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CARRIER-MEDIATED TRANSPORT SYSTEMS OF TETRAETHYLAMMONIUM IN RAT RENAL BRUSH-BORDER AND BASOLATERAL MEMBRANE VESICLES

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Transport of [^{3}H]tetraethylammonium, an organic cation, has been studied in brush-border and basolateral membrane vesicles isolated from rat kidney cortex. Some characteristics of carrier-mediated transport for tetraethylammonium were demonstrated in brush-border and basolateral membrane vesicles; the uptake was saturable, was stimulated by the countertransport effect, and showed discontinuity in an Arrhenius plot. In brush-border membrane vesicles, the presence of an H^+ gradient ($[H^+]_i > [H^+]_o$) induced a marked stimulation of tetraethylammonium uptake against its concentration gradient (overshoot phenomenon), and this concentrative uptake was completely inhibited by $HgCl_2$. In contrast, the uptake of tetraethylammonium by basolateral membrane vesicles was unaffected by an H^+ gradient. Tetraethylammonium uptake by basolateral membrane vesicles was significantly stimulated by a valinomycin-induced inside-negative membrane potential, while no effect of membrane potential was observed in brush-border membrane vesicles. These results suggest that tetraethylammonium transport across brush-border membranes is driven by an H^+ gradient via an electroneutral H^+-tetraethylammonium antiport system, and that tetraethylammonium is transported across basolateral membranes via a carrier-mediated system and this process is stimulated by an inside-negative membrane potential.

Introduction

It is well known that organic anions and cations are actively secreted by the proximal renal tubules [1]. However, it has been difficult to characterize the specific membrane events underlying the transport of organic ions in the intact tubule, because of its complex structure, its being composed of two distinct membranes, luminal brush-border and contraluminal basolateral membranes. In recent years, a methodology for the isolation of brush-border and basolateral membrane vesicles from renal cortex has been developed, and the membrane vesicles have been used in this [2–4] and other [5–10] laboratories for analysis of the transport properties of organic anions. On the other hand, corresponding studies of organic cation transport using isolated membrane vesicles are represented by only a few reports of Ross and co-workers [6,11,12]. Therefore, in order to obtain further information on the molecular mechanisms underlying the secretion of organic cations by proximal tubules, we have studied the uptake of tetraethylammonium by rat renal brush-border and basolateral membrane vesicles.

The present results suggest that brush-border and basolateral membranes contain the carrier-mediated transport systems for tetraethylam-

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Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid.

monium, and that an H^+ gradient (inside > outside) in brush-border membranes and an inside-negative membrane potential in basolateral membranes may be proposed as driving forces for tetraethylammonium transport across proximal tubular cells.

Materials and Methods

Materials

[^{3}H]Tetraethylammonium (93.0 mCi/mmol) was purchased from New England Nuclear (Boston, MA). Percoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Acridine orange was obtained from Merck (Darmstadt, F.R.G.), valinomycin from Sigma (St. Louis, MO). All other chemicals used for the experiments were of the highest purity available.

Isolation of plasma membrane vesicles

According to our previous reports [2–4,13], brush-border and basolateral membrane vesicles were isolated from the renal cortex of male Wistar albino rats (200–230 g) by the method of calcium precipitation and Percoll density gradient centrifugation, respectively. In some experiments, brush-border and basolateral membranes were isolated simultaneously by a combination of Percoll gradient centrifugation and calcium precipitation. Based on the method of preparation of basolateral membranes [13], the crude plasma membrane suspension was mixed with Percoll (10%, v/v) in 0.25 M sucrose/1 mM EDTA/10 mM Tris-HCl (pH 7.5), and this mixture (total volume, 30 ml) was centrifuged at 48 000 × *g* for 30 min. The top 8 ml of the gradient were collected as fraction I, then 5 ml as fraction II, 2 ml as fraction III and 15 ml as fraction IV. In order to remove Percoll particles, fractions II and IV were centrifuged at 100 000 × *g* for 120 min. The pellet obtained from fraction II, as in the previous report [13], was washed once and suspended in a buffer for transport studies by sucking the suspension ten times through a fine needle (0.4 × 19 mm) with a plastic syringe (basolateral membranes). The pellet from fraction IV was suspended in a buffer comprising 10 mM mannitol/2 mM Hepes-Tris (pH 7.1), and homogenized by a glass/Teflon Potter homogenizer with ten strokes at 1000 rev/min. Then, $CaCl_2$ was added to a final concentration of 10 mM. After 15 min, the suspension was diluted 1 : 1 with the same buffer containing 10 mM $CaCl_2$, and centrifuged at 750 × *g* for 12 min (pellet 1, discard), following which the supernatant was centrifuged at 30 000 × *g* for 12 min. The pellet was suspended in a buffer for transport studies, homogenized by a glass/Teflon Potter homogenizer with ten strokes at 1000 rev/min, and centrifuged at 48 000 × *g* for 20 min. The pellet was suspended in the same buffer and centrifuged at 2000 × *g* for 5 min, following which the supernatant was centrifuged at 48 000 × *g* for 20 min. The final pellet was suspended in a buffer for transport studies by sucking the suspension ten times through a fine needle (0.4 × 19 mm) with a plastic syringe (brush-border membranes). Usually, brush-border and basolateral membranes were suspended in a buffer comprising 100 mM mannitol/20 mM Hepes-Tris (pH 7.5). In the H^+-gradient studies for [^{3}H]tetraethylammonium uptake, 20 mM Hepes-Tris was replaced with either 10 mM Mes (pH 6.0) or 10 mM Hepes (pH 7.5).

Transport studies

The uptake of [^{3}H]tetraethylammonium by the freshly isolated membrane vesicles was measured by a rapid filtration technique. In the regular assay, the reaction was initiated rapidly by adding 20 μl buffer, containing [^{3}H]tetraethylammonium plus KCl or NaCl, to 20 μl of membrane vesicle suspension (2–5 mg protein/ml) at 25 °C. At the stated times, the incubation was stopped by diluting a reaction sample with 1 ml of ice-cold stop solution comprising 200 mM LiCl/20 mM Hepes-Tris (pH 7.5) [12]. The tube contents were immediately poured onto Millipore filters (HAWP, 0.45 μm, 2.5 cm diameter) and washed with 5 ml of ice-cold stop solution. The radioactivity of dried filters was determined by a liquid scintillation counting. In separate experiments, nonspecific adsorption was determined by the addition of labeled substrate mixture to 1 ml of ice-cold stop solution containing 20 μl membrane vesicles. This value was subtracted from the uptake data.

