Kor J Physiol (1996) 30(1), 53~62

Transepithelial Transport of Organic Cation and Its Inhibition by Sulfhydryl and Carboxyl Reagents in Opossum Kidney Cell Monolayer

Jae Suk Woo, Se Ok Oh, Jin Sup Jung, Yong Keun Kim and Sang Ho Lee

Department of Physiology, College of Medicine, Pusan National University, Pusan(602-739), Korea

=Abstract=

Transepithelial transport of tetraethylammonium (TEA) was studied in monolayers of opossum kidney cells cultured on permeable membrane filters. [^{14}C]-TEA was transported across the OK cell monolayer from basolateral to apical side by a saturable process which can be stimulated by acidification of the apical medium. The apparent Michaelis-Menten constant (K_m) and the maximum velocity(V_{max}) for the transport were 41 μM and 147 pmole/ mg protein/ min, respectively. The transport was significantly inhibited by unlabelled TEA, amiloride, cimetidine, choline, and mepiperphenidol added to the basolateral side at 1 mM and was slightly inhibited by 5 mM N^1-methylnicotinamide (NMN). Unlabelled TEA added to the apical side stimulated the basolateral-to-apical [^{14}C]-TEA transport, suggesting that the TEA self-exchange mechanism was involved at the apical membrane. Sulfhydryl reagents such as *p*-chloromercuribenzoic acid (PCMB) and *p*-chloromercuribenzene sulfonate (PCMBS) and carboxyl reagents such as N,N'-dicyclohexylcarbodiimide (DCCD) and N-ethoxy-carbonyl-2-ethoxy-1,2-dihydro-quinoline(EEDQ) inhibited the TEA transport at both the basolateral and apical membranes of the OK cell monolayer. These results suggest that OK cell monolayers possess a vectorial transport system for organic cations which is similar to that for organic cation secretion in the renal proximal tubule.

Key Words: Tetraethylammonium transport, Organic cation, Sulfhydryl reagent, Carboxyl reagent, Opossum kidney cell

INTRODUCTION

The use of cultured kidney epithelial cell lines has offered advantages for the study of various renal functions (Handler & Kreisberg, 1991). LLC-PK1 cells derived from pig kidney (Hull et al, 1976) have been used for elucidating transport properties of the proximal tubule at the cellular level. Recently, LLC-PK1 cells were found to have the ability to transport organic cations such as TEA and NMN (McKinney et al, 1988; Fouda et al, 1990; Saito et al, 1992). These organic cations were transported by the organic cation transport system from basolateral to apical side across the LLC-PK1 cell monolayers, which corresponds to the secretion in the renal proximal tubules.

Opossum kidney (OK) cells, an established cell line derived from the American opossum kidney

* This work was supported by a grant from Korea Science & Engineering Foundation(941-0700-017-1).

(Koyama et al, 1978), also have many characteristics of renal proximal tubule cells, including specific transport systems for hexoses, amino acids, and inorganic phosphate (Malmstrom et al, 1987; Van den Bosch et al, 1989), as well as receptors for parathyroid hormone (Teitelbaum & Strewler, 1984). Recently, (Yuan et al, 1991) have demonstrated that the apical membrane of OK cells possesses the transport system for organic cations. However, the characteristics of the system in relation to the known H^+/organic cation exchange in apical membrane and electrically drived uptake in basolateral membrane is not clearly defined yet.

Although the organic cation transport system of the renal proximal tubule has been extensively studied kinetically, little information is available on its molecular structure. The structure of a transporter may be defined only after its isolation and purification. However, the use of group-specific modifying reagents may provide insight into understanding the chemical nature of a transporter. Several chemical groups including sulfhydryl (Sokol et al, 1986; Hori et al, 1987) and carboxyl (Sokol et al, 1987; Kim et al, 1993) groups have been proposed to be associated with the organic cation transport system, but the results are confined to the study in isolated membrane vesicles.

In the present study, we cultured OK cells as monolayers on permeable polycarbonate membrane filters and determined the transepithelial TEA transport and its modification by various organic cations, and sulfhydryl and carboxyl group modifying reagents. The results indicate that OK cell monolayer posesses vectorial transport system for organic cations which is similar to that for organic cation secretion in the renal proximal tubule. It was also indicated that sulfhydryl and carboxyl groups are essential for the transport systems at both the basolateral and apical membranes.

MATERIALS AND METHODS

Cell culture

OK cells obtained from the American Type Culture Collection (ATCC CRL-1840) were grown on plastic dishes in Earle's minimum essential medium containing 10% fetal calf serum without antibiotics in an atmosphere of 5% CO_2-95% air at 37℃. Subcultures were done every 6 days using 0.02% EDTA and 0.05% trypsin. In this study, cells between 60th and 85th passages were used.

