrylamide gel electrophoresis (39). The separated proteins were transferred electrophoretically to a Hybond-P polyvinylidene difluoride transfer membrane (Amersham Biosciences). The membranes were treated with diethylamine to dehydrate and affinity purified anti-OATV1 antibody overnight at 4 °C. Thereafter, horseradish peroxidase-conjugated anti-rabbit IgG was used as the secondary antibody (Jackson ImmunoResearch Laboratories, Inc). The signals were detected using the ECL Plus system (Amersham Biosciences). The specificity of immunoreaction was confirmed by an absorption experiment in the presence of antigen peptide (100 μg/ml).

Immunohistochemistry—Three-micrometer paraffin sections of pig kidney were processed for light microscopic immunohistochemical analysis as described previously (36). The kidney sections were incubated with affinity purified anti-OATV1, antibody (1:1,000) at 4 °C overnight and treated with Envision (+) rabbit peroxidase (DAKO) for 30 min. The immunoreactions were detected with diaminobenzidine (0.8 mM) (40). For absorption experiments, the serial kidney sections were treated with the primary antibody in the presence of antigen peptide (100 μg/ml).

RESULTS

Screening of cDNA Library—Total poly(A)$^+$ RNA was isolated from pig kidney cortex and size-fractionated. When expressed in Xenopus oocytes, the fractions corresponding to 1.7 to 2.2 kilobases exhibited the peak activity of the uptake of 20 μM $[^{14}C]$PAH in high potassium uptake solution: 1.5–2.0-fold of the uptake by control water-injected oocytes. These poly(A)$^+$ RNA fractions were used as a template for construction of a cDNA library. The library was screened for $[^{14}C]$PAH uptake by expression in Xenopus oocytes to isolate a 1,856-base pair cDNA clone that encodes a protein designated as OATV1 (voltage-age-driven organic anion transporter 1). The OATV1 cDNA contains an open reading frame encoding a putative 467-amino acid protein. Twelve transmembrane domains were predicted on the OATV1 amino acid sequence based on SOSUI algorithm (41). The amino acid sequence of OATV1 exhibited the highest identity (60–65%) to that of sodium-dependent phosphate co-transporter type 1 (NPT1) of each species (rat, mouse, rabbit, pig, and human).

FIG. 3. Concentration dependence of OATV1-mediated $[^{14}C]$PAH uptake. a, OATV1-mediated $[^{14}C]$PAH uptake by oocytes expressing OATV1 was measured in a high potassium uptake solution (98 mM K$^+$) and plotted against PAH concentration. The PAH uptake was saturable and fit to the Michaelis-Menten curve. b, Eadie-Hofstee plot analysis was performed on the PAH uptake. $K_v$ and $V_{max}$ values were 4.40 mM and 18.38 pmol/oocyte/min, respectively.

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and human). The homology to amino acid sequences of sodium-dependent phosphate cotransporter type 2 (NPT2) and other OATs is 25–30%.

Tissue Distribution—The Northern blot using the OAT V1 cDNA fragment as a probe showed a strong signal in pig kidney and liver (Fig. 1a). The OATV1 transcript was not detected in brain, lung, small intestine, and skeletal muscle. The major transcript detected in liver and kidney was 3.2 kb. In addition, a faint band at 1.8 kb was also detected in both kidney and liver. The 4.4-kb faint band was detected only in liver.

In Western blot analysis, the antibody raised against the COOH terminus peptide of OATV1 recognized a band of 60 kDa in the membrane protein prepared from total pig kidney and the brush border membrane of pig kidney cortex (Fig. 1b). This band was consistent with a predicted molecular mass of OATV1 protein (51 kDa). The band disappeared in the presence of antigen peptide in the absorption test, confirming the specificity of the immunoreaction (Fig. 1b).

Transport Activity of OATV1—Because the luminal facilitated transport system in pig and rabbit was reported to be markedly dependent on membrane potential (15, 16), we examined the effect of raising external K⁺ on the OATV1-mediated transport. The elevation of external K⁺ depolarizes the plasma membrane of Xenopus oocyte (42). As shown in Fig. 2, the uptake values of [14C]PAH, [14C]urate, [3H]estrone sulfate, and [3H]estradiol-17β-glucuronide by OATV1 expressing oocytes were significantly higher in high potassium solution (98 mM K⁺) compared with those incubated in standard uptake solution (2 mM K⁺), whereas control oocytes showed no difference between these conditions, suggesting that OATV1-mediated transport is dependent on membrane voltage. The uptake of [14C]PAH was saturable and followed Michaelis-Menten kinetics (Fig. 3a) with a $K_m$ value of 4.38 ± 0.96 mM (mean ± S.E. of three separate experiments) (Fig. 3b). $K_m$ values for [14C]urate and [3H]estrone sulfate were 5 and 0.212 mM, respectively (data not shown).