Analytical methods

Protein was determined, after precipitation with ice-cold 10% (w/v) trichloroacetic acid, by the

method of Lowry et al. [14] with bovine serum albumin as a standard. Alkaline phosphatase (EC 3.1.3.1), $(Na^+ + K^+)$-ATPase (EC 3.6.1.3), glucose-6-phosphatase (EC 3.1.3.9) and cytochrome *c* oxidase (EC 1.9.3.1) were assayed by the methods as previously described [13]. γ-Glutamyltransferase (EC 2.3.2.2) was measured as described by Glossmann and Neville [15]. *N*-Acetyl-β-D-glucosaminidase (EC 3.2.1.30) was measured according to Van Hoof and Hers [16], using a reaction mixture containing 0.15% Triton X-100.

Fluorescence spectroscopy

The change in transmembrane ΔpH was visualized by using the fluorescence quenching of Acridine orange. In experiments, 25 μl brush-border membrane vesicles (approx. 15 mg protein/ml) were diluted into 3 ml buffer containing 6 μM Acridine orange. The fluorescence was continuously recorded at 25 °C in a Shimadzu spectrofluorophotometer RF-510 (excitation, 493 nm; emission, 530 nm).

Results

Enzymatic characterization of brush-border membranes isolated by simultaneous preparation with basolateral membranes

In Table I are compiled the specific activities of marker enzymes for brush-border membranes (alkaline phosphatase and γ-glutamyltransferase), basolateral membranes ($(Na^+ + K^+)$-ATPase), endoplasmic reticulum (glucose-6-phosphatase), lysosomes (*N*-acetyl-β-D-glucosaminidase) and mitochondria (cytochrome *c* oxidase) in the homogenate and the final brush-border membranes isolated by simultaneous preparation with basolateral membranes. The specific activities of alkaline phosphatase and γ-glutamyltransferase in brush-border membranes were enriched by a factor of 10.5 and 16.2 relative to those in the homogenate, respectively. In contrast, the contamination by other marker enzymes was small. Thus, the purity of brush-border membranes isolated by simultaneous preparation was almost similar, compared with the membranes by calcium precipitation method of Evers et al. [2–4,17]. The membrane vesicles by both methods also showed similar transport properties for D-glucose (data not shown). In addition, the purity of basolateral membranes was the same as previous report [13].

Characteristics of tetraethylammonium transport by brush-border and basolateral membrane vesicles

As evidence that the vesicular tetraethylammonium represented transport into the intravesicular space, rather than nonspecific binding of tetraethylammonium to the membrane surface, the tetraethylammonium accumulated by the vesicles in 30 min was found to be inversely proportional to the osmolarity of the incubation medium increased by mannitol addition in the presence of 100 mM KCl (Fig. 1). Extrapolating tetraethylammonium uptake to infinite osmolarity, i.e., to zero intravesicular space, suggests that binding comprised 25% and 8% for brush-border and basolateral membrane vesicles respectively, under the incubation conditions normally used. In addition, tetraethylammonium binding under the incubation conditions without KCl was relatively large: 52% for brush-border and 45% for basolateral membrane vesicles (data not shown). Therefore, the following

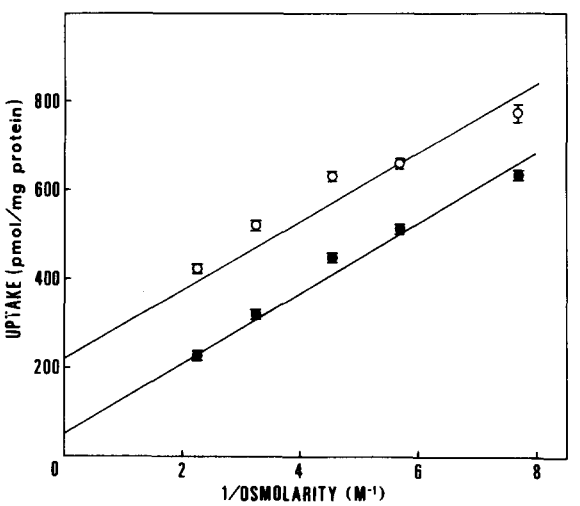

Fig. 1. Effect of osmolarity on tetraethylammonium uptake by brush-border (○) and basolateral membrane vesicles (●). Membrane vesicles, suspended in 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/100 mM KCl, were preincubated at 25 °C for 10 min. The vesicles (20 μl) were incubated with substrate mixture (20 μl) comprising mannitol/20 mM Hepes-Tris (pH 7.5)/100 mM KCl/0.5 mM [^{3}H]tetraethylammonium for 30 min. The osmolarity was varied by addition of mannitol and is shown as the inverse of the osmolarity. Each point represents the mean ± S.E. of four determinations from a typical experiment.

TABLE I

SPECIFIC ACTIVITIES, RECOVERIES AND ENRICHMENT FACTORS FOR MARKER ENZYMES IN BRUSH-BORDER

S.A., specific activity (nmol/min per mg protein) (ΔA_{550}/min per mg protein for cytochrome *c* oxidase). % represents the percentage of the enzyme activity found initially in the homogenate.

	Alkaline phosphatase		γ-Glutamyl transferase		$(Na^+ + K^+)$-ATPase	
	S.A.	%	S.A.	%	S.A.	%
Homogenate	46 ± 3	100	647 ± 37	100	54 ± 5	100
Fraction IV	258 ± 28	12.4 ± 2.5	4661 ± 390	18.3 ± 5.0	170 ± 12	6.7 ± 1.2
Pellet 1	227 ± 15	6.6 ± 0.9	3309 ± 770	8.4 ± 2.9	120 ± 12	3.1 ± 0.2
Brush-border membranes	481 ± 29	2.2 ± 0.6	10474 ± 99	3.0 ± 1.1	73 ± 18	0.4 ± 0.2
	(10.5)		(16.2)		(1.4)	

uptake experiments were carried out in the presence of salts, and the uptake data were not corrected for binding.