Measurement of transepithelial TEA transport

OK cells were seeded on Transwell inserts (Costar, Cambridge, MA) with tissue culture-treated polycarbonate membrane filters (0.4 μm pore size, 13 mm diameter) in Transwell chambers at a cell density of 10^5 cells/cm^2. The cell monolayers were fed fresh medium every 3 days.

The integrity of the monolayers was evaluated by measuring transepithelial resistance using epithelial voltohmmeter (World Precision Instruments, Sarasota FL) and by measuring transepithelial [^{14}C]-mannitol flux.

Transwell inserts with OK cell monolayers were placed in a modified Ussing type diffusion chamber (MRA International, Naples, FL) and then the monolayers were washed and stabilized in Earle's balanced salt solution (EBSS). Mixing of the medium in the diffusion chamber was achieved by 5% CO_2/ 95% O_2 airlift and constant temperature of 37°C was maintained by a water-circulated jacket (Fig. 1). The volume of medium in each side of diffusion chamber was 6 ml. Transport measurement was initiated by adding 100 μM [^{14}C]-TEA to the basolateral or apical side of the monolayers. The monolayers were incubated for a specified period of time and an aliquot (100 μl) of the incubation medium in the opposite side was taken for the measurement of radioactivity. The radioactivity of the

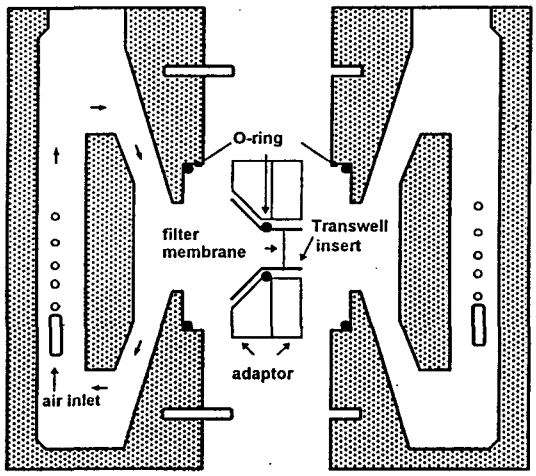

Fig. 1. Sectional diagram of modified Ussing-type diffusion chamber. Transwell inserts containing cell monolayers cultured on permeable polycarbonate membrane filters are mounted between two acrylic half- chambers with the aid of an O-ring. Transport medium is circulated by O_2/CO_2 gas lift and flows in the direction indicated by arrows. Temperature is maintained by water jacket connected to circulating water bath.

collected media was determined in scintillation cocktail with a liquid scintillation counter.

The protein concentration in the cell monolayers was determined by the method of Bradford (Bradford, 1976), using Bio-Rad protein assay kit. Bovine serum albumin was used as a standard.

Data analysis

In this study, all experiments were carried out in a minimum of at least three monolayers of different generations. Data were presented as mean ± S.E. Statistical significance was determined by the unpaired Student's *t*-test. The difference was considered to be significant when P values were less than 0.05. In kinetic studies, the apparent Michaelis-Menten constant (K_m) and maximum transport rate (V_{max}) were estimated from the Michaelis-Menten equation using nonlinear least-squares analysis program, Enzfitter (Biosoft, Cambridge, UK).

Materials

[^{14}C]-TEA bromide was purchased from New England Nuclear Research Products (Boston MA). Cell culture reagents, TEA-chloride, PAH, amiloride, cimetidine, NMN, choline chloride, procainamide, PCMB, PCMBS, DCCD, EDAC, and EEDQ were purchased from Sigma Chemical Co. (St. Louis, MO). Mepiperphenidiol was kindly supplied as gifts by Merck Sharp and Dohme (West Point, PA). Fetal calf serum was from GIBCO Life Technologies (Grand Island, NY).

Abbreviations: TEA, tetraethylammonium; NMN, N^1-methylnicotinamide; PAH, *p*-aminohippuric acid PCMB, *p*-chloromercuribenzoic acid; PCMBS, p-chloromercuribenzene sulfonate; DCCD, N, N′-dicyclohexylcarbo-diimide; EDAC, 1-ethyl-3-methylaminopropyl) carbociimide; EEDQ, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline

RESULTS

Functional integrity of OK cell monolayer cultured on permeable filters

In order to evaluate functional integrity of OK cell monolayer we determined age-dependent changes of transepithelial resistance. Transepithelial resistance increased with culture age and reached its maximum value from 6 to 10 days after seeding ranging from 220 to 300 $\Omega \cdot cm^2$. Although the increase of transepithelial resistance indicated formation of functional tight junction we confirmed it by measuring [^{14}C]-mannitol flux. As mannitol does not enter the cell, [^{14}C]-mannitol transport across the monolayer reflects paracellular leak pathway. Transepithelial [^{14}C]-mannitol flux decreased with culture age and it was inversely proportional to the change of transepithelial resistance (data not shown).

