Kinetic Analysis of *p*-Aminohippurate Transport in the OK Kidney Epithelial Cell Line

Yasushi Habu,[1] Ikuko Yano,[1] Masahiro Okuda,[1] Yukiya Hashimoto,[1] and Ken-ichi Inui[1,2]

Received March 5, 2000; accepted June 5, 2000

KEY WORDS: organic anion; p-aminohippurate; epithelial transport; kinetic analysis; opossum kidney (OK) cell line.

INTRODUCTION

The organic ion transport systems of the renal proximal tubules play important physiological roles in excretion of a wide variety of ionic compounds including endogenous metabolites, drugs, and xenobiotics into the urine (1). These systems are highly effective, and evaluation of the secretion in proximal tubules is necessary for rational design of drug administration schedules, and for detection of drug–drug interactions. Studies of renal membrane vesicles have provided much information about the transport systems in renal basolateral and brush–border membranes (1–2). However, because the renal secretion of organic ions consists of two-transport steps in apical and basolateral membranes, it is essential to study the transport in intact cells. Cultured epithelial cells derived from the kidney have been useful in the study of transcellular transport of solutes, including organic ions, across renal epithelial monolayers. The pig kidney epithelial cell line LLC-PK_1 was shown to be a model for the renal proximal tubular secretion of organic cations (3) and we clarified how apical and basolateral transport steps contribute to transcellular transport of organic cations in LLC-PK_1 cells (4). On the other hand, OK cells, which were established from the American opossum kidney (5), possess the ability to transport *p*-aminohippurate (PAH), an organic anion, in the apical and basolateral membranes (6). Therefore, in the present study we established an *in vitro* model to evaluate membrane transport of organic anions, and to quantitatively evaluate PAH transport at apical and basolateral membranes in OK cells.

MATERIALS AND METHODS

Materials

D-[1-^{3}H(N)]-Mannitol (728.9 GBq/mmol) and *p*-[glycyl-1-^{14}C]-aminohippuric acid (1.5022 GBq/mmol) were obtained from NEN™ Life Science Products, Inc. (Boston, MA). All other chemicals used were of the highest purity available.

Cell Culture

OK cells were maintained as previously described (6), and the cells were used between passages 77 and 88.

Measurement of Cell Volume and Intracellular α-Ketoglutarate Concentration in OK Cells

The volume of OK cells cultured in Transwell™ chambers (Costar, Cambridge, MA) was estimated by the amount of sulfanilamide in the cells at steady-state (7). The amount of α-ketoglutarate (α-KG) in OK cell monolayers cultured in Transwell™ chambers was measured as previously described (8). The concentration of α-KG was calculated with the intracellular volume of OK cell monolayers.

Transepithelial Transport and Accumulation of PAH in OK Cells

Transepithelial transport and cellular accumulation of PAH were measured in OK cell monolayers cultured in Transwell™ chambers. Culture medium was removed and the monolayers were washed once with Dulbecco's phosphate-buffered saline (PBS), supplemented with 5 mM D-glucose or PAH medium. PAH medium was 5, 15, or 50 μM PAH in PBS, containing (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. To reach steady-state, the monolayers were incubated with 2 ml of PAH medium on each side for 30 min at 37°C. Thereafter, apical and basolateral media were aspirated off, 5, 15, or 50 μM [^{14}C]PAH medium (7.5 or 22.5 KBq/ml, 2 ml) was added to one side, and unlabeled PAH medium (2 ml) to the other. At the indicated times, the levels of radioactivity in the opposite side and in the cells were measured. To estimate the paracellular transport and the extracellular trapping of PAH, 5 or 15 μM [^{3}H]mannitol (22.8 or 68.5 KBq/ml) was added to the [^{14}C]PAH medium. Data were obtained from four separate experiments with different cell cultures. Each experiment was carried out using two or three cell monolayers. Radioactivity and protein content were measured as previously described (6,9).

Data Analysis

Transport clearance of PAH in OK cell monolayers was analyzed using a simple model (Fig. 1) as previously described (4). $CL_{A\text{-}B}$ and $CL_{B\text{-}A}$ were analyzed by the two-compartment model using [^{3}H]mannitol transport data simultaneously assayed with [^{14}C]PAH. $CL_{A\text{-}C}$, $CL_{C\text{-}A}$, $CL_{C\text{-}B}$, and $CL_{B\text{-}C}$ of PAH transport were analyzed by a three-compartment model using [^{14}C]PAH net transport and accumulation data. In this analysis, $CL_{A\text{-}B}$ and $CL_{B\text{-}A}$ were fixed to the values obtained using [^{3}H]mannitol data in each condition. The kinetic parameters were calculated with NONMEM software (10) on a FACOM M1800 running on a UXP/M UNIX clone at the Kyoto University Data Processing Center.