Fig. 2 shows the curves for the concentration dependence of tetraethylammonium uptake by brush-border and basolateral membrane vesicles. The relationship between the concentration and the rate of uptake was nonlinear in both mem-

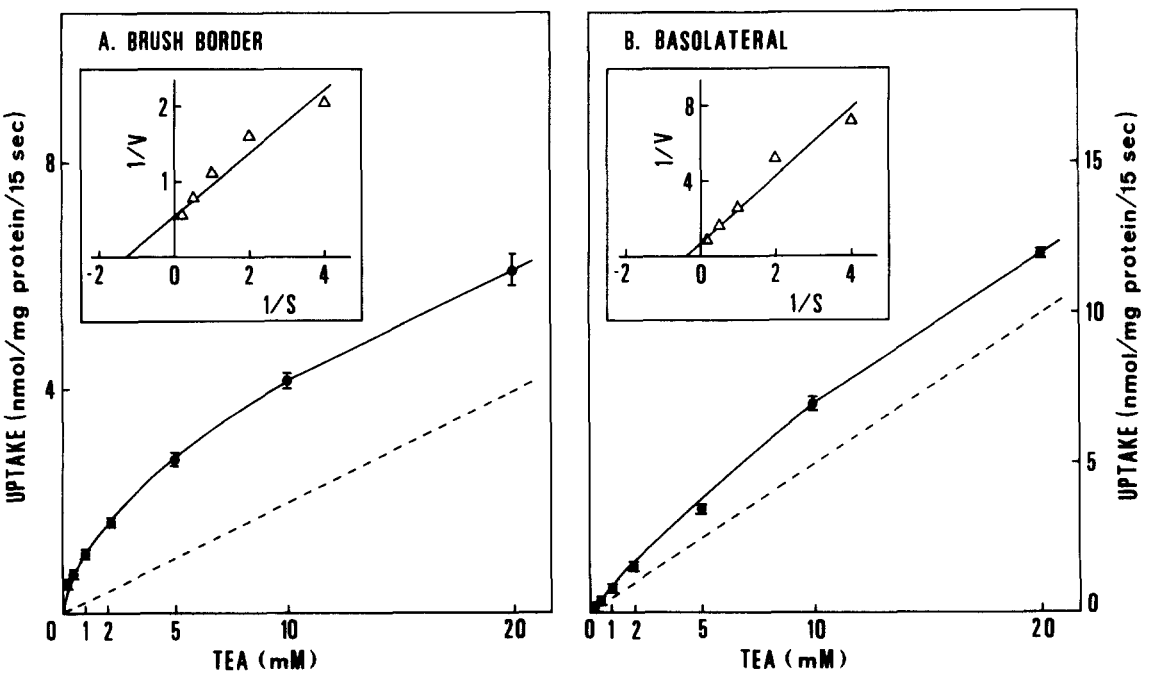

Fig. 2. Concentration dependence of tetraethylammonium (TEA) uptake by brush-border (A) and basolateral membrane vesicles (B). The uptake for 15 s at concentrations between 0.25 and 20 mM was determined. Membrane vesicles were preincubated at 25 °C in 100 mM mannitol and 20 mM Hepes-Tris (pH 7.5) for 10 min. The vesicles (20 μl) were incubated at 25 °C with the substrate mixture (20 μl) comprising 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/200 mM KCl/[^{3}H]tetraethylammonium. The dashed line represents the nonsaturable component and the inset shows the Lineweaver-Burk plot of tetraethylammonium uptake after correction for the nonsaturable component, as described in the text. Each value represents the mean ± S.E. of four determinations from a typical experiment.

MEMBRANES ISOLATED BY SIMULTANEOUS PREPARATION WITH BASOLATERAL MEMBRANES

The values in parentheses represent the ratio of the specific activities in brush-border membranes and the homogenate (enrichment factor). Each value represents the mean ± S.E. for 3–8 experiments.

Glucose-6-phosphatase		*N*-Acetyl-β-D-glucosaminidase		Cytochrome *c* oxidase		Protein (%)
S.A.	%	S.A.	%	S.A.	%	
169 ± 11	100	42 ± 5	100	16.8 ± 4.1	100	100
311 ± 26	4.44 ± 0.59	16 ± 2	0.95 ± 0.05	8.8 ± 2.5	1.25 ± 0.39	2.46 ± 0.25
454 ± 25	4.13 ± 0.48	10 ± 1	0.39 ± 0.07	6.2 ± 0.6	0.59 ± 0.14	1.48 ± 0.15
125 ± 10	0.17 ± 0.03	3 ± 2	0.02 ± 0.01	0.4 ± 0.2	0.005 ± 0.002	0.28 ± 0.1
(0.7)		(0.07)		(0.02)		

branes, providing evidence for saturability. In order to analyze the saturation of tetraethylammonium uptake, the uptake rate was corrected for the non-saturable component. The contribution of the non-saturable uptake could be estimated by employing the straight-line equation generated at higher tetraethylammonium concentrations, and subtracting this amount from the total uptake at each concentration. The values of apparent K_m and V_{max} for tetraethylammonium uptake in brush-border

