p-Aminohippurate Transport at the Apical Membrane in the OK Kidney Epithelial Cell Line

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Purpose. We investigated the characteristics of transport of an organic anion, *p*-aminohippurate (PAH), at the apical membrane in a kidney epithelial cell line OK.

Methods. Efflux and uptake of [^{14}C]PAH across the apical membrane were measured using OK cell monolayers grown on microporous membrane filters.

Results. PAH efflux to the apical side was greater than that to the basolateral side and significantly inhibited by probenecid. Diethyl pyrocarbonate (DEPC), an inhibitor of potential-sensitive organic anion transport, significantly decreased PAH efflux to the apical side. Moreover, PAH efflux to the apical side was significantly decreased on incubation with high potassium buffer, as compared with control condition. Extracellular pH and Cl^- had no effect on PAH efflux across the apical membrane. PAH uptake from the apical side was inhibited by various organic anions, and the inhibition patterns of PAH uptake from the apical and basolateral sides by various dicarboxylates were similar.

Conclusions. These results suggested that PAH efflux to the apical side in OK cells was mediated by a potential-sensitive transport system, but not by an anion exchanger. Moreover, PAH uptake from the apical side was mediated by a specific transport system, which interacts with various organic anions and dicarboxylates.

KEY WORDS: organic anion; membrane transport; opossum kidney cell; potential-sensitive transport; anion exchanger.

INTRODUCTION

The organic anion transport system of the renal proximal tubules plays an important role in the elimination of a wide variety of anionic compounds including endogenous metabolites, drugs and xenobiotics (1–4). Organic anions are taken up in the proximal tubules from the blood and secreted into the luminal fluid. Studies with intact kidneys, renal cortical slices, isolated renal tubules and renal membrane vesicles have provided a great deal of information about the organic anion transport systems. At the basolateral membrane, *p*-aminohippurate (PAH), a typical organic anion, is transported via the PAH/dicarboxylate (physiologically α-ketoglutarate) exchange system (5,6). Recently, organic anion transporter 1 (OAT1, ROAT1) isolated from rat kidney was shown to mediate PAH/dicarboxylate exchange (7,8) and to be localized at the basolateral membrane (9). On the other hand, studies using brush-border membrane vesicles suggested that PAH transport at the apical membrane is mediated by an anion exchanger and/or a potential-sensitive transport system (10–16). PAH transport at the apical membrane had been investigated mostly with brush-border membrane vesicles, but little in intact cells.

The development of cell culture techniques has promoted the study of transcellular transport of solutes such as organic anions. At present, OK cells, established from the American opossum kidney (17), are a unique established kidney epithelial cell line, in which the transcellular transport of PAH occurs unidirectionally from the basolateral to the apical side (18). Moreover, PAH transport system at the basolateral membrane of OK cells shows similar substrate specificity to that in rat renal proximal tubules (19) and is mediated by the PAH/dicarboxylate exchange system (20). We kinetically evaluated the characteristics of PAH transport at the basolateral and apical membranes in OK cells, and showed that PAH is unidirectionally transported from the basolateral side into cells, whereas PAH transport at the apical membrane was bidirectional (21). In this study, we further investigated the characteristics of PAH transport at the apical membrane of OK cells.

MATERIALS AND METHODS

Materials

D-[1-^{3}H(N)]-Mannitol (736.3 GBq/mmol) and *p*-[glycyl-1-^{14}C]-aminohippuric acid (1.9 GBq/mmol) were obtained from NEN™ Life Science Products, Inc. (Boston, MA). *p*-Aminohippuric acid and probenecid were purchased from Sigma Chemical Co. (St. Louis, MO). Benzylpenicillin, methotrexate, uric acid, tetraethylammonium and DEPC were purchased from Nacalai Tesque (Kyoto, Japan). Indomethacin was purchased from Wako Pure Chemicals (Osaka, Japan). All other chemicals used were of the highest purity available.

Cell Culture

OK cells were cultured in medium 199 (Life Technologies, Inc., Rockville, MD) containing 10% fetal bovine serum (Whittaker Bioproducts Inc., Walkersville, MD) without antibiotics, in an atmosphere of 5% CO_2–95% air at 37°C, and subcultured every 7 days using 0.02% EDTA and 0.05% trypsin (18). OK cells were used between passages 81 and 93.

