Kinetic Analysis of Tetraethylammonium Transport in the Kidney Epithelial Cell Line, LLC-PK$_1$

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Purpose. The aims of this study were to establish a kinetic means of analyzing the membrane transport of organic cations in renal epithelial cells, and to simultaneously evaluate drug interactions in apical and basolateral membranes.

Methods. Tetraethylammonium (TEA) transport was measured using LLC-PK$_1$ cell monolayers grown on microporous membrane filters. After incubating the cells with unlabeled TEA or other drugs, apical or basolateral medium was changed to that containing labeled TEA, and transcellular transport and cellular accumulation were measured. Clearance from apical medium to cells (CL_{12}), cells to apical medium (CL_{21}), cells to basolateral medium (CL_{23}) and basolateral medium to cells (CL_{32}) were calculated based on a three compartment model.

Results. TEA was accumulated progressively in the monolayers from the basolateral side and was transported unidirectionally to the apical side. CL_{32} was greater than CL_{12} and CL_{23} was greater than CL_{21}. Therefore, the rate limiting step of TEA transport from the basolateral to the apical medium was the cell-to-apical step. Co-incubation of TEA with procainamide decreased the transport parameters of TEA, CL_{12}, CL_{21} and CL_{32}, whereas that with levofloxacin decreased only CL_{12} and CL_{21}, not affecting the parameters in basolateral membranes.

Conclusions. Using a simple model, we analyzed the transport of organic cation in kidney epithelial cell line, LLC-PK$_1$. This method can be useful for the analysis of cation transport and drug interactions in the apical and basolateral membranes of renal tubules.

KEY WORDS: organic cation; epithelial transport; LLC-PK$_1$; kinetic model; levofloxacin.

INTRODUCTION

Organic cation transport in the renal proximal tubule has been intensively studied using membrane vesicles. For example, tetraethylammonium (TEA), an organic cation, is transported by an H^+ gradient dependent active transporter in brush-border (apical) membranes (1–8) and by a membrane potential dependent facilitating transporter in basolateral membranes (1,3,9). Furthermore, using epithelial cell lines (10–13), renal tubules and other models (7,14–17), organic cation transport from blood to the lumen has been studied. This process consists of two transport-steps in apical and basolateral membranes. It remains still unclear how these steps contribute to the organic cation transport in intact cells. We previously demonstrated the validity of LLC-PK$_1$ cell monolayers as a model for the renal proximal tubular secretion of organic cations (13). In this study, we constructed a simple model to simultaneously analyze TEA transport in apical and basolateral membranes of LLC-PK$_1$ cell monolayers, and then estimated the kinetic parameters of TEA transport at lower apical pH or in the presence of other drugs.

MATERIALS AND METHODS

Materials

D-[1-^{3}H(N)]Mannitol (828.8GBq/mmol) and [1-^{14}C]tetraethylammonium bromide (185 or 124.3 MBq/mmol) were obtained from DuPont-New England Nuclear Research Products (Boston, MA). Levofloxacin was supplied by Daiichi Seiyaku Co. (Tokyo, Japan). All other chemicals were of the highest purity available.

Cell Culture

LLC-PK$_1$ cells were maintained as described (13), and the cells were used between passages 217 and 220.

Transport Study

Transepithelial transport and accumulation of [^{14}C]TEA were measured in monolayers cultured in Transwell chambers as described (13), except collagen-uncoated filters were used. The composition of the incubation medium was as follows: 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, 5 mM MES (pH 6.0) or HEPES (pH 7.4). The pH of the medium was adjusted with a solution of HCl or NaOH. Culture medium was removed from both sides of the monolayers, and the monolayers were washed once with TEA medium (50 μM TEA in the incubation medium, pH 7.4) and incubated with 2 ml of TEA medium in each side for 30 min at 37°C. When transport was measured in apical medium at pH 6.0, TEA medium in the apical side was changed after 25 min to TEA medium (pH 6.0) and the monolayers were incubated for a further 5 min. Thereafter, apical and basolateral media were aspirated, and then 50 μM [^{14}C]TEA medium (2 ml) was added to one side, and unlabeled TEA 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 [^{14}C]TEA, 5 μM [^{3}H]mannitol (4.6 GBq/mmol) was added to the 50 μM [^{14}C]TEA medium. To examine drug interactions, 2.5 mM procainamide or 1 mM levofloxacin was added to labeled and unlabeled TEA medium. Data were obtained from four separate experiments with different cell cultures. Each experiment was carried out using two or three cell monolayers. The cell volume was estimated by the amount of sulfanilamide in the cell at steady-state (3.4 μl/mg protein) (18). Radioactivity and protein content (0.67 ± 0.01 mg/well) were measured as described (13).

