Kinetics of Organic Ion Transport Across Rabbit Renal Brush Border and Basolateral Membrane Vesicles

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=국문초록=

가토 신피질 Brush Border Membrane과 Basolateral Membrane Vesicle에서 유기이온의 이동에 대한 동력학적 분석

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가토 신피질에서 분리한 brush border membrane (BBM)과 basolateral membrane vesicle (BLM)에서 유기 음이온인 *p*-aminohippuric acid (PAH)와 유기 양이온인 tetraethylammonium (TEA)의 이동에 대한 동력학적 분석을 하였다. BLM에서 PAH에 대한 Km과 Vmax값은 각각 0.34±0.02 mM과 0.22±0.07 nmol/mg protein/20s였으며 BBM에서 각 값은 8.46±0.57 mM과 4.43±0.40 nmol/mg protein/20s였다. BLM에서 용액내 Na의 제거는 PAH에 대한 Km 값에는 영향없이 Vmax 값을 변화시켰다. BBM에서 TEA 이동에 대한 Km과 Vmax 값은 각각 0.55±0.15 mM과 1.04±0.23 n mol/mg protein/20s였으며 BLM에서 각 값은 0.46±0.04 mM과 0.61±0.06 nmol/mg protein/20s였다.

BLM에서 측정한 유기 이온들의 이동에 대한 Km 값이 신절편이나 분리된 tubule에서 보고된 값과 일치함을 보였으며 이러한 결과는 신세뇨관 세포막을 통한 유기 이온들의 이동 특성이 membrane vesicle을 분리하는 과정에서 변하지 않았음을 가르킨다.

Key Words: Kinetics, Organic ion, Membrane vesicles, Kidney cortex

INTRODUCTION

It is well known that organic anions and cations are actively secreted by the proximal tubules (Weiner, 1973). However, their molecular mechanisms have not yet been elucidated.

It has been difficult to characterize the specific membrane events underlying transport of organic ions in intact epithelial preparations because of the complexities involved in studying transtubular transport processes.

Recently, by employing the technique of membrane vesicle isolation, brush-border (BBM) and basolateral membrane (BLM) can be isolated separately and the metabolic effect also can be eliminated by the removal of the cytoplasmic components. Because of such advantages of isolated membrane vesicles in comparision to intact cell or tissues, this approach has been used extensively in studies for properties of membrane-bound transport systems. However, in order to get the isolated membrane

vesicles a long procedure of preparation is required in completely artificial solutioins. Consequently, alterations in permeability properties are possible and inactivation of transport system can occur (Murer & Kinne, 1980). Ulrich (1973) also pointed out that the major disadvantage of isolated membrane vesicles is the heterogeneity of vesicles which constitutes a particular problem in determination of kinetic parameters for transported solutes or of the potency of inhibitors. Therefore, it is of importance to establish that the kinetic parameters of organic ion transport in isolated membrane vesicles correspond with those found in intact cells.

The present study was undertaken to estimate the kinetic parameters of *p*-aminohippurate (PAH) and tetraethylammonium (TEA) transport by BLM and BBM vesicles isolated from rabbit renal cortex, and to compare with the values previously reported for the intact cell preparations.

METHODS

Meterials

^{3}H-PAH (5.2 mCi/mmol), ^{14}C-TEA (4.5 mCi/mmol) and ^{14}C-D-glucose (14.4 mCi/mmol) were purchased from New England Nuclear (Boston, MA). Percoll was obtained from Pharmacia, Fine Chemicals (Sweden). Valinomycin and probenecid from Sigma (St. Louis, MO). Darstine (mepiperphendiol) from Merk, Sharp and Dohem (West Point, Pa). All other chemicals were of the highest grade available.

Membrane isolation

BBM and BLM vesicles were prepared from rabbit kidney cortex by a Percoll density gradient according to the method of Goldinger et al (1984).