Transport Measurements

The cellular uptake and efflux of PAH were measured in OK cell monolayers cultured in Transwell® chambers (Costar, Cambridge, MA). The composition of incubation buffer was as follows (in mM): 145 NaCl, 3 KCl, 1 $CaCl_2$, 0.5 $MgCl_2$, 5 D-glucose and 5 HEPES (pH 7.4). After removal of the culture medium, cell monolayers were washed once and preincubated for 10 min with incubation buffer at 37°C. Thereafter, PAH uptake was measured as previously described (20). D-[^{3}H]Mannitol was used to correct for extracellular trapping and nonspecific uptake of PAH in each experiment. Protein content was determined by the method of Bradford (22) with bovine γ-globulin as the standard.

For measurement of PAH efflux from OK cells, the cell monolayers were preincubated with 2 ml of incubation buffer at the apical membrane and with 2 ml of incubation buffer

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containing 15 μM [^{14}C]PAH (28.5 kBq/ml) and 15 μM [^{3}H]mannitol (68.5 kBq/ml) at the basolateral membrane at 37°C for 30 min. At the end of the preincubation period, the cell monolayers were washed rapidly 3 times with 1.5 ml of ice-cold incubation buffer. Then, 2 ml of incubation buffer was added to the apical and basolateral sides, and the cell monolayers were incubated for the specified periods. Then, aliquots of the buffer from the apical and basolateral sides were collected and were replaced with equal volume of incubation buffer. At the end of the incubation period, the filter was washed rapidly 3 times with ice-cold incubation buffer. Then, the cell monolayers on the filters were solubilized in 0.5 ml of 1 N NaOH and radioactivity was counted for determination of the intracellular remaining PAH.

To investigate the effects of extracellular Cl^- or pH on PAH efflux, we substituted Cl^- for gluconate or prepared the incubation buffer with HEPES (pH 7.4, 8.0) or MES (pH 5.4, 6.4). To examine the effects of membrane potential on PAH efflux, high potassium buffer (in mM: 3 NaCl, 145 KCl, 1 $CaCl_2$, 0.5 $MgCl_2$, 5 D-glucose and 5 HEPES, pH 7.4), was added to the Transwell® chamber. In the study of the effects of diethyl pyrocarbonate (DEPC) on PAH efflux, Dulbecco's phosphate-buffered saline (in mM: 137 NaCl, 3 KCl, 8 Na_2HPO_4, 1.5 KH_2PO_4, 1 $CaCl_2$ and 0.5 $MgCl_2$) supplemented with 5 mM D-glucose (PBS(G)) was used before the beginning of PAH efflux. First, OK cell monolayers were washed and preincubated with PBS(G) (pH 7.4) at 37°C for 10 min. Then, they were incubated at 25°C with PBS(G) (pH 6.0) in the presence of [^{14}C]PAH and [^{3}H]mannitol at the basolateral side for 20 min. Thereafter, the buffer at the apical side was aspirated and cells were incubated at 25°C with PBS(G) (pH 6.0) or PBS(G) (pH 6.0) containing 0.5 mM DEPC for 10 min. At the end of the incubation period, the filter was washed rapidly 3 times with ice-cold incubation buffer. Then, PAH efflux and intracellular remaining PAH were measured at 37°C with incubation buffer as described above.

Statistical Analysis

Statistical significance of differences between mean values was calculated using the paired or non-paired *t* test. Multiple comparisons were performed using Scheffé's test after one-way ANOVA.

RESULTS

Effects of Probenecid on PAH Efflux and Intracellular Remaining PAH in OK Cells

Figure 1 shows the time course of PAH efflux and intracellular remaining PAH in OK cells. PAH efflux to the apical side was greater than that to the basolateral side, and amount of intracellular remaining PAH decreased rapidly. Probenecid, an inhibitor of the organic anion transport system, added to the extracellular compartment significantly decreased PAH efflux to both the apical and basolateral sides and increased intracellular remaining PAH.