Kinetic Analysis

CL_{13} and CL_{31} (Fig. 1) were analyzed by the two compartment model using [^{3}H]mannitol transport data simultaneously

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ABBREVIATIONS: TEA, tetraethylammonium; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; MES, 2-(N-morpholino) ethanesulfonic acid.

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assayed with [^{14}C]TEA. When a drug was applied to apical (basolateral) medium,

$$dX_1/dt = -K_{13}*X_1 + K_{31}*X_3 \quad (1)$$

$$dX_3/dt = -K_{31}*X_3 + K_{13}*X_1 \quad (2)$$

(X_1, X_3 = the amount of drug in each compartment,

K_{13}, K_{31} = the rate constant

(CL = K/compartment volume))

CL_{13} and CL_{31} were not significantly different and did not change under the various conditions studied in this report (data not shown). CL_{12}, CL_{21}, CL_{23}, CL_{32} were analyzed by a three compartment model using [^{14}C]TEA transport data corrected by [^{3}H]mannitol data. CL_{13} and CL_{31} were fixed in this analysis to the data obtained under each condition. When a drug was applied to apical (basolateral) medium,

$$dX_1/dt = -K_{12}*X_1 + K_{21}*X_2 - K_{13}*X_1 + K_{31}*X_3 \quad (3)$$

$$dX_2/dt = -K_{21}*X_2 + K_{12}*X_1 - K_{23}*X_2 + K_{32}*X_3 \quad (4)$$

$$dX_3/dt = -K_{32}*X_3 + K_{23}*X_2 - K_{31}*X_3 + K_{13}*X_1 \quad (5)$$

(X_1, X_2, X_3 = the amount of drug in each compartment,
K_{12}, K_{21}, K_{23}, K_{32}, K_{13}, K_{31} = the rate constant
(CL = K/compartment volume))

At apical pH 6.0, transport data were obtained only at 5 minutes, to avoid the effect of the change in intracellular pH. CL_{23} and CL_{32} were fixed to the values obtained under the same conditions except for the apical pH.

Data were analyzed using NONMEM software on a FACOM M1800 running under a UXP/M UNIX clone at the Kyoto University Data Processing Center (19).

Data are expressed as means ± SE. Statistical significance between mean values was calculated using a non-paired *t* test provided that the variances between groups were similar. If this is not the case, Mann-Whitney's U-test was applied.

RESULTS

Kinetic Analysis of TEA Transport

TEA transport in LLC-PK$_1$ cell monolayers was analyzed using a simple model (Fig. 1). To avoid changing parameters during the study, unlabeled TEA, procainamide or levofloxacin was added to the incubation medium. During the initial 30 minutes, less than 4.1% of the labeled TEA was transported to the opposite side, and less than 1.2% labeled TEA was accumulated in the cells. Thus, the total TEA concentration in apical and basolateral medium was assumued as constant during the experiments. TEA clearance across each membrane was calculated under each condition (Table 1), and based on the mean values of four separate experiments, transport was simulated (Figs. 2, 3, 4). Despite the simplicity of the model, the simulation curves fit the observed data (symbols) well, suggesting the adequacy of the model.

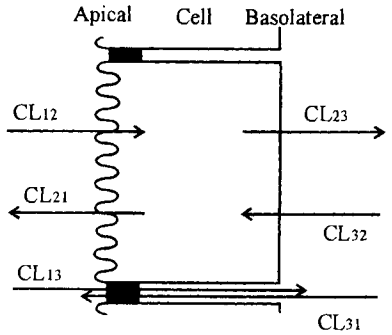

Fig. 1. Three compartment transport model. CL_{12}, CL_{21}, CL_{23} and CL_{32} represent clearance across membranes from apical to cell, from cell to apical, from cell to basolateral, from basolateral to cell directions, respectively. CL_{13} and CL_{31} represent clearance through paracellular routes from apical to basolateral, from basolateral to apical directions, respectively.