Adult rabbits were killed by a blow to the neck, and the kidneys were removed and perfused with an ice-cold solution containing (mM) 140 NaCl, 10 KCl, and 1.5 $CaCl_2$. The cortex was dissected, minced and homogenized in 250 mM sucrose and 10 mM triethanolamine, pH 7.6, (sucrose buffer) to make a 10% (wt/vol) homogenate with a tissue homogenizer (20 strokes). The homogenate was centrifuged at 1,200 *g* for 10 min (Sorvall SS-34 rotor), and the supernatant was pipetted out and recentrifuged at 9,500 *g* for 15 min. The supernatant and the uper fluffy layer of the pellet were separated from the lower dark pellet and homogenized gently with a tissue homogenizer (5 strokes). This suspension was recentrifuged at 25,000 *g* for 30 min. The fluffy layer was carefully taken and suspended in sucrose buffer to a total volume of 26.5 ml. This crude plasma membrane suspension was mixed with Percoll of 3.5 ml and then centrifuged at 31,000 *g* for 45 min. The spontaneously formed gradient was divided from top to bottom. The first 5 ml were discarded, the next 10 ml were collected as BLM and the last 15 ml as BBM. Each fraction was centrifuged at 65,000 *g* for 60 min (Sorvall OTD-75B) to remove the Percoll from preparation. The membrane vesicles were resuspended in an appropriate vesicle buffer by passing it several times through a 25 gauge needle. The BBM were further purified by a Ca^{2+} -precipitation procedure (Kinsella et al, 1979a). Membrane vesicles were suspended in a buffer containing 100 mM mannitol and 20 mM Hepes/Tris, pH 7.4. Before using the membranes were incubated to preload with the intravesicular buffer at 37℃ for 30 min.

Marker enzyme assays

Na-K-ATPase activity was assayed according to the method of Jϕrgensen & Skou (1971), and used as a marker for BLM. Alkaline phosphatase activity, a marker for BBM, was measured by the method of Linhardt & Walter (1963). Protein concentration was determined by the method of Bradford (1976).

Transport Studies

Uptake was determined by a rapid filtration technique similar to that described by Berner and Kinne

(1976). Briefly, vesicles were added to prewarmed (25℃) buffers containing the radioactive substrates. The composition of buffers is indicated in the figure legends. At appropriate time intervals, 100 μl were taken and quickly filtered under vacuum through presoaked Millipore filters (0.45 μm pore size). Filters were washed with 5 ml of ice-cold stop solution being of similar composition as the uptake media but without substrates. Filters were dissolved in 1 ml of 2-methoxyethanol, added 10 ml of scintillation fluid and the amount of radioactivity taken up by vesicles was determined by liquid scintillation spectrometer (Packard Tricarb 300 C). When required, non-specific filter binding in PAH and glucose transport was determined by incubating vesicles in distilled water containing ^{3}H-PAH or ^{14}C-glucose and filtering as described above. TEA binding was determined by filtering the uptake medium containing ^{14}C-TEA without vesicles. All experiments for kinetics were performed in the presence of valinomycin with 20 mM K^{+} inside and outside the vesicles to abolish potential difference across the vesicle membranes.

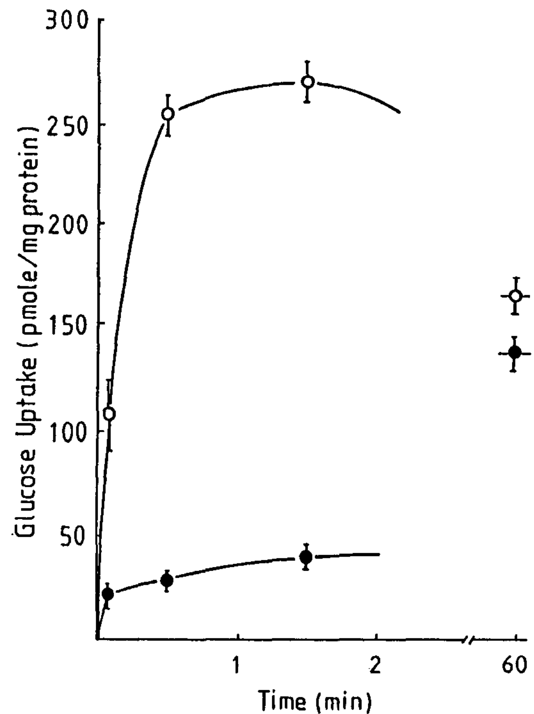

Fig. 1. The time course of D-glucose uptake by rabbit renal BBM in the presence of 100 mM NaCl (○) or 100 mM KCl (●) in the medium. Vesicles were preloaded with 100 mM mannitol and 20 mM Hepes/Tris (pH 7.4). Uptake was assayed at 25°C in a medium containing 100 mM mannitol, 20 mM Hepes/Tris (pH 7.4), 100 mM NaCl or 100 mM KCl, and 50 μM (^{14}C)-D-glucose. Each point represents mean ± S.E. of four determinations.