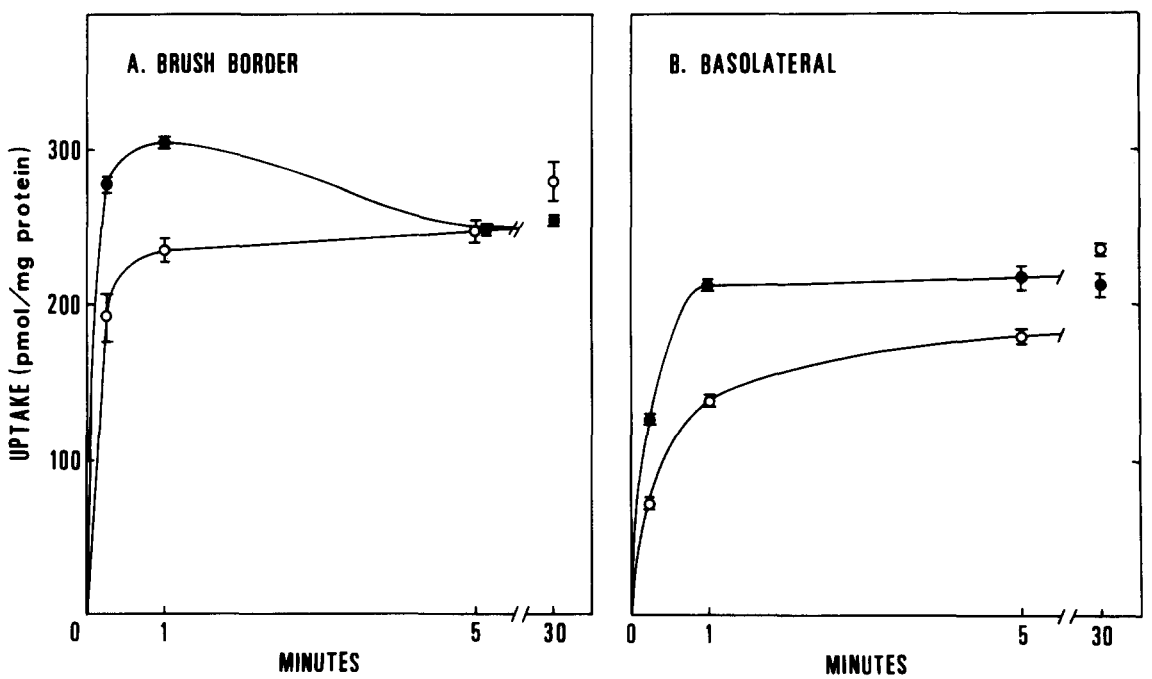

Fig. 3. Countertransport effect on tetraethylammonium uptake by brush-border (A) and basolateral membrane vesicles (B). Membrane vesicles were preincubated in 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/100 mM KCl, with (●) or without (○) 1 mM unlabeled tetraethylammonium for 30 min, and then the aliquots (20 μl) were incubated with the substrate mixture (120 μl) comprising 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/100 mM KCl/0.1 mM [^{3}H]tetraethylammonium for the indicated periods. Each point represents the mean ± S.E. of three determinations from a typical experiment.

membranes were 0.8 mM and 7.4 nmol/mg protein per min, and in basolateral membranes were 2.5 mM and 5.6 nmol/mg protein per min, respectively. These values were compatible with those of Kinsella et al. [6] who found a higher affinity and capacity of N^1-methylnicotinamide transport in brush-border membranes as compared with basolateral membranes.

Fig. 3 shows the effect of countertransport on tetraethylammonium uptake by brush-border and basolateral membranes. Membrane vesicles preloaded with high concentration of unlabeled tetraethylammonium showed enhancement of [^{3}H]tetraethylammonium accumulation by countertransport in both membranes, suggesting carrier-mediated transport of tetraethylammonium.

Furthermore, in order to confirm the existence of carrier-mediated transport of tetraethylammonium in brush-border and basolateral membranes, we studied the temperature dependence of tetraethylammonium uptake. As previously described [2,18], the Arrhenius plot for the uptake via a carrier-mediated transport system is biphasic. In Fig. 4, the Arrhenius plots for the uptake of tetraethylammonium by brush-border and basolateral membrane vesicles are both seen to be biphasic (apparent transition temperature, 27 °C), with activation energies of 5.8 and 19.7 kcal/mol in brush-border membranes, 2.3 and 13.0 kcal/mol in basolateral membranes, respectively. These results suggest the possibility that brush-border and basolateral membranes contain a carrier-mediated transport system for tetraethylammonium, independently.

Driving force for tetraethylammonium transport

In order to estimate the driving force for tetraethylammonium transport, the effect of an Na^+ gradient on tetraethylammonium uptake by brush-border and basolateral membrane vesicles

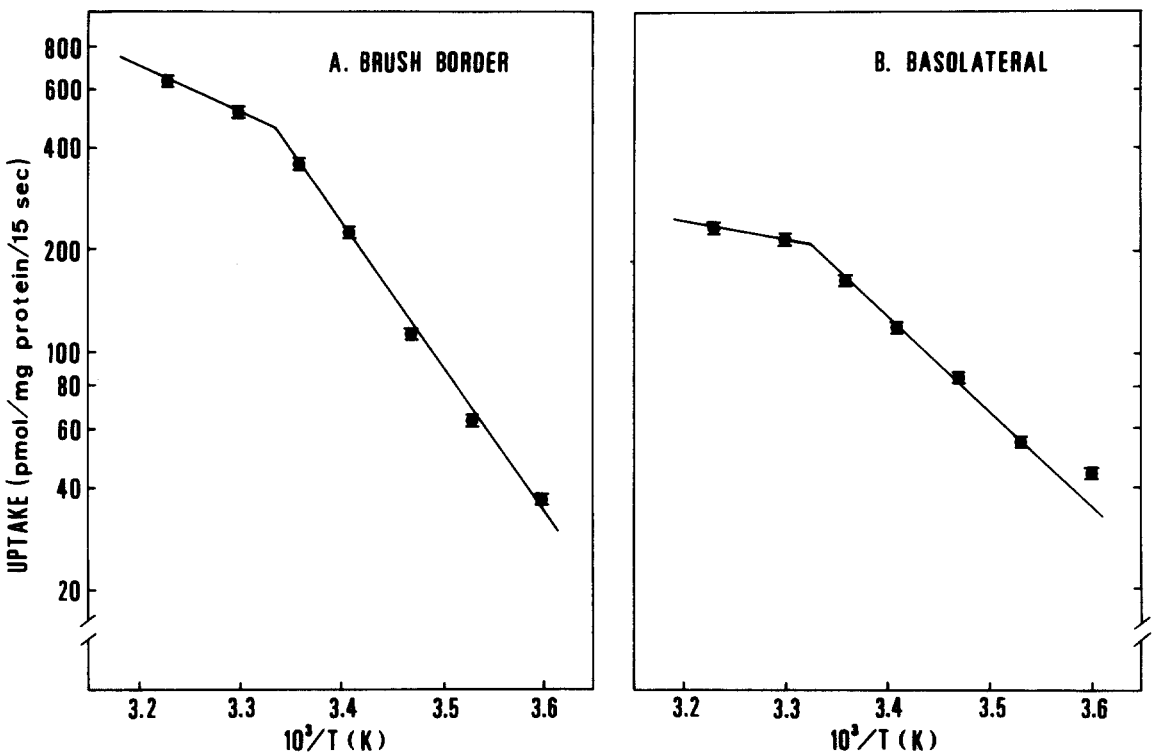

Fig. 4. Temperature dependence of tetraethylammonium uptake by brush-border (A) and basolateral membrane vesicles (B). Membrane vesicles (20 μl), suspended in 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/100 mM KCl, were incubated at 5, 10, 15, 20, 25, 30 and 37 °C for 15 s with the substrate mixture (20 μl) comprising 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/100 mM KCl/0.5 mM [^{3}H]tetraethylammonium. Each point represents the mean ± S.E. of four determinations from a typical experiment.