Effects of DEPC Preincubation on PAH Efflux to the Apical Side and Intracellular Remaining PAH in OK Cells

We investigated the effects of DEPC on PAH efflux to the apical side in OK cells. DEPC pretreatment did not significantly affect PAH accumulation before the initiation of PAH efflux (control, 47.0 ± 11.7; DEPC pretreatment, 39.7 ± 11.5 pmol/mg protein; mean ± SE of three monolayers). As shown in Fig. 2, DEPC pretreatment significantly decreased PAH efflux to the apical side, accompanied with an increase

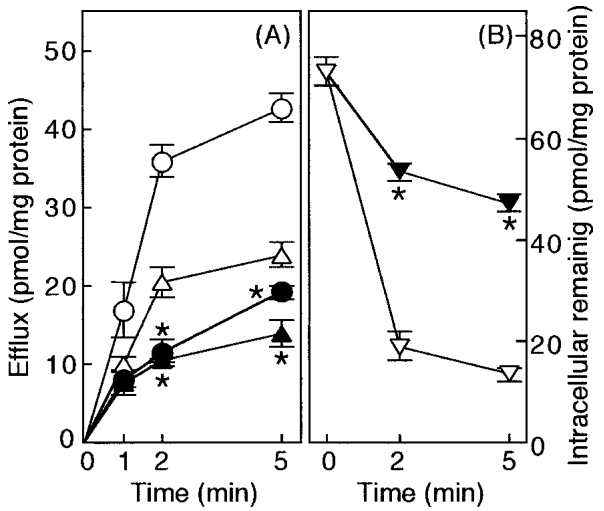

Fig. 1. Effects of probenecid on PAH efflux (A) and intracellular remaining PAH (B) in OK cells. [^{14}C]PAH (15 μM) and D-[^{3}H]mannitol (15 μM) were added to the basolateral side of the monolayers. After incubation for 30 min at 37°C, the monolayers were washed and PAH efflux to the apical (○, ●) and basolateral (△, ▲) sides and intracellular remaining PAH (▽, ▼) were measured at the indicated times in the absence (control, open symbols) or presence of 5 mM probenecid (solid symbols). The data at 2 min represent cumulative values. Each point represents the mean ± SE of three monolayers from a typical experiment. *$p < 0.05$, significantly different from control.

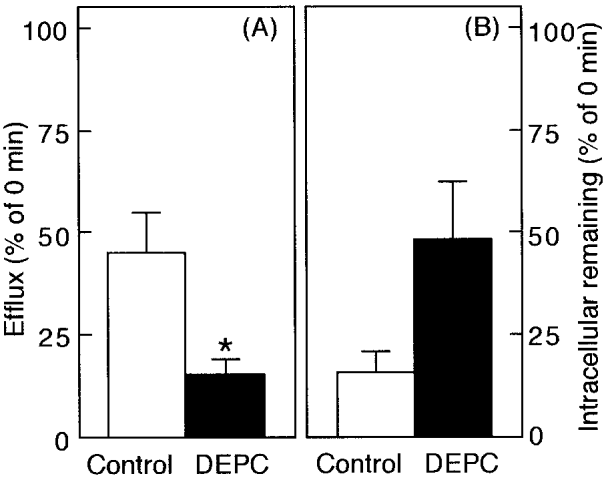

Fig. 2. Effects of DEPC preincubation on PAH efflux to the apical side (A) and intracellular remaining PAH (B) in OK cells. [^{14}C]PAH and D-[^{3}H]mannitol were added to the basolateral side of the monolayers. After incubation for 20 min, buffer on the apical side was replaced with fresh buffer (open columns) or that containing 0.5 mM DEPC (solid columns) and cells were incubated for 10 min. Efflux to the apical side and intracellular remaining PAH at 0 min and 2 min were measured. Each column represents the mean ± SE of three monolayers from a typical experiment. *$p < 0.05$, significantly different from control.

in the amount of intracellular remaining PAH, although this was not significant.