RESULTS

Characteristics of Vesicles

The purity of BLM was controlled by examining the acitivity of Na-K-ATPase as a marker enzyme, and that of BBM by the assay of alkaline phos-

Table 1. Specific activity for marker enzymes in BLM and BBM

	Na–K–ATPase (μ mol/mg/hr.)	Enrichment (fold)	Alkaline phsophatase (μ mol/mg/hr.)	Enrichment (fold)
Homogenate	4.80 ± 0.89		1.25 ± 0.12	
	(4)		(6)	
Basolateral membrane	108.50 ± 5.13	22.97	0.88 ± 0.09	0.70
	(4)		(6)	
Brush-border	5.44 ± 0.25	1.13	30.25 ± 2.14	24.20
	(4)		(4)	

Enrichment indicates the ratio of specific activities relative to the homogenate. Data represent mean ± S.E. Number of experiments is given in patenthesis.

phatase as a marker enzyme. The activity of Na-K-ATPase in the fraction containing BLM was enriched 23-fold and the activity of alkaline phosphatase in the fraction containing BBM was enriched 24-fold as compared with those in the homogenate (Table 1). These results are similar to values obtained by others (Boumendil-Podevein & Podevin, 1983; Goldinger et al. 1984).

The purity of the membrane preparation was also evaluated functionally by determining Na-dependent D-glucose uptake in BBM. The Na-dependent uptake was increased about ten times compared to the Na-independent uptake at 30 s, showing overshoot phenomenon over the equilibrium value (Fig. 1). These results, together with the marker enzyme studies, provided strong evidence that BLM and BBM were highly purified.

To demonstrate that organic ion influx was due to intravesiclular accumulation rather than nonspecific binding to membrane vesicles the effect of varying osmolarity on organic ion transport was examined (Fig. 2). When the equilibrium uptake (60 min) of PAH in BLM was determined as a function of increasing sucrose concentration in incubation medium, PAH accumulation was inversely proportional to the osmolarity of the incubation medium (Fig. 2A). Extrapolating PAH uptake to infinite osmolarity revealed that binding was about 10%.