Transcellular Transport of TEA

As shown in Figure 2, the transport of TEA from basolateral to apical was much greater than that from apical to basolateral, and more TEA accumulated into cells from the basolateral, than from the apical medium. The clearance from basolateral medium to cells (CL_{32}) was much greater than that from apical medium (CL_{12}) (Table 1). At the apical pH 7.4, the clearance from cells to basolateral medium (CL_{23}) was larger than that to apical medium (CL_{21}). Thus, the rate limiting step of transcellular transport of TEA from the basolateral to the apical medium, was cell-to-apical step at apical pH 7.4.

When the apical pH was changed from 7.4 to 6.0, TEA transport from the basolateral to the apical side was increased, while the cellular accumulation of TEA was decreased (Fig. 2 A, B). As shown in Table 1, CL_{21} value was much increased at pH 6.0, compared with that at pH 7.4. CL_{32} value was much greater than CL_{12} value at apical pH 6.0, being the same as that at apical pH 7.4 (Table 1). At the apical pH 6.0, CL_{21} value was larger than CL_{23} value. However, the value of CL_{21} was much lower than that of CL_{32}. The predicted intracellular concentration at apical pH 6.0 was 230 μM (predicted accumulation was 0.53 nmol/well and intracellular volume was 2.3 μl/well). Therefore, the intracellular concentration was 4.6-fold that of the basolateral medium, while the value of CL_{32} was 8-fold that of CL_{21}. Thus, the rate limiting step of TEA transport from the basolateral, to the apical medium, was the cell-to-apical step even at apical pH 6.0.

Effect of Procainamide on TEA Transport

Procainamide transport in renal epithelial cell membranes has been intensively studied (20–22). Procainamide interacts with the apical H^+/organic cation antiport (21,22) and the basolateral potential-dependent, organic cation transport systems (13,20). We measured TEA transport in the presence of procainamide (Fig. 3). Transcellular transport of TEA from basolateral to apical (A) was decreased, and the accumulation of TEA from both the basolateral (B) and the apical (D) sides were decreased in the presence of procainamide (Fig. 2, 3). As shown in Table 1, CL_{12} and CL_{32} values were decreased in the presence of procainamide at apical pH 7.4 and 6.0. On the other hand, the CL_{21} value was decreased in the presence of procainamide only at apical pH 6.0.

Effect of Levofloxacin on TEA Transport

Ofloxacin and levofloxacin, pyridone carboxylic acid antibacterial drugs, interact with the apical H^+/organic cation anti-

Table 1. Clearance of Tetraethylammonium in LLC-PK$_1$

Condition	CL_{12}	CL_{21}	CL_{23}	CL_{32}
Control				
Apical pH = 7.4	1.89 ± 0.29	0.31 ± 0.01	0.69 ± 0.04	9.02 ± 1.15
Apical pH = 6.0	1.40 ± 0.30	1.2 ± 0.09[a]		
Procainamide				
Apical pH = 7.4	0.81 ± 0.06[a]	0.26 ± 0.03	0.92 ± 0.15	3.43 ± 0.22[b]
Apical pH = 6.0	0.55 ± 0.06[c]	0.67 ± 0.07[d]		
Levofloxacin				
Apical pH = 7.4	0.80 ± 0.04[a]	0.10 ± 0.01[b]	0.58 ± 0.06	5.79 ± 0.72
Apical pH = 6.0	0.60 ± 0.10[c]	0.32 ± 0.05[d]		

Note: Values are means ± SE from 4 separate experiments. In each experiment, clearance values were calculated as described in Materials and Methods. CL_{12}: clearance from apical medium to cell (μl/min/mg protein). CL_{21}: clearance from cell to apical medium (μl/min/mg protein). CL_{23}: clearance from cell to basal medium (μl/min/mg protein). CL_{32}: clearance from basal medium to cell (μl/min/mg protein).
[a,b] Significant difference from control (apical pH = 7.4).
[c,d] Significant difference from control (apical pH = 6.0).
[a,c] $P < 0.05$.
[b,d] $P < 0.01$.