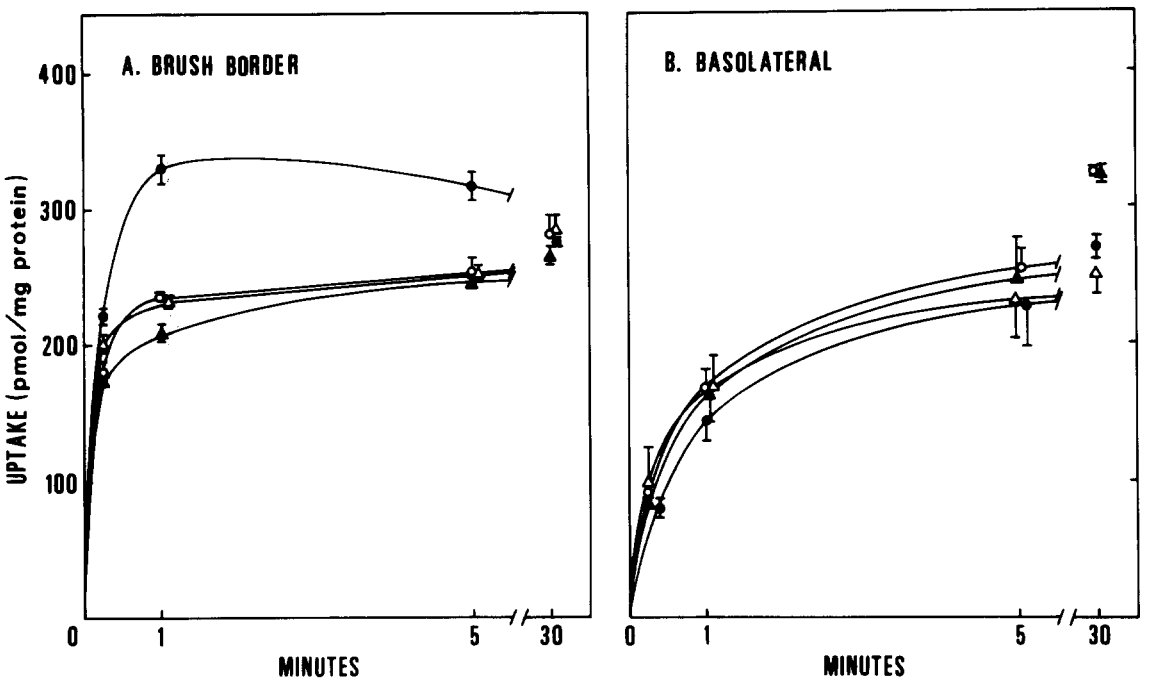

Fig. 5. Effect of various ionic conditions on tetraethylammonium uptake by brush-border (A) and basolateral membrane vesicles (B). Membrane vesicles (20 μl), suspended in 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5) and either 100 mM KCl (○, ▲) or 100 mM NaCl (●, △), were incubated with the substrate mixture (80 μl) comprising 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 0.125 mM [^{3}H] tetraethylammonium and either 100 mM KCl (○, ●) or 100 mM NaCl (△, ▲). Each point represents the mean ± S.E. of 1–2 experiments performed in duplicate determinations.

was studied. As shown in Fig. 5, tetraethylammonium uptake by brush-border membrane vesicles was markedly stimulated in the presence of an Na^+ gradient ($[Na^+]_i > [Na^+]_o$), while there was not stimulative effect of an Na^+ gradient on tetraethylammonium uptake by basolateral membranes. As previously reported by Holohan and Ross [12], the stimulative effect of an Na^+ gradient ($[Na^+]_i > [Na^+]_o$) on tetraethylammonium uptake by brush-border membrane vesicles could be due to the two functionally linked antiport systems, Na^+-H^+ antiport and H^+-organic cation antiport. Although they reported that it was necessary to decrease the buffering capacity inside the vesicles (0.1 mM Hepes) in order to produce the stimulative effect of an Na^+ gradient, the stimulation of tetraethylammonium uptake by Na^+ gradient was clearly observed in our experimental conditions with higher buffering capacity (20 mM Hepes-Tris).

Furthermore, we examined the H^+ movement under the same experimental conditions as in Fig. 5 using Acridine orange. Acridine orange is a weak base and can be used to estimate changes in intravesicular pH [19–21]. When brush-border membrane vesicles, preloaded with 100 mM NaCl, were diluted into buffer containing 6 μM Acridine orange and 100 mM KCl, a time-dependent drop in Acridine orange fluorescence occurred (Fig. 6), indicating an intravesicular uptake of Acridine orange due to an Na^+-gradient-driven intravesicular acidification. Addition of NaCl to the external buffer produced a rapid increase in Acridine orange fluorescence, as described by Warnock et al. [19]. In contrast, by diluting Na^+-preloaded vesicles into buffer containing Acridine orange and the same concentration of Na^+, or by diluting K^+-preloaded vesicles into buffer containing Acridine orange and NaCl or KCl, no change of fluorescence was observed (data not shown).

As intravesicular acidification of brush-border membrane vesicles was confirmed in the presence of $[Na^+]_i$ and $[K^+]_o$, we studied the effect of an H^+ gradient on tetraethylammonium uptake (Fig.

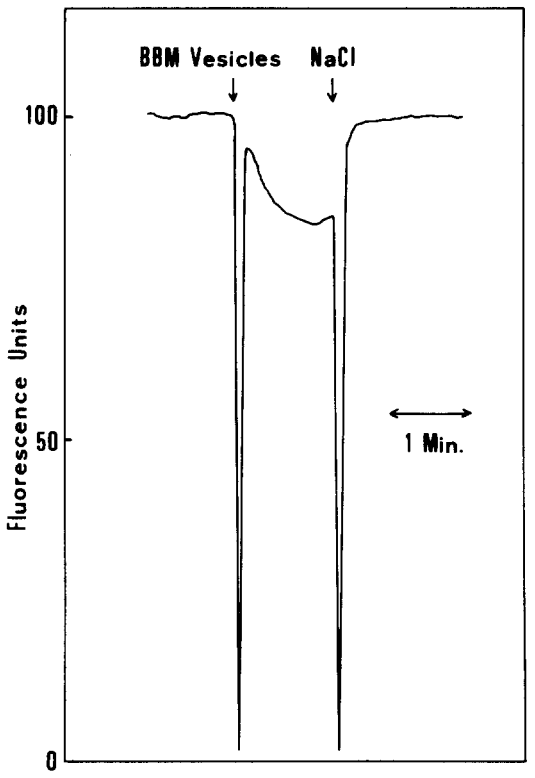

Fig. 6. Uptake of Acridine orange by brush-border membrane (BBM) vesicles. External buffer contained 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 6 μM Acridine orange and 100 mM KCl. Acridine orange fluorescence was continuously recorded as described in the text. At the first arrow, brush-border membrane vesicles (25 μl), suspended in 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/100 mM NaCl, were added into 3 ml external buffer. After quenching of Acridine orange fluorescence had been observed, 1 M NaCl (77 μl; final concentration, 25 mM) was added at the second arrow, and then the Acridine orange fluorescence increased to a final steady state level.