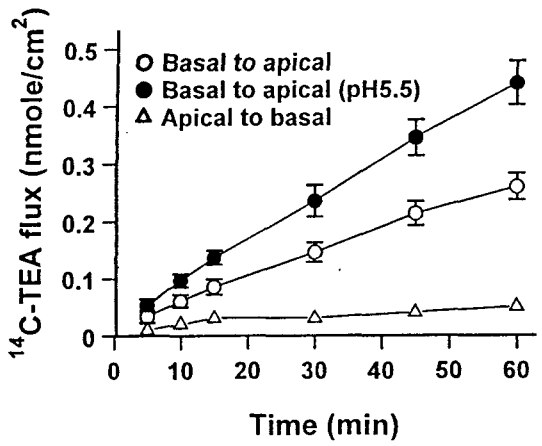

Fig. 2. Transepithelial transport of tetraethylammonium (TEA) in OK cell monolayers grown on permeable filters. The OK cell monolayers were incubated in Earle's balanced salt solution (EBSS) containing 100 μM [^{14}C]-TEA added to the apical (△) or basolateral(○, ●) side at 37°C. The basolateral-to-apical transport of TEA was measured at the apical pH of 7.4(○) or 5.5(●). Appearance of [^{14}C]-TEA at the opposite side was plotted against incubation time. Each point is mean± S.E. of the values determined in 4 monolayers.

Table 1. The apparent Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) of the transcellular [^{14}C]-TEA transport

To determine the kinetic parameters of basolateral to apical [^{14}C]-TEA transport, transport rates were determined for 10 min as a function of TEA concentration (0.01 to 1.6 mM) in the presence and absence of 0.5 mM cimetidine. The K_m and V_{max} were estimated from the Michaelis-Menten equation using nonlinear least-squares analysis program, Enzfitter (Biosoft, Cambridge, UK).

	K_m (μM)	V_{max} (pmole/mg protein/min)
− cimetidine	41.3±4.7	146.5±16.2
+ cimetidine	227.2±21.4*	128.4±12.1

Mean ± S.E. of 6 determinations in 3 monolayers
*, Significantly different from the control($p<0.005$)

Transepithelial transport of [^{14}C]-TEA

The basolateral-to-apical and apical-to-basolateral transepithelial transport of [^{14}C]-TEA were measured in OK cell monolayers cultured for 6 to 10 days. As shown in Fig. 2, the basolateral-to-apical transport of [^{14}C]-TEA increased almost linearly during 60-min incubation. The apical-to-basolateral [^{14}C]-TEA transport was significantly lower than basolateral-to-apical transport. If nonspecific paracellular flux of [^{14}C]-TEA were assumed to be similar to that of [^{14}C]-mannitol one could calculate pure transcellular transport of [^{14}C]-TEA by subtracting [^{14}C]-mannitol flux from the total transepithelial [^{14}C]-TEA transport. When the apical-to-basolateral [^{14}C]-TEA flux was corrected for the paracellular flux estimated by [^{14}C]-mannitol flux, the value became negligible. These results indicate that there exist of unidirectional transcellular transport of organic cations in OK cell monolayer. It is generally accepted that organic cation transport in the apical membrane is mediated by a H^+/organic cation exchange mechanism. In this regard, we determined the effect of acidification of apical medium on the basolateral-to-apical [^{14}C]-TEA transport. When apical medium was acidified to pH 5.5, TEA transport was significantly stimulated. This observation showed that the transport of TEA across the monolayer can be stimulated by acidifying the medium on the apical side, i.e., the apical to basolaterally directed H^+ gradient acts as a driving force for the transport.

To determine the kinetic parameters of the basolateral-to-apical [^{14}C]-TEA transport, the transport rates were determined as a function of TEA concentration which was varied from 0.01 to 1.6 mM. The transport rates were determined for 10 min, as the basolateral-to-apical [^{14}C]-TEA transport increased linearly during the first 10 min(Fig. 2). In same experiments, we tested the effect of cimetidine (0.5 mM), a well known competitive inhibitor of TEA transport, on the the kinetics of [^{14}C]-TEA transport. The apparent Michaelis-Menten constant (K_m) and