Characteristic of OATV1-mediated Organic Anion Transport—The PAH uptake by OATV1-expressing oocytes was also measured in various uptake solutions with different ionic contents. Replacement of Na⁺ with Li⁺ or choline did not affect [14C]PAH uptake (Fig. 4a), indicating that OATV1-mediated [14C]PAH transport was not dependent on Na⁺ in the uptake solution. The replacement of Na⁺ with K⁺ or Rb⁺ increased the OATV1-mediated transport (Figs. 2 and 4a). Fig. 4b further shows the dependence of OATV1-mediated [14C]PAH uptake on K⁺ concentration, indicating that raising extracellular K⁺ increased PAH transport in a concentration-dependent manner. Fig. 4a also shows the effect of the replacement of Cl⁻ with

![Fig. 4. Ion and pH dependence of OATV1-mediated transport.](http://www.jbc.org/)

**a**, the measurement of OATV1-mediated [14C]PAH uptake (20 μM) was performed in the standard uptake solution (NaCl) and the uptake solution in which NaCl was replaced by choline Cl, LiCl, KCl, RbCl, sodium gluconate, potassium gluconate, NaBr, or NaF for control non-injected oocytes (open column) and OATV1 cRNA-injected oocytes (closed column). **b**, the dependence of [14C]PAH uptake on extracellular K⁺ concentration was evaluated in the oocytes expressing OATV1. The concentrations of potassium in the bath solution were varied over a range of 0–100 mM in the Na⁺-free condition in which [KCl] plus [choline-Cl] are equal to 100 mM. **c**, Cl⁻ dependence of OATV1-mediated [14C]PAH uptake was examined in a high potassium concentration (K⁺ = 100 mM). The concentrations of Cl⁻ in the bath solution were in the range of 0–100 mM ([KCl] plus [potassium gluconate] is equal to 100 mM). **d**, pH dependence of OATV1-mediated [14C]PAH uptake was measured in high potassium uptake solution ([K⁺] = 98 mM) with varied pH to 5.5, 6.5, 7.4, and 8.5. *, **, and *** indicate statistical significance (p < 0.05, p < 0.01, and p < 0.001, respectively) compared with the uptake in standard uptake solution (NaCl) (unpaired Student’s t test).
other anions. PAH uptake was higher in OATv1-expressing oocytes incubated in potassium gluconate solution than that in KCl uptake solution. PAH uptake was also higher in sodium gluconate, NaBr, or NaF solution than that in NaCl solution. As shown in Fig. 4c, PAH transport was dependent on Cl− concentration. In the chloride-free solution and the solution containing 5 mM chloride, OATv1 expressing oocytes showed a significant elevation of PAH uptake (Fig. 4c). The PAH uptake did not show any remarkable pH dependence within the pH range of 5.5 and 8.5 (Fig. 4d). We also found that urate transport by OATv1 showed the same properties as those of PAH transport (data not shown).

Voltage-dependent PAH Transport—Fig. 4 showed that PAH uptake by OATv1-expressing oocytes was affected by the changes of K+ or Cl− concentration in the uptake solution. It seemed that PAH uptake is dependent on membrane voltage change generated by the alteration of K+ and Cl− concentrations. To confirm the voltage dependence of PAH transport by OATv1, we performed electrophysiological measurement of PAH-induced currents under the voltage clamp condition. PAH application to the bath medium induced outward current in the oocytes expressing OATv1, whereas no significant current was elicited by 5 mM PAH in the control oocytes. The PAH-induced current was dependent on membrane voltage (Fig. 5). The electric currents induced by 5 mM PAH were increased when the holding potential was elevated from −60 to +40 mV as shown in Fig. 5.