port system as reported previously (23,24). We therefore analyzed TEA transport in the presence of levofloxacin. As shown in Figure 4, the TEA transport from the basolateral to the apical (A) and vise versa (C) were much decreased compared with the control (Fig. 2). However, the accumulation of TEA from basolateral (B) and from apical (D) were not significantly decreased. CL_{12} and C_{21} values were decreased in apical membranes (Table 1), whereas levofloxacin did not significantly affect the CL_{32} value in basolateral membranes.

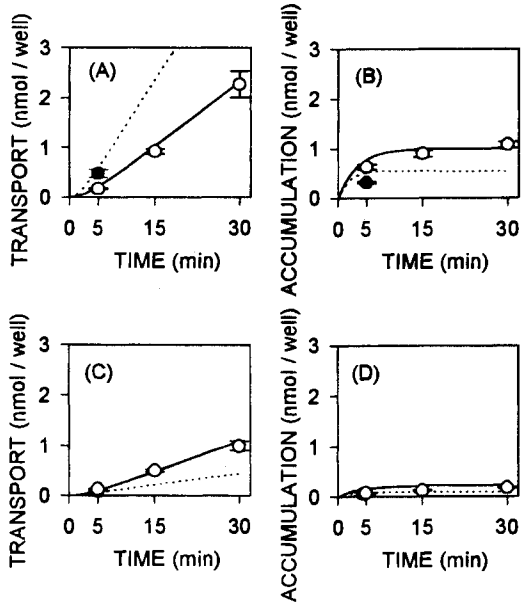

Fig. 2. Transcellular transport of [^{14}C]TEA from basolateral to apical (A), apical to basolateral (C), and cellular accumulation of [^{14}C]TEA from basolateral (B), from apical (D). The concentration of [^{14}C]TEA was 50 μM. Data were obtained from four separate experiments (○, apical pH 7.4; ● apical pH 6.0). Curves are predicted using the mean CL values obtained from four separate experiments (solid curve, apical pH 7.4; broken curve, apical pH 6.0).

DISCUSSION

To estimate how transport in apical and basolateral membranes contribute to the net transcellular transport of TEA in LLC-PK$_1$ cell monolayers, we analyzed TEA transport based on a simple, three-compartment model. We also examined the effect of apical pH, and the drug interaction of procainamide or levofloxacin on the transport of TEA in this model.

The transport of TEA in apical membranes of LLC-PK$_1$ cell monolayers is mediated by the H^+/organic cation antiport system (2,11–13) and in basolateral membranes, TEA may be transported by the potential dependent organic cation transport system (13). In this study, the net transport of TEA proceeded in the basolateral-to-apical direction and it was much increased

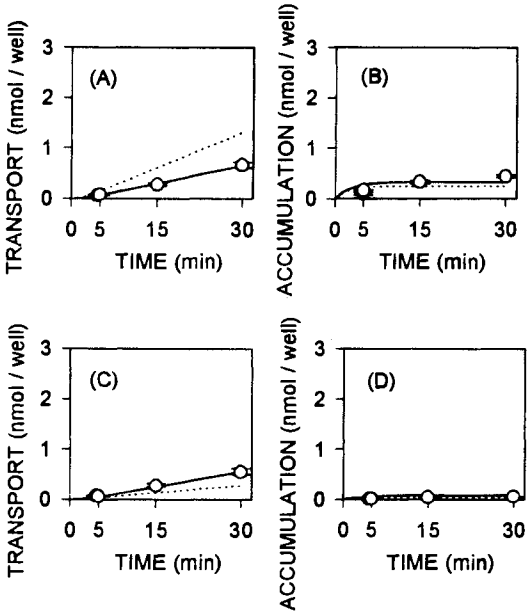

Fig. 3. Transcellular transport and cellular accumulation of [^{14}C]TEA in the presence of 2.5 mM procainamide. Symbols and curves are the same as those shown in Fig. 2.