Effects of Membrane Potential on PAH Efflux to the Apical Side in OK Cells

We investigated the effect of membrane potential on PAH efflux to the apical side by means of changing the composition of incubation buffer. When OK cells were incubated with high potassium buffer, PAH efflux to the apical side was significantly decreased compared with control buffer (control, 40.6 ± 6.1; high potassium buffer, 30.5 ± 4.6% of intracellular PAH at 0 min; mean ± SE of three experiments; $p < 0.05$). In the same experiments, PAH efflux to the basolateral side was not changed by the incubation with high potassium buffer (control, 20.7 ± 3.5; high potassium buffer, 19.8 ± 2.8% of intracellular PAH at 0 min; mean ± SE of three experiments).

Effects of Extracellular pH and Chloride Ions on PAH Efflux to the Apical Side and Intracellular Remaining PAH in OK Cells

In previous studies of brush-border membrane vesicles, PAH was shown to be transported by an anion exchanger, which interacts with OH^- or Cl^- (10,11,15). Therefore, we investigated the effects of extracellular pH and Cl^- on PAH efflux to the apical side in OK cells. No stimulation of PAH efflux by alkalinization of the buffer was observed (Fig. 3 A and B). The substitution of gluconate for Cl^- in the incubation buffer did not significantly affect PAH efflux to the apical side or intracellular remaining PAH (Fig. 3 C and D).

Effects of Inhibitors on PAH Uptake from the Apical Side in OK Cells

We investigated the effects of various ionic compounds on PAH transport from the apical side into OK cells (Fig. 4). Various organic anions, i.e., unlabeled PAH, benzylpenicillin, probenecid and indomethacin, strongly inhibited PAH uptake, and methotrexate and uric acid showed moderate inhibitory effects. On the other hand, tetraethylammonium, an organic cation, did not significantly inhibit PAH uptake from the apical side.

Effects of Various Dicarboxylates on PAH Uptake from the Apical Side in OK Cells

We previously reported the inhibition pattern of PAH uptake at the basolateral membrane by various dicarboxylates with different carbon chain lengths (19). Therefore, we investigated the effects of various dicarboxylates on PAH uptake from the apical side (Fig. 5). Dicarboxylates with 3 or 4 carbon atoms (malonate, succinate) had no effect on PAH uptake from the apical side. However, those with 5 or 6 carbon atoms (glutarate, α-ketoglutarate, adipate) strongly inhibited PAH uptake from the apical side and the inhibition was weaker for a longer molecule with 7 carbon atoms (pimelate). The inhibitory effect increased again with increasing number of carbon atoms (suberate, azelate).

DISCUSSION

The organic anion transport system at the apical membrane has been characterized by investigations with brush-border membrane vesicles (10–16). These reports suggested PAH transport at the apical membrane was mediated by an anion exchanger and/or potential-sensitive system. However, PAH transport at the apical membrane in intact cells is not well understood. OK cells are a unique kidney epithelial cell line in which transcellular transport of PAH corresponding to renal secretion was demonstrated (18). Therefore, we investigated the characteristics of PAH transport across the apical membrane in an intact kidney epithelial cell line OK.

As shown in Fig. 1, PAH was effluxed preferentially to the apical side and PAH efflux was inhibited by probenecid, suggesting that PAH efflux to the apical side was a specifically mediated process in OK cells. Studies with brush-border membrane vesicles suggested that PAH was transported via a potential-sensitive transport system (12–15) and diethyl pyro-