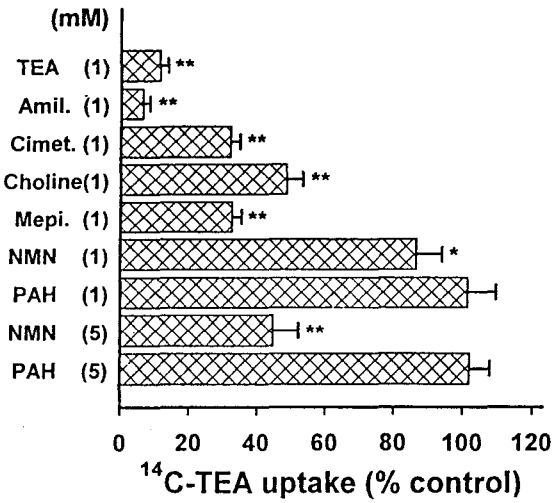

*Fig. 3. Effects of cationic and anionic compounds at the basolateral side on the transepithelial TEA transport in OK cell monolayers grown on permeable filters. The OK cell monolayers were incubated in Earle's balanced salt solution (EBSS) containing 100 μM [^{14}C]-TEA added to the basolateral side in the absence and presence of various cationic or anionic compounds at the same side. The basolateral-to-apical transport of [^{14}C]-TEA was determined by measuring appearance of [^{14}C]-TEA at the apical side (pH 7.4) for 10 min. Data were expressed as % of the control value. Each column represents mean±S.E. of the values determined in 4 monolayers. *, Significantly different from the control (P<0.05); **, (P<0.01).*

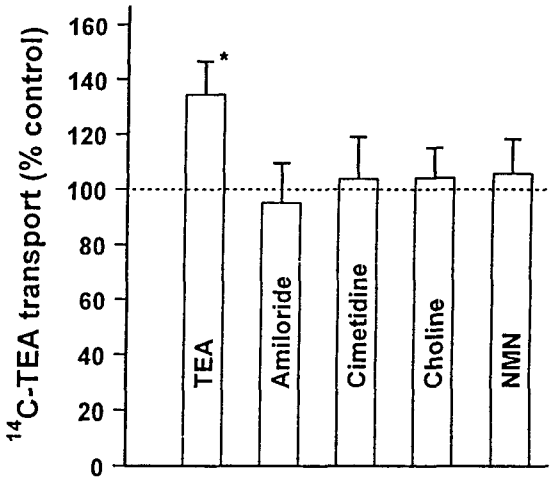

*Fig. 4. Effect of various cationic compounds at the apical side on the transepithelial TEA transport in OK cell monolayers grown on permeable filters. The OK cell monolayers were incubated in Earle's balanced salt solution (EBSS) containing 100 μM [^{14}C]-TEA added to the basolateral side in the absence and presence of various cationic compounds at the opposite side. The basolateral-to-apical transport of [^{14}C]-TEA was determined by measuring appearance of [^{14}C]-TEA at the apical side (pH 7.4) for 10 min. Data were expressed as % of the control value. Each column represents mean±S.E. of the values determined in 4 monolayers. *, Significantly different from the control (P<0.01)*

maximum transport rate (V_{max}) were estimated from the Michaelis-Menten equation using nonlinear least-squares analysis program, Enzfitter (Biosoft, Cambridge, UK) were shown in Table 1.

Effect of organic ions

In order to determine the substrate specificity of the transport system, effects of various organic cations and an organic anion PAH on the [^{14}C]-TEA transport were determined. As shown in Fig. 3, TEA, amiloride, cimetidine, choline and mepiperphenidol added to the basolateral side drastically inhibited [^{14}C]-TEA transport for 10 min at concentration of 1 mM. In contrast, NMN showed a comparable inhibition only at a high concentration (5 mM). An organic anion PAH did not inhibit [^{14}C]-TEA transport even at 5 mM. These results indicated the presence of a common transport mechanism for organic cations in the basolateral membrane of OK cell. The results also suggested that passive diffusion played a minor role in the uptake of TEA across the basolateral membrane of the OK cell.

Fig. 4 dipicts effects of organic cations in apical medium on the basolateral-to-apical [^{14}C]-TEA transport. Unlabelled TEA significantly stimulated the basolateral-to-apical [^{14}C]-TEA transport (P<0.01),

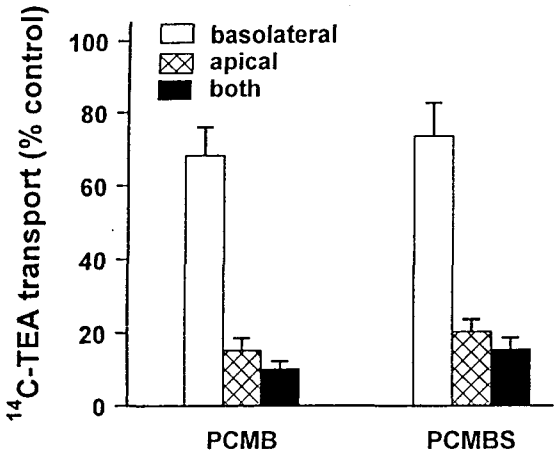

Fig. 5. Effect of sulfhydryl reagents on the transepithelial TEA transport in OK cell monolayers grown on permeable filters. Either apical or basolateral or both sides of the OK cell monolayers were pretreated with 0.1 mM PCMB or PCMBS for 30 min. The monolayers were then washed with reagent-free Earle's balanced salt solution (EBSS), and incubated in EBSS containing 100 μM [^{14}C]-TEA added to the basolateral side. The basolateral-to-apical transport of [^{14}C]-TEA was determined by measuring the appearance of [^{14}C]-TEA at the apical side (pH 7.4) for 10 min. Data were expressed as % of the control value. Each column represents mean±S.E. of the values determined in 3 monolayers.