Characteristic of OATv1-mediated PAH Efflux—According to the voltage-driven transport of PAH, in the physiological condition PAH is supposed to be transported from the intracellular compartment to the extracellular compartment along the electrical gradient. As shown in Fig. 6a, OATv1-expressing oocytes preloaded with [14C]PAH showed time-dependent efflux of radioactivity when incubated in the standard uptake solution. The voltage dependence of PAH transport was also observed for the efflux experiments. The OATv1-expressing oocytes showed a reduction of PAH efflux from the oocytes to the extracellular medium when the oocytes were incubated in high potassium uptake solution, which generates inside positive potential (Fig. 6b). The effect of pH on [14C]PAH efflux was examined in the standard uptake solution with varied pH. The efflux of [14C]PAH from the oocytes expressing OATv1 was dependent on extracellular pH. [14C]PAH efflux was elevated by incubating the oocytes in the uptake solution with higher pH, whereas it was reduced by lowering the pH of the uptake solution (Fig. 6c). To determine whether OATv1 is an exchanger or a facilitated transporter, the efflux of radioactivity from the oocytes preloaded with [14C]PAH was compared in the absence and presence of extracellular PAH. The efflux of radioactivity was not affected by the extracellularly applied PAH (Fig. 6d), consistent with the property of the transporters mediates facilitated diffusion. The efflux of [125I]lactate and [3H]estrone sulfate also showed the identical properties compared with that of [14C]PAH (data not shown).

Substrate Selectivity of OATv1—The substrate selectivity of OATv1 was investigated by inhibition experiments in which the uptake of 20 μM [14C]PAH was measured in the presence of high concentrations of inhibitor. The concentrations of inhibitors were 1 and 5 mM except for ochratoxin A (0.1 mM) and 5-nitro-2-(3-phenylpropylamino)benzoate (0.1 and 1 mM). The PAH uptake was highly inhibited by estrone sulfate, probenecid, 5-nitro-2-(3-phenylpropylamino)benzoate, bumetanide, and furosemide but not by D- and L-lactate, glutarate, and tetraethylammonium (Fig. 7a). Some sulfate and glucuronide conjugates also inhibited OATv1-mediated [14C]PAH uptake. The inhibitory effect on [14C]PAH uptake was stronger for sulfate conjugates than that for glucuronide conjugates (Fig. 5, b and c). The inhibition of [14C]PAH uptake by unlabeled PAH was shown to be competitive in a double reciprocal plot analysis with a Ki value of 0.83 mM. Urate and estrone sulfate also competitively inhibited PAH uptake with Ki values of 1.87 mM and 17.13 μM, respectively (data not shown). Because OATv1 exhibited sequence similarity to NPT1 that was reported to transport both phosphate and organic anions, we examined whether OATv1 transports [32P]phosphate. In the condition in which we detected the [32P]phosphate (100 μM) uptake by rat NaPi type IIa, Na+/phosphate transporter, and OATv1-expressing oocytes did not show a detectable amount of [32P]phosphate uptake (data not shown).

Immunohistochemical Analysis—The immunohistochemical analysis of pig kidney revealed that OATv1 protein was localized in the apical membrane of proximal tubules. The immunoreactivity was detected only in the cortex but not in the medulla portion of pig kidney (Fig. 8). The immunostaining at the apical membrane of proximal tubule completely disappeared in the absorption experiments in which the tissue sections were treated with primary antibody in the presence of antigen peptide (Fig. 8c), confirming the specificity of immunoreactions.

DISCUSSION

In the present study, we identified a novel protein, OATv1, from pig kidney cortex. The amino acid sequence of OATv1 showed the highest similarity to human sodium/phosphate co-
transporter type 1 (human NPT1) that was previously reported to transport phosphate and organic anions. When expressed in Xenopus oocytes, OATv1 exhibited the character of a multispecific organic anion transporter. This transporter mediated the transport of PAH, urate, and estrone sulfate (Fig. 2). Various organic anions but not organic cations interacted with PAH transport by OATv1 (Fig. 7). We named this transporter OATv1 (voltage-driven organic anion transporter 1) because it mediates a facilitated diffusion driven by membrane voltage. The PAH transport was shown to be sodium-independent and chloride-sensitive, as reported for human NPT1 expressed in HEK293 cells (22). In contrast to human NPT1 (43), OATv1 expressed in Xenopus oocytes did not show the uptake of phosphate (data not shown). OATv1, furthermore, differed from human NPT1 in the dependence on pH and K<sup>+</sup>/H<sup>+</sup>. The PAH uptake by human NPT1 expressed in HEK293 cells was increased by lowering extracellular pH, whereas OATv1-mediated PAH uptake was not affected by changing the extracellular pH. In contrast to OATv1, the response to high K<sup>+</sup> medium was not observed for human NPT1. Because of these differences in the functional properties of the transporters, we could not, at this moment, conclude whether OATv1 is an orthologue or paralogue of human NPT1. Furthermore, even though OATv1 exhibited similar substrate selectivity to that of OAT family members, it could not be classified into the OAT family because of its low sequence similarity (25–30%) to members of the OAT family.