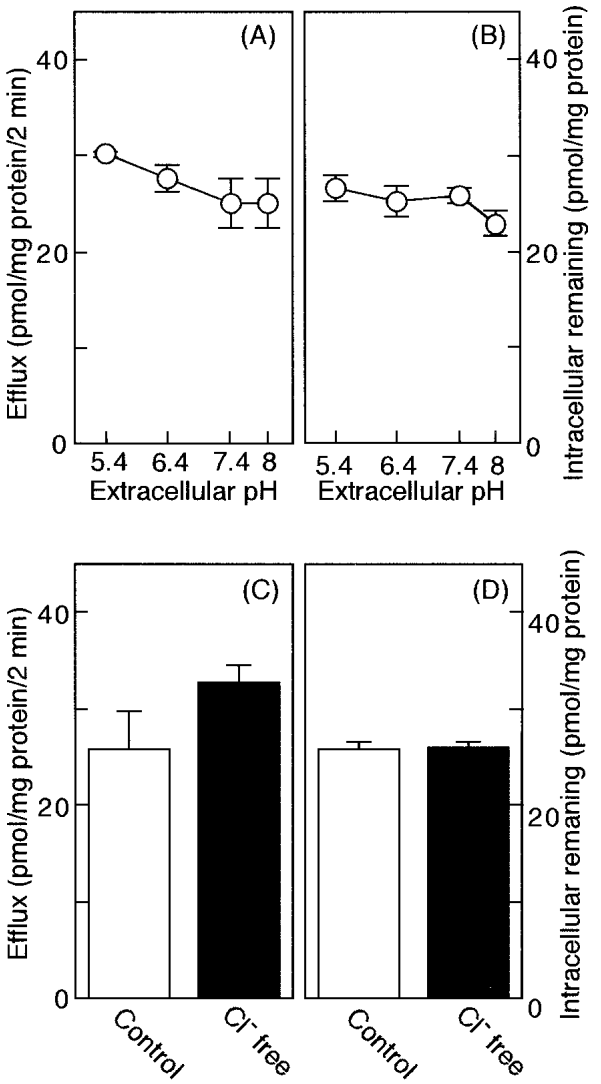

Fig. 3. Effects of extracellular pH (A, B) or Cl^- (C, D) on PAH efflux to the apical side (A, C) and intracellular remaining PAH (B, D) in OK cells. After incubation for 30 min with [^{14}C]PAH and D-[^{3}H]mannitol, cells were washed and incubated for 2 min with incubation buffer prepared at various pHs or substituted Cl^- for gluconate (solid columns). Efflux to the apical side and intracellular remaining PAH at 2 min were measured. Each point and column represent the mean ± SE of three monolayers from a typical experiment.

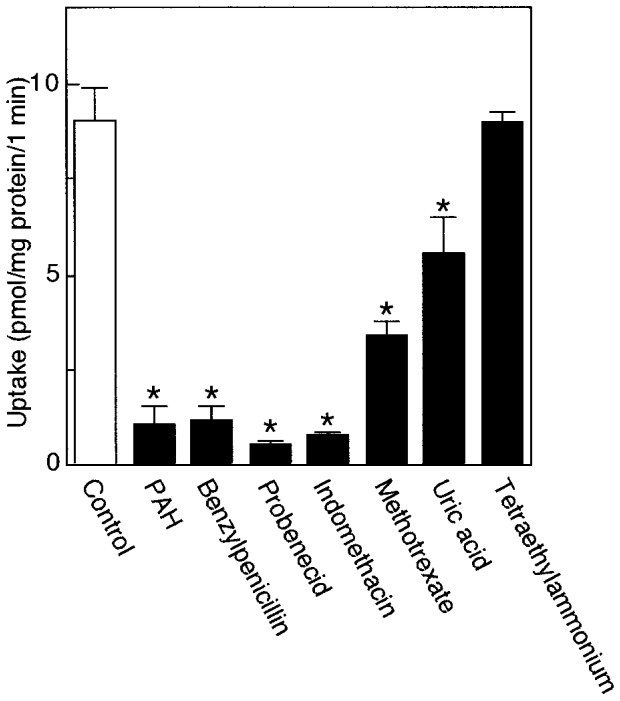

Fig. 4. Effects of inhibitors on PAH uptake from the apical side in OK cells. [^{14}C]PAH (15 μM) and D-[^{3}H]mannitol (15 μM) were added to the apical side of monolayers, and PAH uptake at 1 min was measured in the absence (control; open column) or presence of 1 mM inhibitors (solid columns). Each column represents the mean ± SE of three monolayers from a typical experiment. *$p < 0.05$, significantly different from control.

carbonate (DEPC) selectively inhibited potential-sensitive PAH transport but not the anion exchanger (16). The pretreatment of OK cells with DEPC significantly decreased PAH efflux to the apical side, and PAH efflux to the apical side was significantly decreased on incubation with high potassium buffer in comparison with controls. Therefore, we considered that DEPC-sensitive PAH efflux to the apical side in OK cells might be the potential-sensitive PAH transport. In contrast to our results, in microperfusion using rat intact kidney, PAH efflux were not affected by changes of membrane potential using high potassium buffer (23). We did not know the exact reason for these differences, but OK cells might be useful to evaluate characteristics of potential-sensitive PAH transport.