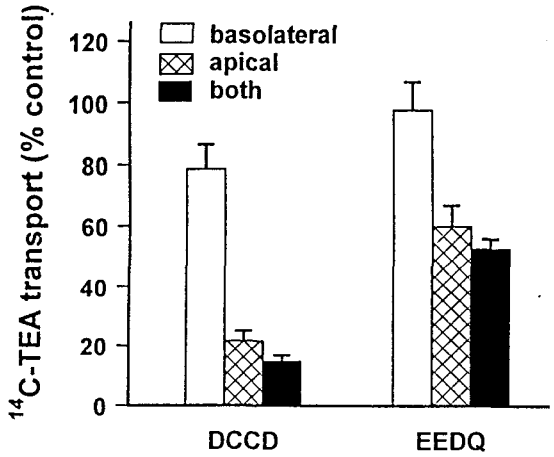

Fig. 6. Effect of carboxyl reagents DCCD and EEDQ on the transepithelial TEA transport by OK cell monolayers grown on permeable filters. Either apical or basolateral or both sides of the OK cell monolayers were pretreated with 0.1 mM of DCCD or EEDQ for 30 min. The monlayers were then washed with reagent-free Earle's balanced salt solution (EBSS), and incubated in EBSS containing 100 μM [^{14}C]-TEA added to the basolateral side. The basolateral- to-apical transport of [^{14}C]-TEA was determined by measuring appearance of [^{14}C]-TEA at the apical side (pH 7.4) for 10 min. Data were expressed as % of the control value. Each column represents mean±S.E. of the values determined in 3 monolayers.

Table 2. The concentrations of sulfhydryl (PCMB and PCMBS) and carboxyl (DCCD and EEDQ) reagents to produce 50% inhibition (IC_{50}) of the basolateral to apical [^{14}C]-TEA transport

	IC_{50} (μM)	
	Basolateral	Apical
PCMB	471.7±72.4	21.3±3.6*
PCMBS	584.1±64.6	30.2±4.1*
DCCD	842.6±96.2	38.4±3.7*
EEDQ	2041.2±248.1	126.2±16.4*

Mean ± S.E. of 5 determinations in 3 monolayers
*, Significantly different from each other (P<0.005)

indicating an existence of the TEA-TEA self exchange mechanism. Other cations, such as amiloride, cimetidine, choline or NMN, did not show any significant effect.

Effect of sulfhydryl and carboxyl reagents

Fig. 5 shows the effect of sulfhydryl group reagents PCMB and PCMBS on the basolateral to apical [^{14}C]-TEA transport. OK cell monolayers were preincubated for 30 min in the presence of PCMB or PCMBS in either apical, basolateral or both sides of the monolayer. The drugs were then removed by washing the monolayer with drug-free EBSS and the basolateral-to-apical [^{14}C]-TEA transport was determined for 10 min. Pretreatment

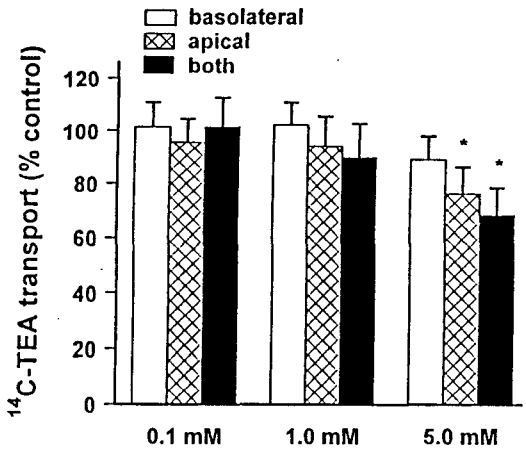

*Fig. 7. Effect of a carboxyl reagent, EDAC on the transepithelial TEA transport in OK cell monolayers grown on permeable filters. Either apical or basolateral or both sides of the OK cell monolayers were pretreated with 0.1 mM of EDAC for 30 min. The monlayers were then washed with reagent-free Earle's balanced salt solution (EBSS), and incubated in EBSS containing 100 μM [^{14}C]-TEA added to the basolateral side. The basolateral-to-apical transport of [^{14}C]-TEA was determined by measuring appearance of [^{14}C]-TEA at the apical side (pH 7.4) for 10 min. Data were expressed as % of the control value. Each column represents mean±S.E. of the values determined in 3 monolayers. *, Significantly different from the control (P<0.05).*