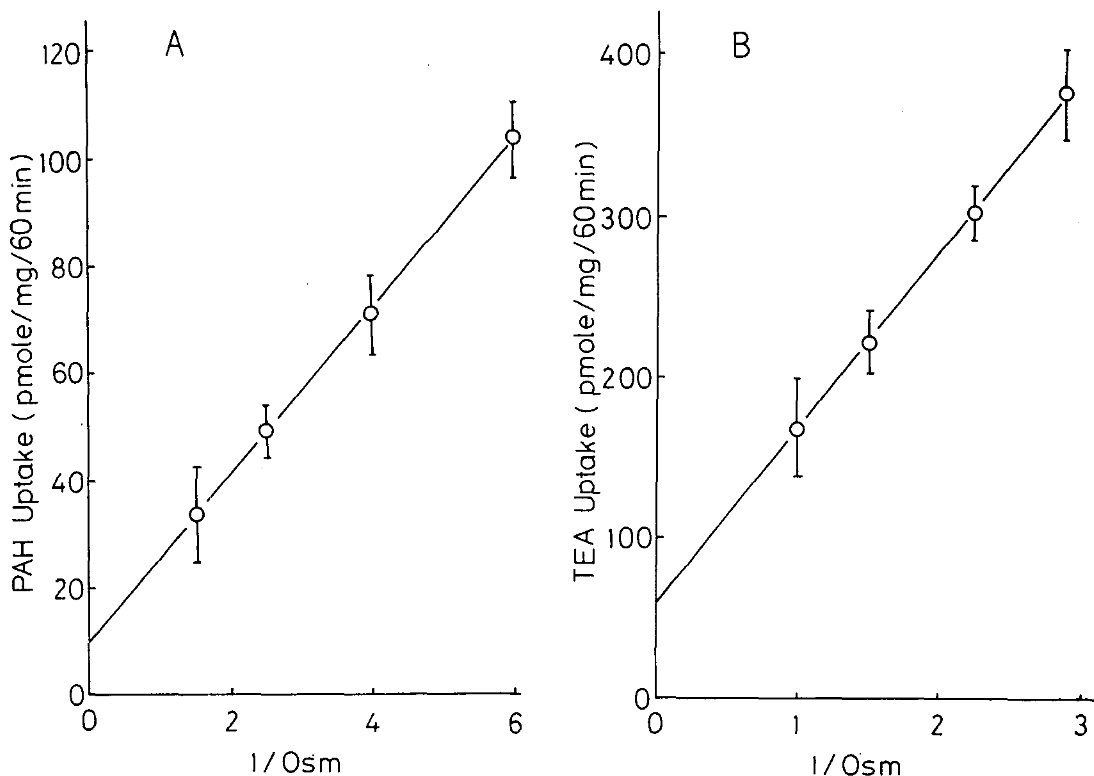

Fig. 2. Effect of osmolarity on PAH uptake by BLM (A) and TEA uptake by BBM (B). Vesicles were preloaded with 100 mM mannitol and 20 mM Hepes/Tris (pH 7.4). PAH uptake was determined after 60 min at 25°C in medium containing 50 μM (^{3}H)–PAH, 20 mM Hepes/Tris (pH 7.4), 100 mM NaCl and various concentrations of mannitol. TEA uptake was determined after 60 min in a medium containing 50 μM (^{14}C)–TEA, 20 mM Hepes/Tris (pH 7.4), 100 mM KCl and various concentrations of mannitol. Each point represents mean ± S. E. of three determinations.

These findings indicate that membrane vesicles are osmotically active and that PAH is transported into an intravesicular space. A similar result for TEA with BBM is shown in Fig. 2B. demonstrating that less than 15% of TEA uptake was due to binding.

Kinetics of PAH transport

For kinetic analysis of PAH transport, it is necessary to distinguish a carrier-mediated transport from nonspecific uptake through a leak pathway or simple diffusion which was uninhibitable by a maximum concentration of probenecid. However, the concentrations of probenecid used to evaluate the rate of diffusional PAH transport in vesicle experiments varied from 1.0 to 10 mM according to authors (Berner & Kinne, 1976; Kinsella et al., 1979b; Kasher et al., 1983; Tse et al., 1983; Goldinger et al., 1984). Therefore, in order to ascertain a concentration of probenecid producing a maximum inhibition on carrier-mediated PAH transport but without a nonspecific effect, the uptake of D-glucose and PAH for 20 s in BLM was determined in the presence of varying concentrations of probenecid (Fig. 3). At 2 mM, probenecid inhibited PAH uptake by about 50 % without a significant effect on D-glucose uptake. On the other hand, at the higher concentrations of 5 and 10 mM, probenecid showed greater inhibitory

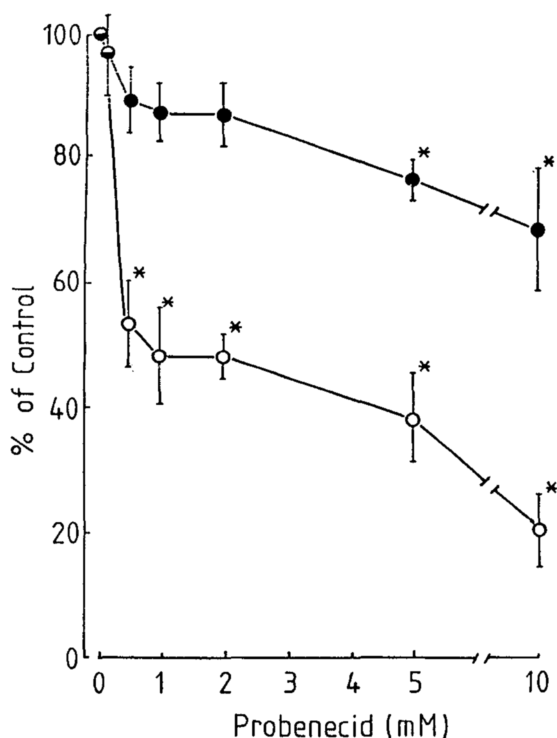

Fig. 3. Effect of probenecid on PAH (○) and D-glucose (●) uptakes by BLM. Vesicles were preloaded with 100 mM mannitol, 20 mM KCl and 20 mM Hepes/Tris (pH 7.4). Uptake was determined after 20 s in a medium containing 100 mM mannitol, 20 mM KCl, 20 mM Hepes/Tris (pH 7.4), 100 mM NaCl, 10 μM valinomycin, 50 μM (^{3}H)–PAH or (^{14}C)–D–glucose and various concentrations of probenecid. Each point represents mean ± S.E. of three determinations.
* P < 0.05 compared with control value without probenecid.