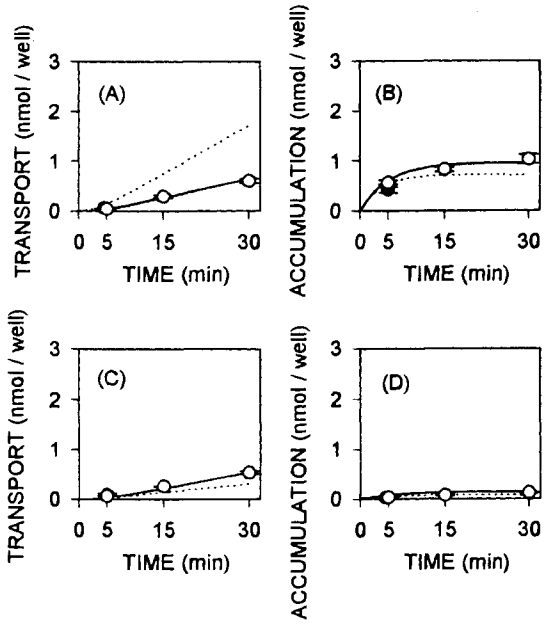

Fig. 4. Transcellular transport and cellular accumulation of [^{14}C]TEA in the presence of 1 mM levofloxacin. Symbols and curves are the same as those shown in Fig. 2.

at apical pH 6.0, compared with that at pH 7.4. Present results corresponded with previous data (13), in spite of the different experimental condition, in which cells were incubated initially with unlabeled TEA. The increase of CL_{21} value at apical pH 6.0 agreed with the characteristics of the H^+/organic cation antiport system in apical H^+ gradient (1–8). Furthermore, we defined the rate limiting step of TEA transport in LLC-PK_1 cell monolayers. Transport from the cell to the apical side was the rate limiting step under all conditions examined in this study, even at apical pH 6.0.

Procainamide and levofloxacin have been reported to interact with the H^+/organic cation antiport system in apical membranes in the kidney (20–24). The decreased CL_{12} and CL_{21} values induced by procainamide and levofloxacin indicated that the transport system in apical membranes works in both directions, and that procainamide and levofloxacin interact with this system working in each direction. Because unlabeled TEA was preloaded, trans-stimulation may accelerate the transport (1,4–8).

Procainamide have also been reported to interact with the organic cation transport system in basolateral membranes in the kidney (20). In the presence of procainamide, CL_{32} value was decreased, but CL_{23} value was not affected. These results indicated that the transport system in basolateral membranes works mostly in a basolateral to cell direction. This unidirectional transport may be due to an overwhelming driving force from the basolateral side to the cell (inside-negative potential).

In the presence of levofloxacin, on the other hand, the values of CL_{32} and CL_{23} were not significantly decreased. These are remarkable characteristics of levofloxacin clarified in this analysis. While there are many drugs which interact to the organic cation transporters in both apical and basolateral membranes, levofloxacin is the unique drug which interacts organic cation transporter mostly in apical membranes, in consistent with previous data (24). Because of these characteristics, levofloxacin will let accumulate other drugs in the cells, which interact with the organic cation transport systems both in apical and basolateral membranes like TEA or procainamide.

Thus, we constructed a simple model with which to kinetically examine the transport characteristics of organic cations in apical and basolateral membranes. Some kinetic models of drug transport in cells have been described (25,26), but our analysis is superior in two respects. First, present model does not restrict the direction of transport systems, so that it can analyze the bi-directional transport phenomena. This is important, because the transport direction of the secondary active transport system is changeable under various conditions. Second, as the concentrations of substrates in each compartment are not changed during the experiments, this analysis can exclude nonlinear expression. This is a fundamental method that can be widely applied to other substrates and transport systems.

In conclusion, our model offers a simple means of analyzing the transport of drugs in apical and basolateral membranes separately, even in intact epithelial cells. This method mathematically clarified the characteristics of the transport of TEA, as well as TEA interactions with other drugs, especially the unique characteristics of levofloxacin in LLC-PK_1 cell monolayers.

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