Although OATv1-mediated PAH transport was Na<sup>+</sup>-independent, it was affected by altering extracellular K<sup>+</sup> and Cl<sup>-</sup> (Fig. 4). When the extracellular concentration of K<sup>+</sup> was increased or that of Cl<sup>-</sup> was decreased, the PAH uptake mediated by OATv1 was elevated. This suggests that the PAH transport was altered by the action of membrane voltage. Based on the Goldman-Hodgkin and Katz equation, the increase in extracellular K<sup>+</sup> or decrease in extracellular Cl<sup>-</sup> results in the elevation of membrane potential (toward the positive direction). This membrane depolarization is proposed to promote the uptake of negatively charged PAH via OATv1 once the net charge of the fully loaded carrier is negative (24). Moreover, the results from electrophysiological measurements showed that the membrane depolarization increased the outward current associated with the PAH uptake into the oocytes expressing OATv1 (Fig. 5), supporting the idea...
that the PAH transport by OAT\textsubscript{V1} is a voltage-driven transport.

To prove that the transport via OATV1 is a facilitated transport of organic anions, the properties of the efflux of PAH mediated by OATV1 was examined. It was shown in the present study that the efflux of PAH was not stimulated by the application of PAH in the bath solution (extracellular medium), suggesting that PAH transport via OATV1 is not trans-stimulated. Therefore, together with the results from electrophysiological measurements demonstrating the voltage dependence of the outward current associated with PAH transport (Fig. 5), it is suggested that OATV1-mediated transport involves electrogenic facilitated diffusion driven by membrane voltage, rather than the exchange of substrate organic anions.

We found that PAH efflux by OAT\textsubscript{V1} expressed in oocytes was increased by elevating extracellular pH and decreased by lowering extracellular pH (Fig. 6c). This may be consistent with the idea of PAH/OH\textsuperscript{-} exchange that has been proposed for the PAH exit path through the apical membrane of renal proximal tubules (12, 13). We attempted to clarify whether OAT\textsubscript{V1} mediates the PAH/OH\textsuperscript{-} exchange by incubating the oocytes expressing OAT\textsubscript{V1} in the medium containing sodium acetate or NH\textsubscript{4}Cl that causes intracellular acidification (44–46). PAH uptake was, however, not altered significantly by these maneuvers (data not shown). There are two possible explanations for this observation; the first is that OAT\textsubscript{V1} exclusively mediates facilitated transport and a PAH/OH\textsuperscript{-}/H\textsubscript{2}O\textsuperscript{2+} exchange mode is not involved for OAT\textsubscript{V1}. The second is that the voltage-driven PAH transport is much more predominant than the PAH/OH\textsuperscript{-}/H\textsubscript{2}O\textsuperscript{2+} exchange mode, so that it masked the PAH/OH\textsuperscript{-} exchange mode in the experimental conditions. Further investigation is necessary to understand the action of extracellular pH on PAH efflux via OAT\textsubscript{V1}.

OAT\textsubscript{V1} mediated the transports of PAH, urate, sulfate, and glucuronide conjugates of steroid hormones in a sodium-independent manner. In addition, OAT\textsubscript{V1} interacted with various anionic compounds such as nonsteroidal anti-inflammatory drugs and diuretics. Therefore, OAT\textsubscript{V1} is a multispecific organic anion transporter, overlapping substrate selectivity with members of the OAT family. Most of the substrates of OAT1 (6,
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porters OAT1 and OAT3 in exchange for intracellular dicarboxylates (6–9). OAT1 is highly expressed at the basolateral side of the S2 segment of the proximal tubule (40), whereas OAT3 is expressed throughout the proximal tubule (52). In this study, OATV1 was shown to be expressed in the apical membrane of all the segments of the proximal tubule. Therefore, we propose that organic anions are taken up by the epithelial cells of renal proximal tubules via OAT1 and OAT3 and then exit from the epithelial cell via OATV1. Large organic anions with bulky moieties that are not substrates of OAT1 may be transported out of the epithelial cells via MRP2, an ABC transporter, using energy from ATP hydrolysis. It was previously reported that the secretion of PAH via the apical membrane of the proximal tubule might also be mediated by a PAH/glutarate (18) or PAH/OH− exchanger (12, 13). Therefore, in addition to OAT1, other unidentified transporters may also participate in the excretion of organic anions through the epithelium of the renal proximal tubules.

We have identified a novel Na+-independent voltage-driven organic anion transporter, OATV1. It localizes at the apical membrane of the proximal tubule. This transporter might play an important role in the renal excretion of drugs, xenobiotics, and their metabolites in the form of organic anions.

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Identification of a Novel Voltage-driven Organic Anion Transporter Present at Apical Membrane of Renal Proximal Tubule
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