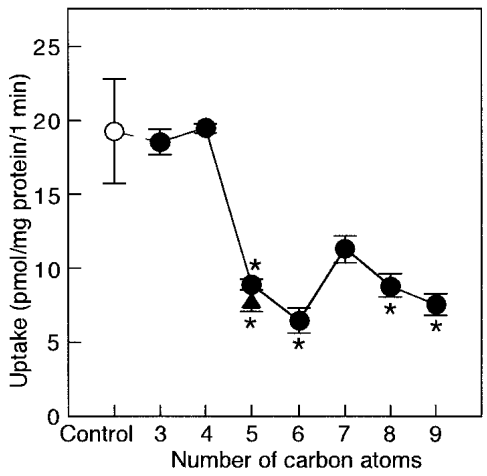

Fig. 5. Effects of various dicarboxylates on PAH uptake from the apical side in OK cells. [^{14}C]PAH uptake from the apical side was measured as described in Fig. 4 in the absence (control; ○) or presence of 1 mM α-ketoglutarate (▲) and other dicarboxylates (COOH-$(CH_2)_X$-COOH) (●) with 3 (malonate), 4 (succinate), 5 (glutarate), 6 (adipate), 7 (pimelate), 8 (suberate) and 9 (azelate) carbon atoms. Each point represents the mean ± SE of three monolayers from a typical experiment. *$p < 0.05$, significantly different from control.

In brush-border membranes, PAH is also transported by an anion exchanger that transports inorganic anions such as OH^- and Cl^- (10,11,15). Therefore, we investigated the effects of extracellular pH and Cl^- on PAH efflux in OK cells. As shown in Fig. 3, extracellular pH and Cl^- had no effect on PAH efflux to the apical side in OK cells and the exchanger appeared to be absent in OK cells. Ullrich and Rumrich (23) reported that PAH efflux to the apical side was not trans-stimulated by Cl^- and OH^- in microperfusion using rat intact kidney, which was consistent with our results. The reason for the difference between the investigations with brush-border membrane vesicles and those with intact cells is unclear. The anion exchanger was reported to be different between species, and to be present in dogs and rats (10,11,15), but absent in rabbits and pigs (12,13) and all these species that do not have the anion exchanger secrete organic anions. Thus, the anion exchanger does not appear to be an obligatory component of secretion.

We further performed inhibition studies of PAH transport from the apical side into OK cells. Probenecid, uric acid, and penicillin inhibited PAH influx at brush-border membrane vesicles (11). PAH uptake from the apical side of OK cells was inhibited by probenecid, uric acid and benzylpenicillin and other organic anions examined also significantly inhibited PAH uptake from the apical side, but tetraethylammonium, an organic cation, did not. These results indicated that PAH was transported from the apical side into cells via a specifically mediated system that recognized various organic anions. Previously, we kinetically evaluated membrane transport of PAH in OK cells and showed that the value of PAH influx clearance was similar to that of PAH efflux clearance at the apical membrane (21). Because membrane potential should work effectively for PAH efflux, potential-sensitive PAH transport system could not be responsible for bidirectional PAH transport at the apical membrane and we considered that PAH efflux and influx at the apical membrane was mediated by distinct transport systems in OK cells.

The interaction of basolateral PAH transporter with dicarboxylates of various chain lengths was investigated in rat proximal tubules (24), across the basolateral membrane in OK cells (19) or in rat organic anion transporter 1 (rOAT1)-expressing oocytes (25). In this study, we showed the inhibition pattern of PAH uptake across the apical membrane by dicarboxylates, which was similar to that across the basolateral membrane in OK cells (19). Organic anion transporter 1 exclusively localizes at the basolateral membrane of rat and human proximal tubules (9,26). However, experiments using bovine and human brush-border membranes indicated that the PAH/α-ketoglutarate exchange system can function in the same fashion in the basolateral membrane (27,28). Therefore, our results in OK cells could be explained by the following two possibilities. First, OAT1 homologue may localize both at the basolateral and apical membranes in OK cells, although

OAT1 homologue in OK cells is not identified. Second, PAH uptake at the apical membrane in OK cells may be mediated by a transporter that interacts with dicarboxylates and is not OAT1 homologue.