of basolateral surface with 0.1 mM PCMB or PCMBS inhibited the [^{14}C]-TEA transport to 68 and 73% of the control value, respectively. When the apical surface of the monolayers was pretreated with 1 mM PCMB or PCMBS the [^{14}C]-TEA transport was drastically inhibited to 15 and 20% of the control value, respectively. The inhibition was most pronounced when both sides of the monolayers were pretreated with these reagents. The concentrations of PCMB and PCMBS for 50% inhibition of the transport (IC_{50}) were calculated from dose-dependent inhibition curves and listed in Table 2. The IC_{50} values for PCMB were 472 and 21 μM, and those for PCMBS were 584 and 30 μM in the basolateral and apical side, respectively. These observations indicate that sulfhydryl groups play an important role in both the apical and basolateral TEA transport systems, but the apical system is more sensitive than the basolateral system.

Fig. 6 presents the effect of carboxyl group reagents DCCD (a carbodiimide derivative) and EEDQ (a quinoline derivative) on the basolateral to apical [^{14}C]-TEA transport. Both DCCD and EEDQ inhibited the [^{14}C]-TEA transport. As in PCMB and PCMBS, the inhibitory effects of DCCD and EEDQ were more profound when added to the apical side. The IC_{50} values are presented in Table 2.

Fig. 7 depicts the effect of a hydrophilic carbodiimide EDAC on the [^{14}C]-TEA transport. Unlike DCCD and EEDQ, EDAC did not inhibit the [^{14}C]-TEA transport at 0.1 - 1 mM. At 5 mM, it showed a slight inhibition when added to the apical side.

DISCUSSION

The modified Ussing type diffusion chamber we used in this study was proved to be an excellent tool in determining transepithelial solute transport. Although recent researches (Jourdain et al, 1993; Kim et al, 1993) demonstrated that transepithelial transport might be successfully determined using Transwell chamber(Costar, Cambridge, MA), the lack of stirring might create an unstirred water layer and lead to a misunderstanding of transport parameters. In the system we used here, the transport buffers in both sides were mixed by gas lift. This method has been proved to reduce the thickness of unstirred water layer effectively without affecting the integrity of monolayers (Hori et al, 1993).

The results in this study demonstrated that OK cell monolayers possess vectorial transport system for TEA. The transcellular transport of TEA is accomplished through two processes, basolateral uptake and apical efflux. It has been well documented that TEA is transported across the renal

brush-border membranes by a H^+-organic cation antiport system (Kinsella et al, 1979; Holohan & Ross, 1981; Takano et al, 1984; Dantzler et al, 1989; Hidalgo etl al, 1991). In this study, the basolateral to apical [^{14}C]-TEA transport was significantly stimulated when the apical medium was acidified to pH 5.5. Although this may not provide direct evidence for the existence of a H^+-TEA antiport system, it suggests strongly that the inwardly-directed H^+ gradient could serve as a driving force for TEA transport at the apical membrane. Further studies using isolated apical membrane vesicles from OK cells are required to prove the existence of the H^+-TEA antiport system in OK cell membrane. The inwardly directed H^+ gradient in the proximal tubule is generated predominantly by the Na^+-H^+ exchanger in the brush-border membranes, which depends on the electrochemical gradient of Na^+ produced by the Na^+-K^+ exchange pump in the basolateral membranes (Jung et al, 1989). Such a Na^+-H^+ exchanger also operates in OK cells (Kinsella & Aronson, 1980; Pollock et al, 1986; Gennari et al, 1992). Therefore, the H^+ gradient produced by a Na^+-H^+ exchange system in the apical membrane of OK cells might be responsible for the TEA transport at the apical pH of 7.4 in the present study.

The basolateral-to-apical TEA transport was a saturable process which could be competitively inhibited by another organic cation, cimetidine. The apparent K_m and V_{max} values obtained in this study were 41 μM and 147 pmole/mg protein/min. It is difficult to compare these kinetic parameters with those obtained in membrane vesicles, because the kinetic parameters in this study reflect characteristics of the transcellular transport, not the transmembrane transport. When compared with the kinetic parameters observed in LLC-PK_1 cell monolayer (Kim et al, 1993), the K_m and V_{max} values of the present study were slightly lower than those in LLC-PK_1 cell (K_m, 67 μM; V_{max}, 222 pmole/mg protein/min).