7). In brush-border membranes, the presence of an H^+ gradient ($[H^+]_i > [H^+]_o$) induced a marked stimulation of tetraethylammonium uptake against its concentration gradient (overshoot phenomenon). Accumulation of tetraethylammonium in brush-border membrane vesicles was maximal at approx. 30 s and then decreased, indicating efflux of tetraethylammonium. The final level of tetraethylammonium uptake in the presence of the H^+ gradient ($[H^+]_i > [H^+]_o$) was identical to that attained in the absence of the gradient and in the presence of the reverse gradient ($[H^+]_i < [H^+]_o$), suggesting that an equilibrium had been established. In contrast, an H^+ gradient was ineffective in the stimulation of tetraethylammonium uptake by basolateral membrane vesicles, although the external pH slightly affected the tetraethylammonium uptake.

Fig. 8 shows the effect of $HgCl_2$ on tetraethylammonium uptake by brush-border membrane vesicles. The concentrative uptake of tetraethylammonium driven by H^+ gradient was completely inhibited by 0.1 mM $HgCl_2$. This result suggests that the H^+-gradient-dependent tetraethylammonium transport in brush-border membranes is a specific carrier-mediated process. The tetraethylammonium uptake (0.25 mM) by basolateral membrane vesicles was also inhibited by $HgCl_2$: control, 174 ± 19; 0.1 mM $HgCl_2$, 128 ± 4 pmol/mg protein per 15 s (mean ± S.E. of four determinations).

Furthermore, it is important to clarify the role of membrane potential as a driving force for tetraethylammonium uptake by brush-border and basolateral membrane vesicles. Valinomycin in the presence of a K^+ gradient ($[K^+]_i > [K^+]_o$) was employed to produce an inside-negative membrane potential. In order to confirm the anticipated alteration of the membrane potential (inside-negative), we observed the stimulation of D-glucose uptake by brush-border membrane vesicles under the same experimental conditions (data not shown) [22]. As shown in Fig. 9, tetraethylammonium uptake by brush-border membrane vesicles was unaffected by valinomycin, suggesting electroneutral antiport of H^+ and tetraethylammonium. In contrast, a valinomycin-induced inside-negative membrane potential stimulated significantly the initial rate of tetraethylammonium uptake by basolateral membrane vesicles (Student's *t*-test, $P < 0.05$ at 0.25 min). The effect of membrane potential on tetraethylammonium uptake by basolateral membrane vesicles was also examined by applying different anion gradients such as SCN^-, Cl^- and SO_4^{2-}. Anion permeability to biological membrane generally follows in the order of $SCN^- > Cl^- > SO_4^{2-}$ [23]. Tetraethylammonium uptake (0.25 mM) by basolateral membrane vesicles was higher when Cl^- was replaced by a more permeant anion, SCN^-, and lower when Cl^- was replaced by a less permeant anion, SO_4^{2-}: 100 mM KSCN, 313 ± 5; 100 mM KCl, 258 ± 8; 50 mM

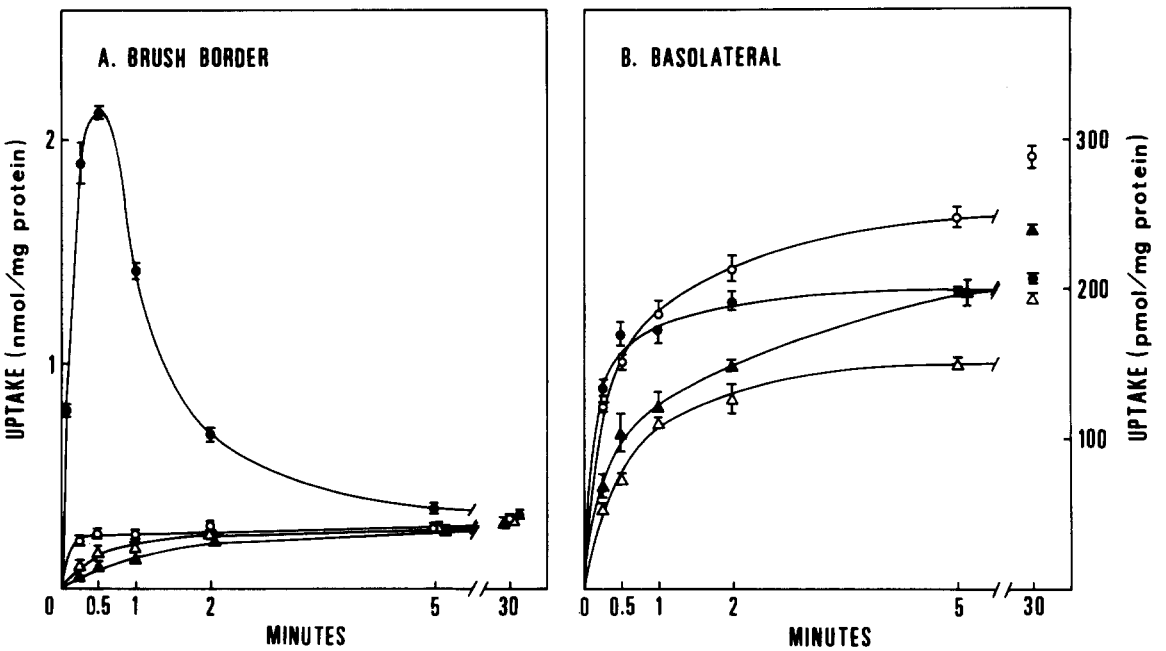

Fig. 7. Effect of H^+ gradient on tetraethylammonium uptake by brush-border (A) and basolateral membrane vesicles (B). Membrane vesicles (20 μl), suspended in 100 mM mannitol, 100 mM KCl and either 10 mM Hepes (pH 7.5) (○, ▲) or 10 mM Mes (pH 6.0) (●, △), were incubated with the substrate mixture (80 μl) comprising 100 mM mannitol, 100 mM KCl, 0.125 mM [^{3}H]tetraethylammonium and either 10 mM Hepes (pH 7.5) (○, ●) or 10 mM Mes (pH 6.0) (△, ▲). Each point represents the mean ± S.E. of two or three experiments performed in two or three determinations for A, and of three determinations from a typical experiment for B.