Recently, some transporters that transport PAH and are localized at the apical membrane of renal proximal tubules were cloned (29,30). NPT1, characterized as human type 1 sodium-dependent inorganic phosphate transporter, transported organic anions such as PAH, benzylpenicillin and uric acid (29). They speculated that PAH transport via NPT1 could be voltage-sensitive. The apical multidrug resistance protein MRP2 was a part of the PAH transport systems in the renal proximal tubule (30). However, the contributions of these transporters to organic anion transport under physiologic conditions are unclear and further studies at the molecular level are needed to understand the mechanisms of renal secretion of organic anions.

In conclusion, we demonstrated that PAH transport at the apical membrane was mediated by specific processes in OK cells. The efflux of intracellular PAH occurred preferentially to the apical side, and this process was mediated by a potential-sensitive transport system. However, PAH efflux to the apical side was not mediated by an anion exchanger in OK cells. We also proposed the presence of a specific transport system for PAH uptake across the apical membrane in OK cells. These results should contribute to our understanding of the transport mechanisms of organic anions in the kidney.

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REFERENCES

1. J. B. Pritchard and D. S. Miller. Renal secretion of organic anions and cations. *Kidney Int.* **49**:1649–1654 (1996).
2. K. Inui and M. Okuda. Cellular and molecular mechanisms of renal tubular secretion of organic anions and cations. *Clin. Exp. Nephrol.* **2**:100–108 (1998).
3. K. Inui, S. Masuda, and H. Saito. Cellular and molecular aspects of drug transport in the kidney. *Kidney Int.* **58**:944–958 (2000).
4. K. J. Ullrich. Specificity of transporters for "organic anions" and "organic cations" in the kidney. *Biochim. Biophys. Acta* **1197**:45–62 (1994).
5. H. Shimada, B. Moewes, and G. Burckhardt. Indirect coupling to Na^+ of *p*-aminohippuric acid uptake into rat renal basolateral membrane vesicles. *Am. J. Physiol.* **253**:F795–F801 (1987).
6. J. B. Pritchard. Coupled transport of *p*-aminohippurate by rat kidney basolateral membrane vesicles. *Am. J. Physiol.* **255**:F597–F604 (1988).
7. T. Sekine, N. Watanabe, M. Hosoyamada, Y. Kanai, and H. Endou. Expression cloning and characterization of a novel multispecific organic anion transporter. *J. Biol. Chem.* **272**:18526–18529 (1997).
8. D. H. Sweet, N. A. Wolff, and J. B. Pritchard. Expression cloning and characterization of ROAT1. The basolateral organic anion transporter in rat kidney. *J. Biol. Chem.* **272**:30088–30095 (1997).
9. A. Tojo, T. Sekine, N. Nakajima, M. Hosoyamada, Y. Kanai, K. Kimura, and H. Endou. Immunohistochemical localization of multispecific renal organic anion transporter 1 in rat kidney. *J. Am. Soc. Nephrol.* **10**:464–471 (1999).
10. S. E. Guggino, G. J. Martin, and P. S. Aronson. Specificity and modes of the anion exchanger in dog renal microvillus membranes. *Am. J. Physiol.* **244**:F612–F621 (1983).
11. T. G. Steffens, P. D. Holohan, and C. R. Ross. Operational modes of the organic anion exchanger in canine renal brush-border membrane vesicles. *Am. J. Physiol.* **256**:F596–F609 (1989).
12. F. Martinez, M. Manganel, C. Montrose–Rafizadeh, D. Werner, and F. Roch–Ramel. Transport of urate and *p*-aminohippurate in rabbit renal brush-border membranes. *Am. J. Physiol.* **258**: F1145–F1153 (1990).