Data are expressed as means ± S.E. Statistical analysis between mean values was performed using Sheffé's test following ANOVA. Statistical significance in kinetic analysis was evaluated by likelihood ratio test using the minimum values of the objective function (–2 log likelihood) produced by NONMEM (10). *P* values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

To study transport of organic anions across apical and basolateral membranes in renal proximal cells, we analyzed

[1] Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan.
[2] To whom correspondence should be addressed. (e-mail: inui@kuhp.kyoto-u.ac.jp)

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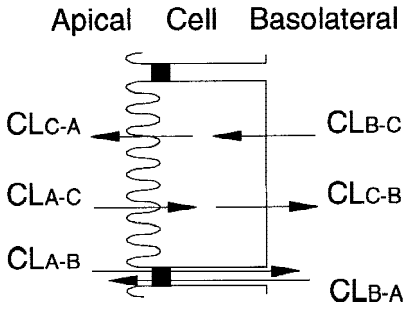

Fig. 1. Three-compartment transport model. $CL_{A\text{-}C}$, $CL_{C\text{-}A}$, $CL_{C\text{-}B}$, and $CL_{B\text{-}C}$ represent clearance across membranes from the apical medium to cells, from cells to the apical medium, from cells to the basolateral medium and from the basolateral medium to cells, respectively. $CL_{A\text{-}B}$ and $CL_{B\text{-}A}$ represent clearance through the paracellular route in the apical to basolateral, and the basolateral to apical direction, respectively.

PAH transport based on a simple three-compartment model. We also examined the concentration dependence of PAH transport using this model.

The transport and accumulation of PAH by OK cells were measured at steady-state with 5 μM PAH. As shown in Figure 2A, the basolateral-to-apical transport of PAH was greater than that of mannitol. On the other hand, the apical-to-basolateral transport of PAH was less than that of mannitol. Therefore, PAH was transported in the secretory direction. When PAH was accumulated from the apical or basolateral side, the amounts of PAH in OK cells were 0.0849 ± 0.0260 or 0.306 ± 0.077 % of dose at 60 min, and the intracellular concentration reached about 0.74 or 2.7-fold higher than the extracellular concentration, respectively.

PAH clearance across each membrane was calculated at 5 μM PAH (Table 1) using the intracellular volume of 2.40 ± 0.06 μl/mg protein obtained by analyzing sulfanilamide accumulation. In this analysis, we evaluated the paracellular flux of PAH by mannitol transport. The mean values of $CL_{A\text{-}B}$ and $CL_{B\text{-}A}$ were estimated as 3.60 ± 0.15 and 3.40 ± 0.15 μl/min/mg protein, respectively. $CL_{A\text{-}C}$ and $CL_{C\text{-}A}$ were similar under all conditions examined, while $CL_{B\text{-}C}$ was much larger than $CL_{C\text{-}B}$. Thus, the characteristics of PAH transport were different between the apical and basolateral membranes. The characteristics of organic anion transport across the basolateral membrane were reported to be essentially the same in all vertebrate species studied to date (11). Recently, organic anion transporter 1 (OAT1, ROAT1) was isolated from rat kidney (12–13) and the characteristics of PAH uptake across basolateral membrane in OK cells were shown to be similar to those of OAT1 (14). On the other hand, the detailed characteristics of organic anion transport across the apical membrane are not known since they appear to be different between species (1). OK cells are a uniquely established kidney epithelial cell line in which organic anion transport across the apical and basolateral membranes are specifically mediated processes (6). Therefore, OK cells appear to be a useful model to examine organic anion secretion in intact epithelial cells.

The results of kinetic analysis showed the unidirectional transport of PAH at the basolateral membrane in OK cells. Since α-KG is considered to be the primary physiological driving force of organic anion transport at the basolateral membrane (15), we measured intracellular concentration of α-KG in OK cells. Intracellular α-KG concentrations after incubation with 0, 5, 15, and 50 μM PAH were 1.36 ± 0.04, 1.31 ± 0.02, 1.36 ± 0.09, and 1.13 ± 0.05 mM, respectively,