To determine the selectivity of TEA transport in OK cell monolayers, we examined the effect of various compounds on the basolateral-to-apical [^{14}C]-TEA transport. The transport was significantly inhibited by organic cations, such as unlabelled TEA, amiloride, cimetidine, choline, and mepiperphenidol, added to the basolateral medium at 1 mM, but was not affected by an organic anion PAH. NMN showed an weak inhibitory effect even at a relatively high concentration (5 mM). Previous studies in apical membrane vesicles isolated from rabbit renal cortex (Rafizadeh et al, 1977; Wright, 1985; Jourdain et al, 1993) and in isolated perfused tubules of snake (Gisclon et al, 1987) and rabbit (Takano et al, 1984) have demonstrated that a variety of organic cations can stimulate the transport of labelled organic cations. (Yuan et al, 1991) have also demonstrated that unlabelled TEA and NMN can accelerate the influx as well as the efflux of [^{14}C]-TEA in OK cell monolayers, as we have observed in OK cell monolayers cultured on plastic culture plate (unpublished results). In contrast, (McKinney et al, 1988) observed that the influx of [^{3}H]-TEA in LLC-PK1 cells was not stimulated by preloading the cells with unlabelled TEA. In our transepithelial transport studies using OK cell monolayers cultured on permeable membrane filters, the TEA added to the apical medium trans-stimulated the basolateral-to-apical [^{14}C]-TEA transport, indicating an involvement of TEA self-exchange mechanism. The discrepancy of the NMN effect on the TEA transport in apical membranes and cell monolayers is not clear. The apical uptake or efflux studies reflect characteristics of the apical membrane alone, whereas the transepithelial transport comprises both the uptake across the basolateral membrane and subsequent processes, including the exit through the apical membrane and intracellular events. Therefore, if the organic cation transport system in the apical and basolateral membrane had different affinities to NMN, the effect of NMN on TEA transport will be different according to the experimental protocols. Further studies using isolated membrane vesicles may be needed to elucidate

the mechanism of the discrepancy. The mechanism of TEA self-exchange has been studied previously in rabbit renal apical membrane vesicles and appears to involve an increase in the maximum turnover of the exchanger without an effect on the binding (Dantzler & Brokl et al, 1988).

The basolateral-to-apical TEA transport across the OK cell monolayer was inhibited by sulfhydryl reagents PCMB and PCMBS, added to either the apical or basolateral side. The IC_{50} values were about 20 times higher in the basolateral side (472 and 584 μM for PCMB and PCMBS, respectively) than in the apical side (21 and 30 μM for PCMB and PCMBS, respectively). Since PCMBS which cannot penetrate the cell membrane (Wright & Wunz et al, 1987) showed a similar potency of inhibition compared with PCMB, these reagents seem to react with the sulfhydryl groups at the outer surface of the basolateral or apical membranes. Accordingly, these findings may suggest that sulfhydryl groups are essential for the organic cation transport system at both the basolateral and apical membranes of the OK cell although the system in apical membrane is more sensitive to these agents. It has been reported that sulfhydryl reagents specifically inhibit the H^+ gradient-dependent transport of TEA in renal brush-border membranes and that the sulfhydryl group may be localized on the external side of the brush-border membrane vesicles (Hori et al, 1987). Considering the membrane vesicles to be oriented right-side-out, the present findings are consistent with the suggestion that the functional sulfhydryl groups of the organic cation transporter at the apical membranes of the OK cell monolayers are localized at outer surface.

Inhibition of the TEA transport by DCCD or EEDQ in this study suggest that carboxyl groups are also involved in the organic cation transport in the OK cell monolayer. As was for sulfhydryl reagents, the inhibitory effects of DCCD and EEDQ were more potent when added to the apical side of the monolayer. Another carbodiimide, a hydrophilic carboxyl group modifier EDAC did not induce a significant inhibition of TEA transport. These results may suggest that the carboxyl groups modified by carbodiimides in the present study are located at hydrophobic sites. The mechanism for the different sensitivities of the basolateral and apical membrane systems to sulfhydryl and carboxyl reagents are not clear at present, but might suggest that the apical system is rate-limiting step for the basolateral-to-apical transcellular TEA transport.

In summary, the present study demonstrated that TEA is transported across the OK cell monolayer from a basolateral-to-apical side by a saturable process. This process is stimulated by acidification of the apical medium and inhibited by sulfhydryl or carboxyl group modifying agents in the basolateral or apical medium.

REFERENCES

Bradford MM (1976) 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

Dantzler WH & Brokl OH (1988) TEA transport by snake renal tubules: choline effects, countertransport, H^+-TEA exchange. *Am J Physiol* **255,** F167-F176

Dantzler WHO, Brokl OH and Wright SH (1989) Brush-border TEA transport in intact proximal tubules and isolated membrane vesicles. *Am J Physiol* **256,** F290-F297

Fouda AK, Fauth C & Roch-Ramel F (1990) Transport of organic cations by kidney epithelial cell line LLC-PK1. *J Pharmacol Exp Ther* **252,** 286-292

Gennari FJ, Helmle KC & Murer H (1992) Influence of extracellular pH and perfusion rate on Na^+/H^+ exchange in cultured opossum kidney cells. *Pflügers Arch* **420,** 153-158

Gisclon L, Wong FM & Giacomini KM (1987) Cimetidine transport in isolated luminal membrane vesicles. *Am J Physiol* **253,** F141-F150