13. D. Werner, F. Martinez, and F. Roch–Ramel. Urate and *p*-aminohippurate transport in the brush border membrane of the pig kidney. *J. Pharmacol. Exp. Ther.* **252**:792–799 (1990).
14. W. Krick, N. A. Wolff, and G. Burckhardt. Voltage-driven *p*-aminohippurate, chloride, and urate transport in porcine renal brush-border membrane vesicles. *Pflügers Arch.* **441**:125–132 (2000).
15. K. Ohoka, M. Takano, T. Okano, S. Maeda, K. Inui, and R. Hori. *p*-Aminohippurate transport in rat renal brush-border membranes: a potential-sensitive transport system and an anion exchanger. *Biol. Pharm. Bull.* **16**:395–401 (1993).
16. M. Takano, J. Nagai, T. Okano, K. Hirozane, M. Yasuhara, and R. Hori. Differential sensitivity of organic anion transporters in rat renal brush-border membrane to diethyl pyrocarbonate. *Biochim. Biophys. Acta* **1192**:190–196 (1994).
17. H. Koyama, C. Goodpasture, M. M. Miller, R. L. Teplitz, and A. D. Riggs. Establishment and characterization of a cell line from the American opossum (*Didelphys virginiana*). *In Vitro* **14**:239–246 (1978).
18. R. Hori, M. Okamura, A. Takayama, K. Hirozane, and M. Takano. Transport of organic anion in the OK kidney epithelial cell line. *Am. J. Physiol.* **264**:F975–F980 (1993).
19. J. Nagai, M. Takano, K. Hirozane, M. Yasuhara, and K. Inui. Specificity of *p*-aminohippurate transport system in the OK kidney epithelial cell line. *J. Pharmacol. Exp. Ther.* **274**:1161–1166 (1995).
20. J. Nagai, I. Yano, Y. Hashimoto, M. Takano, and K. Inui. Efflux of intracellular α-ketoglutarate via *p*-aminohippurate/dicarboxylate exchange in OK kidney epithelial cells. *J. Pharmacol. Exp. Ther.* **285**:422–427 (1998).
21. Y. Habu, I. Yano, M. Okuda, Y. Hashimoto, and K. Inui. Kinetic analysis of *p*-aminohippurate transport in the OK kidney epithelial cell line. *Pharm. Res.* **17**:1155–1157 (2000).
22. M. M. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
23. K. J. Ullrich and G. Rumrich. Luminal transport step of *para*-aminohippurate (PAH): transport from PAH-loaded proximal tubular cells into the tubular lumen of the rat kidney in vivo. *Pflügers Arch.* **433**:735–743 (1997).
24. K. J. Ullrich, G. Rumrich, G. Fritzsch, and S. Klöss. Contraluminal *para*-aminohippurate (PAH) transport in the proximal tubule of the rat kidney. II. Specificity: Aliphatic dicarboxylic acids. *Pflügers Arch.* **408**:38–45 (1987).
25. Y. Uwai, M. Okuda, K. Takami, Y. Hashimoto, and K. Inui. Functional characterization of the rat multispecific organic anion transporter OAT1 mediating basolateral uptake of anionic drugs in the kidney. *FEBS Lett.* **438**:321–324 (1998).
26. M. Hosoyamada, T. Sekine, Y. Kanai, and H. Endou. Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. *Am. J. Physiol.* **276**:F122–F128 (1999).
27. C. Schmitt and G. Burckhardt. *p*-Aminohippurate/2-oxoglutarate exchange in bovine renal brush-border and basolateral membrane vesicles. *Pflügers Arch.* **423**:280–290 (1993).
28. F. Roch–Ramel, B. Guisan, and L. Schild. Indirect coupling of urate and *p*-aminohippurate transport to sodium in human brush-border membrane vesicles. *Am. J. Physiol.* **270**:F61–F68 (1996).
29. H. Uchino, I. Tamai, K. Yamashita, Y. Minemoto, Y. Sai, H. Yabuuchi, K. Miyamoto, E. Takeda, and A. Tsuji. *p*-Aminohippuric acid transport at renal apical membrane mediated by human inorganic phosphate transporter NPT1. *Biochem. Biophys. Res. Commun.* **270**:254–259 (2000).
30. I. Leier, J. Hummel–Eisenbeiss, Y. Cui, and D. Keppler. ATP-dependent *para*-aminohippurate transport by apical multidrug resistance protein MRP2. *Kidney Int.* **57**:1636–1642 (2000).