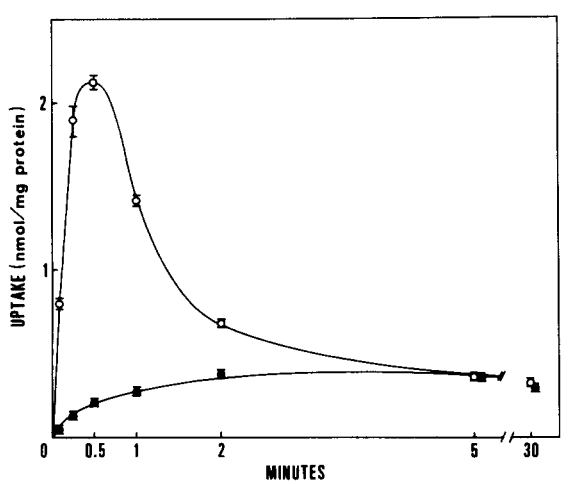

Fig. 8. Effect of $HgCl_2$ on tetraethylammonium uptake by brush-border membrane vesicles. Membrane vesicles (20 μl), suspended in 100 mM mannitol/10 mM Mes (pH 6.0)/100 mM KCl, were incubated with the substrate mixture (80 μl) comprising 100 mM mannitol/10 mM Hepes (pH 7.5)/100 mM KCl/0.125 mM [^{3}H]tetraethylammonium in the presence (●) or absence (○) of 0.125 mM $HgCl_2$. Each point represents the mean ± S.E. of 1–3 experiments performed in two or three determinations.

K_2SO_4, 241 ± 3 pmol/mg protein per 15 s (mean ± S.E. of three determinations).

Discussion

The present results demonstrate the existence of carrier-mediated transport systems for tetraethylammonium in brush-border and basolateral membranes isolated from rat renal cortex. In both membranes, the uptake of tetraethylammonium is saturable, undergoes a countertransport effect, and shows discontinuity in an Arrhenius plot. Holohan and Ross [11] reported that N^1-methylnicotinamide was transported via a carrier in brush-border membranes and via a gated channel in basolateral membranes isolated from dog renal cortex. It is difficult to determine the terminology used to describe transport processes. As previously described by Smedt and Kinne [18] and by ourselves [2], the Arrhenius plot for the uptake via a carrier-mediated transport system was biphasic. Okamoto et al. [24] discussed that if an ion is conducted via a

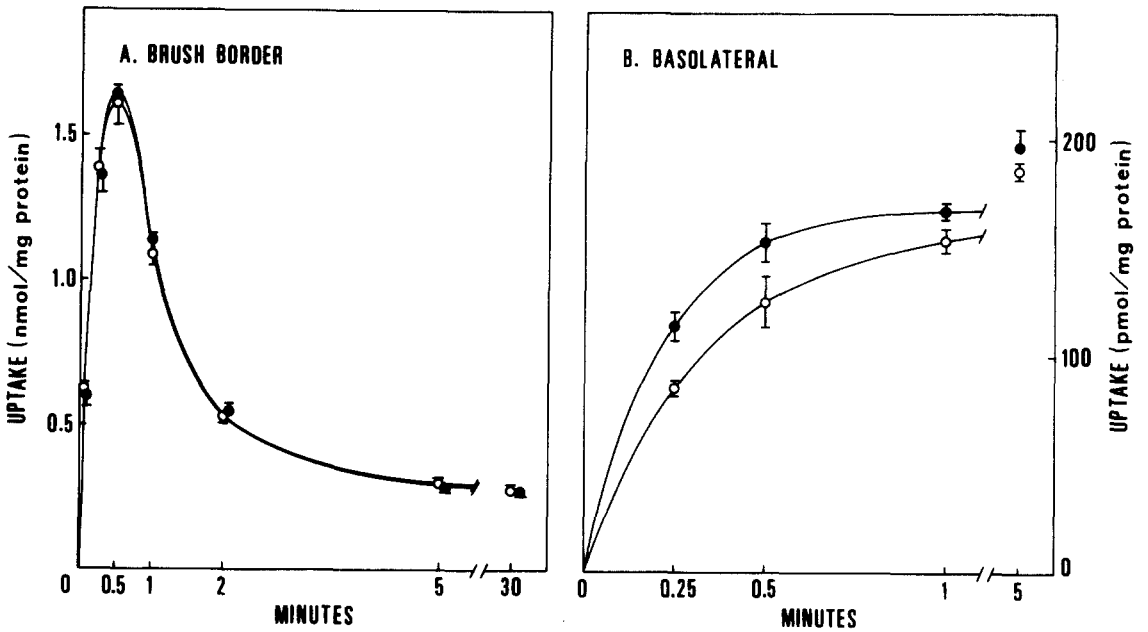

Fig. 9. Effect of valinomycin on tetraethylammonium uptake by brush-border (A) and basolateral membrane vesicles (B). (A) Membrane vesicles (20 μl), suspended in 100 mM mannitol/10 mM Mes (pH 6.0)/100 mM KCl, were incubated with substrate mixture (80 μl) comprising 100 mM mannitol/10 mM Hepes (pH 7.5)/100 mM CsCl/0.125 mM [^{3}H]tetraethylammonium in the presence (●) or absence (○) of valinomycin (8.7 μg/mg protein). (B) Membrane vesicles (20 μl), suspended in 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/100 mM KCl, were incubated with substrate mixture (80μl) comprising 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/100 mM NaCl/0.125 mM [^{3}H]tetraethylammonium in the presence (●) or absence (○) of valinomycin (7.3 μg/mg protein). Each point represents the mean ± S.E. of three determinations from a typical experiment.

carrier, such as monactin, the transport is affected by temperature, which controls the fluidity of lipids in the membrane, and that if it is conducted via a channel, such as gramicidin A, the effect of fluidity is rather small [25]. On the basis of these criteria, the present results suggest that the transport systems of tetraethylammonium in brush-border and basolateral membranes appear to be carriers because the Arrhenius plots are biphasic.

In brush-border membranes, an H^+-gradient produced a transient accumulation of tetraethylammonium above the equilibrium level. At the peak of the overshoot, the accumulation of tetraethylammonium reached more than 6-fold the equilibrium value, although Holohan and Ross showed only a 2-fold overshoot for N^1-methylnicotinamide in dog brush-border membrane vesicles [12]. This discrepancy may be due to the differences in animal species, organic cations and/or the membrane permeability for H^+.