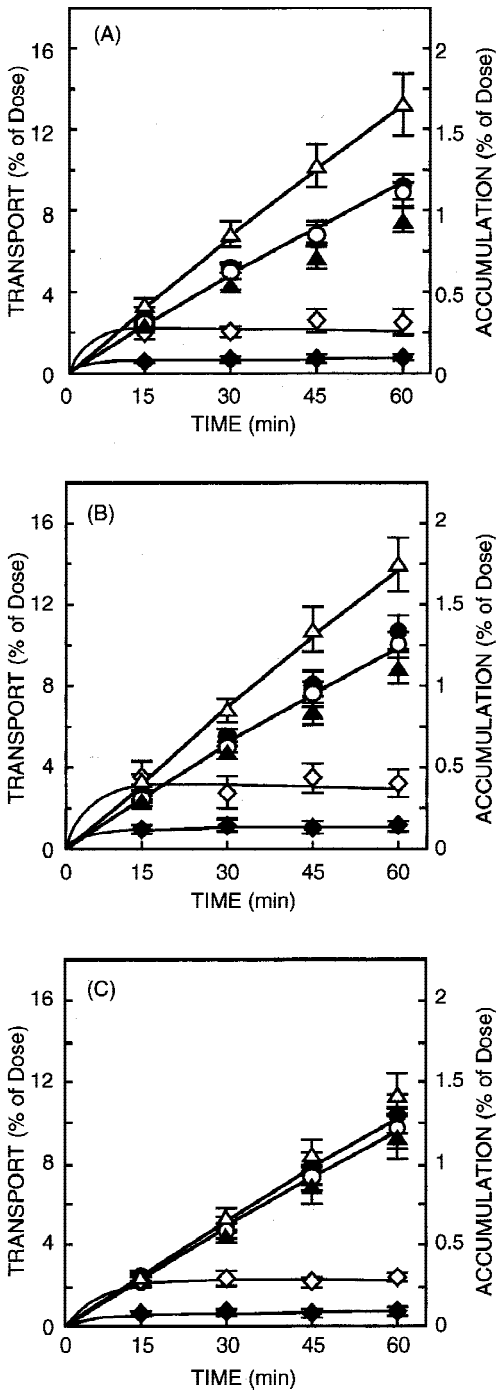

Fig. 2. Transport of PAH from apical to basolateral medium (▲) and from basolateral to apical medium (△), and cellular accumulation of PAH from apical medium (◆) and from basolateral medium (◇). Paracellular flux of mannitol from the apical (●) and basolateral medium (○). Extracellular concentration of PAH was 5 μM (A), 15 μM (B) or 50 μM (C). The lines represent the estimated transport and accumulation using the mean clearance values obtained from 4 separate experiments. Each point represents the mean ± S.E. of 4 separate experiments.

Table 1. PAH Clearance in OK Cells

PAH	Clearance (μl/min/mg protein)			
	CL_{A-C}	CL_{C-A}	CL_{C-B}	CL_{B-C}
5 μM	0.383 ± 0.095	0.726 ± 0.077	5.02E–13 ± 4.82E–13	1.685 ± 0.161
	0.383 ± 0.095	0.726 ± 0.077	0	1.685 ± 0.161
15 μM	0.405 ± 0.090	0.541 ± 0.026	3.39E–11 ± 3.38E–11	1.819 ± 0.287
	0.405 ± 0.090	0.541 ± 0.026	0	1.819 ± 0.287
50 μM	0.214 ± 0.033	0.260 ± 0.046	1.14E–1 ± 9.09E–2	0.946 ± 0.015
	0.202 ± 0.040	0.323 ± 0.056*	0	0.856 ± 0.057*

Note: Values are means ± S.E. from 4 separate experiments. In each experiment, clearance values were calculated as described in Materials and Methods. The values in the lower row were obtained from analysis in which CL_{C-B} was fixed as 0. CL_{A-C}, clearance from the apical medium to cells; CL_{C-A}, clearance from cells to the apical medium; CL_{C-B}, clearance from cells to the basolateral medium; CL_{B-C}, clearance from the basolateral medium to cells.

* $P < 0.05$, significantly different from 5 μM PAH.

obtained from 4 monolayers in a typical experiment. Pritchard measured α-KG levels in rat renal slices and estimated extramitochondrial cytoplasmic α-KG concentration (15). Applying a similar hypothesis, our observations indicated that the extramitochondrial cytoplasmic α-KG concentration in OK cells would be approximately 600 μM. We reported that the rate of α-KG efflux from OK cells into the basolateral medium (0.5 ml) was approximately 1.5 nmol/cm^2/60 min, which corresponded to 14 μM α-KG in basolateral medium (8). Thus, α-KG concentration in basolateral medium was much smaller than the intracellular α-KG concentration, and the unidirectional PAH transport across the basolateral membrane may be due to an overwhelming driving force.

To evaluate the concentration dependence of PAH transport, the transport and accumulation were measured at steady-state with 15 and 50 μM PAH. The characteristics of PAH transport at 15 μM (Fig. 2B) were similar to those at 5 μM PAH (Fig. 2A). On the other hand, transport of PAH at 50 μM approached that of mannitol (Fig. 2C). Therefore, transcellular transport of PAH was partially saturated at a concentration of 50 μM. Then, we calculated PAH clearance at 15 and 50 μM PAH. Since the value of CL_{C-B} was negligible, we fixed CL_{C-B} as 0 (Table 1). Log likelihood ratio test showed that CL_{C-B} was not significantly different from 0 in all experiments. Each clearance at 15 μM PAH was nearly equivalent to that at 5 μM PAH. At 50 μM PAH, CL_{A-C}, CL_{C-A}, and CL_{B-C} were about half of those at 5 μM. Therefore, PAH transport at both membranes seemed to have high affinity. The Michaelis constant was 64.0 μM estimated from the basolateral uptake of PAH in OK cells (6), which was not markedly different from our results.

In conclusion, our model offers a simple means of analyzing the transport of anionic drugs across apical and basolateral membranes simultaneously in intact epithelial cells. This method clarified the kinetic characteristics of PAH transport across both membranes in OK cells.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan and by grants from the Japan Research Foundation for Clinical Pharmacology and the Uehara Memorial Foundation.

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