Handler JS & Kreisberg JI (1991) *Biology of renal cells in culture.* In The Kidney ed. by Brenner BM and Rector FC, Saunders, Pa, p110-131

Hidalgo IJ, Hillgren KM, Grass GM & Borchard RT (1991) Characterization of the unstirred water layer

in Caco-2 cell monolayers using a novel diffusion apparatus. *Pharamceutical Res* **8,** 222-227

Holohan PD & Ross CR: Mechanism of organic cation transport in kidney plasma membrane vesicles. 2. △pH studies. *J Pharmacol Exp Ther* **216,** 294-298

Hori R, Maegawa H, Okano T, Takano, M & Inui KI (1987) Effect of sulfhydryl reagents on tetraethylammonium transport in rat renal brush border membranes. *J Pharmacol Exp Ther* **241,** 1010-1016

Hori R, Okamura M, Takayama A, Hirozane K & Takano M (1993) Transport of organic anion in the OK kidney epithelial cell line. *Am J Physiol* **246,** F975-F980

Hull RN, Cherry WR & Weaver GW (1976) The origin and characteristics of a pig kidney cell strain, LLC-PK1. *In Vitro* **12,** 670-677

Jourdain M, Amiel C & Friedlander G (1993) Modulation of Na-H exchange activity by angiotensin II in opossum kidney cells. *Am J Physiol* **263,** C1141-C1146

Jung JS, Kim YK & Lee SH (1989) Characteristics of tetraethylammonium transport in rabbit renal plasma-membrane vesicles. *Biochem* J **259,** 377-383

Kim YK, Kim TI, Jung DK, Jung JS & Lee SH (1993) Inhibition of H^+/organic cation antiport by carboxyl reagents in rabbit renal brush-border membrane vesicles. *J Pharmacol Exp Ther* **266,** 500-505

Kinsella JL, Holohan PD Pessah HI & Ross CR (1979) Transport of organic ions in cortical luminal and antiluminal membrane vesicles. *J Pharmacol Exp Ther* **209,** 443-450

Kinsella JL & Aronson PS (1980) Properties of the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Am J Physiol* **238,** F461-F469

Koyama HC Goodpasture C, Miller MM, Teplitz RL & Riggs AD (1978) Establishment and characterization of a cell line from the American opossum (*Didelphys virginiana*). *In Vitro* **14,** 239-246

Malmstrom K, Stange G & Murer H (1987) Identification of proximal tubular transport functions in the established kidney cell line, OK. *Biochim Biophys Acta* **902,** 269-277

McKinney TD DeLeon C & Speeg KV, Jr (1988) Organic cation uptake by cultured renal epithelium. *J Cell Physiol* **137,** 513-520

Pollock AS, Warnock DG & Strewler GJ (1986) Parathyroid hormone inhibition of Na^+-H^+ antiporter activity in a cultured renal cell line. *Am J Physiol* **250,** F217-F223

Rafizadeh C, Roch-Ramel F & Schäli C (1977) Tetraethylammonium transport in renal brush border membrane vesicles of the rabbit. *J Pharmacol Exp Ther* **240,** 308-313

Saito HM, Inui YK & Hori R (1992) Transcellular transport of organic cation across monolayers of kidney epithelial cell line LLC-PK1. *Am J Physiol* **262,** C59-C66

Sokol PP, Holohan PD & Ross CR (1986) Essential disulfide and sulfhydryl groups for organic cation transport in renal brush-border membranes. *J Biol Chem* **261,** 3282-3287

Sokol PP, Holohan PD & Ross CR (1987) N,N'-dicyclohexylcarbodiimide inactivates organic cation transport in renal brush-border membranes. *J Pharmacol Exp Ther* **243,** 455-459

Takano M, Inui KI, Okano T, Saito H & Hori R (1984) Carrier-mediated transport systems of tetraethylammonium in rat renal brush-border and basolateral membrane vesicles. *Biochim Biophys Acta* **773,** 113-124

Teitelbaum AP & Strewler GJ (1984) Parathyroid hormone receptors coupled to cyclic adenosine monophosphate formation in an established renal cell line. *Endorinology* **114,** 980-985

Van den Bosch L, De Smedt H & Borghgraef R (1989 Characteristics of Na^+-dependent hexose transport in OK, an established renal epithelial cell line. *Biochim Biophys Acta* **979,** 91-98

Wright SH (1985) Transport of N^1-methylnicotinamide across brush border membrane vesicles from rabbit kidney. *Am J Physiol* **249,** F903-F911

Wright SH & Wunz TM (1987) Transport of tetraethylammonium by rabbit renal brush-border and basolateral membrane vesicles. *Am J Physiol* **253,** F1040-F1050

Yuan G, Ott RJ, Salgado C & Giacomini KM (1991) Transport of organic cations by a renal epithelial cell line (OK). *J Biol Chem* **266,** 8978-8986