An Na^+ gradient ($[Na^+]_i > [Na^+]_o$) also stimulated tetraethylammonium uptake by brush-border membrane vesicles. We have demonstrated H^+ movement driven by the Na^+ gradient using Acridine orange, which had been used to examine pH gradients in several different vesicular systems [19–21]. Therefore, the stimulative effect of an Na^+ gradient on tetraethylammonium uptake by brush-border membrane vesicles could be due to the two functionally linked antiport systems, Na^+-H^+ antiport and H^+-tetraethylammonium antiport. It is important to examine whether the Na^+ gradient itself can be the driving force for tetraethylammonium uptake by brush-border membrane vesicles. However, the stimulative effect of the Na^+ gradient is very small compared with that of the H^+ gradient and it seems unlikely that the Na^+ gradient itself is also the driving force for tetraethylammonium uptake. The stimulative effect of an Na^+ gradient and an H^+ gradient on tetraethylammonium uptake was not observed in basolateral membranes, suggesting that there was

no contamination of brush-border membranes into basolateral membrane preparation, and that the transport systems for tetraethylammonium in both membranes were different.

It is noteworthy that the concentrative uptake of tetraethylammonium by brush-border membrane vesicles was completely inhibited by $HgCl_2$. This result provides a strong evidence that the stimulatory effect of ΔpH is due to the H^+ gradient and tetraethylammonium is transported via a specific H^+-tetraethylammonium antiport system in brush-border membrane vesicles. The inhibitory effect of $HgCl_2$ on tetraethylammonium uptake was also observed in basolateral membranes. As previously described [3], $HgCl_2$ had no effect on the uptake of organic anion, *p*-aminohippurate, in brush-border membranes. Therefore, $HgCl_2$ can be a useful probe as a specific inhibitor with which to study the transport mechanism of organic cations in renal plasma membranes.

In renal epithelial cells, the intracellular compartment has a more negative electrical potential than the luminal and contraluminal compartment [26], and the transport processes of many solutes across plasma membranes are influenced by this membrane potential [2,22,27]. In this study, valinomycin-induced inside-negative membrane potential stimulated tetraethylammonium uptake by basolateral membrane vesicles. On the other hand, membrane potential had no effect on tetraethylammonium uptake by brush-border membrane vesicles, suggesting that the antiport of H^+ and tetraethylammonium was electroneutral and that the stoichiometry might be 1 : 1.

In conclusion, tetraethylammonium is transported from blood to cell across basolateral membranes via a carrier-mediated system and this process is stimulated by the intracellular negative potential. Tetraethylammonium transport from cell to urine across brush-border membranes is driven by an H^+ gradient via an electroneutral H^+-tetraethylammonium antiport system. This H^+ gradient could be created by an Na^+-H^+ antiport system and/or an ATP-driven H^+-pump in brush-border membranes [19,28–30].

These results can represent useful information to further the study of transport mechanisms of organic cations in the kidney.

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References

1 Weiner, I.M. (1973) in Handbook of Physiology, Section 8: Renal Physiology (Orloff, J. and Berliner, R.W., eds.), pp. 521–554, American Physiological Society, Washington, DC
2 Hori, R., Takano, M., Okano, T., Kitazawa, S. and Inui, K. (1982) Biochim. Biophys. Acta 692, 97–100
3 Inui, K., Okano, T., Takano, M., Kitazawa, S. and Hori, R. (1983) Biochem. Pharmacol. 32, 621–626
4 Inui, K., Okano, T., Takano, M., Saito, H. and Hori, R. (1984) Biochim. Biophys. Acta 769, 449–454
5 Berner, W. and Kinne, R. (1976) Pflügers Arch. 361, 269–277
6 Kinsella, J.L., Holohan, P.D., Pessah, N.I. and Ross, C.R. (1979) J. Pharmacol. Exp. Ther. 209, 443–450
7 Kippen, I., Hirayama, B., Klinenberg, J.R. and Wright, E.M. (1979) Biochim. Biophys. Acta 556, 161–174
8 Eveloff, J., Kinne, R. and Kinter, W.B. (1979) Am. J. Physiol. 237, F291–F298
9 Blomstedt, J.W. and Aronson, P.S. (1980) J. Clin. Invest. 65, 931–934
10 Sheikh, M.I. and Møller, J.V. (1982) Biochem. J. 208, 243–246
11 Holohan, P.D. and Ross, C.R. (1980) J. Pharmacol. Exp. Ther. 215, 191–197
12 Holohan, P.D. and Ross, C.R. (1981) J. Pharmacol. Exp. Ther. 216, 294–298
13 Inui, K., Okano, T., Takano, M., Kitazawa, S. and Hori, R. (1981) Biochim. Biophys. Acta 647, 150–154
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
15 Glossmann, H. and Neville, D.M., Jr. (1972) FEBS Lett. 19, 340–344
16 Van Hoof, F. and Hers, H.G. (1968) Eur. J. Biochem. 7, 34–44
17 Evers, C., Haase, W., Murer, H. and Kinne, R. (1978) Membrane Biochem. 1, 203–219
18 Smedt, H.D. and Kinne, R. (1981) Biochim. Biophys. Acta 648, 247–253
19 Warnock, D.G., Reenstra, W.W. and Yee, V.J. (1982) Am. J. Physiol. 242, F733–F739
20 Lee, H.C. and Forte, J.G. (1978) Biochim. Biophys. Acta 508, 339–356
21 Moriyama, Y., Takano, T. and Ohkuma, S. (1982) J. Biochem. 92, 1333–1336
22 Beck, J.C. and Sacktor, B. (1978) J. Biol. Chem. 253, 5531–5535
23 Lever, J.E. (1977) J. Biol. Chem. 252, 1990–1997
24 Okamoto, H., Sone, N., Hirata, H., Yoshida, M. and Kagawa, Y. (1977) J. Biol. Chem. 252, 6125–6131

25 Kranse, S., Eisenman, G. and Szabo, G. (1971) Science 174, 412–415
26 Giebisch, G. and Windhager, E.E. (1973) in Handbook of Physiology, Section 8: Renal Physiology (Orloff, J. and Berliner, R.W., eds.), pp. 315–376, American Physiological Society, Washington, DC
27 Fass, S.J., Hammerman, M.R. and Sacktor, B. (1977) J. Biol. Chem. 252, 583–590
28 Kinsella, J.L. and Aronson, P.S. (1980) Am. J. Physiol. 238, F461–F469
29 Kinne-Saffran, E., Beauwens, R. and Kinne, R. (1982) J. Membrane Biol. 64, 67–76
30 Bichara, M., Paillard, M., Leviel, F., Prigent, A. and Garden, J.P. (1983) Am. J. Physiol. 244, F165–F171