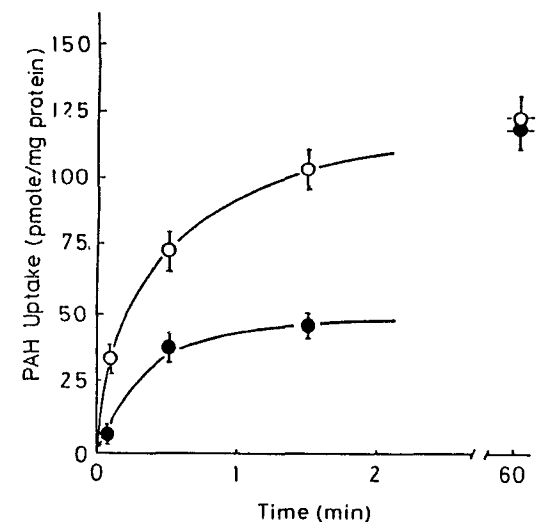

Fig. 4. The time course of PAH uptake by rabbit renal BLM. Vesicles were preloaded with 100 mM mannitol and 20 mM Hepes/Tris (pH 7.4). Uptake of 50 μM (^{3}H)–PAH was determined in a medium containing 100 mM mannitol, 20 mM Hepes/Tris (pH 7.4) and 100 mM NaCl in the presence (●) and absence (○) of 2 mM probenecid. Each point represents mean ± S.E. of four determinations.

potency (60-70% inhibition) but with a significant inhibitory effect on D-glucose uptake. In the following studies the noncarrier-mediated component of PAH uptake was therefore determined in the presence of 2 mM probenecid. Kippen et al. (1979) reported in rabbit renal BBM that 1 mM probenecid significantly inhibited D-glucose uptake (11%) by the effect which was likely due to nonspecific membrane effect.

The effect of probenecid on the uptake of PAH in BLM was determined in the presence of an inwardly Na gradient. As shown in Fig. 4, the uptake of PAH after 30 s was inhibited about 50% by 2 mM probenecid, indicating the presence of uptake through a carrier-mediated transport. The rate of probenecid-sensitive PAH transport for 20 s was determined at different substrate concentrations in the presence of an inwardly 100 mM Na gradient in BLM and BBM. Kinetic parameters of PAH uptake were obtained from Lineweaver-Burk plots (Figs 5 and 6). The values of apparent Km and Vmax for PAH uptake in BLM were 0.35±0.02 mM and 0.

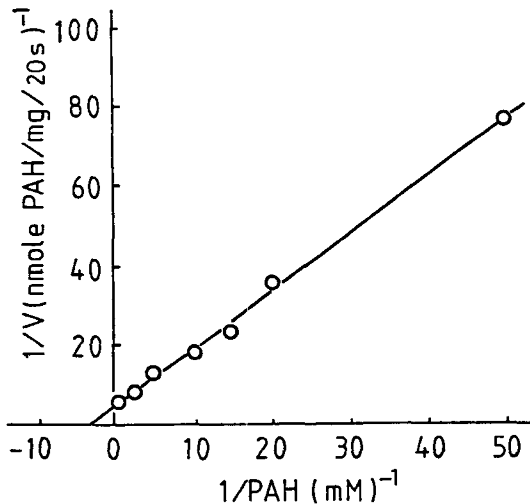

Fig. 5. Lineweaver-Burk plot of probenecid-sensitive PAH uptake by BLM. Vesicles were prepared as described in Fig. 3. Uptake was determined after 20 s in a medium containing 100 mM mannitol, 20 mM KCl, 20 mM Hepes/Tris (pH 7.4), 100 mM NaCl, 10 μM valinomycin and various concentrations of (^{3}H)—PAH in the presence and absence of 2 mM probenecid. Each point represents mean of four determinations.

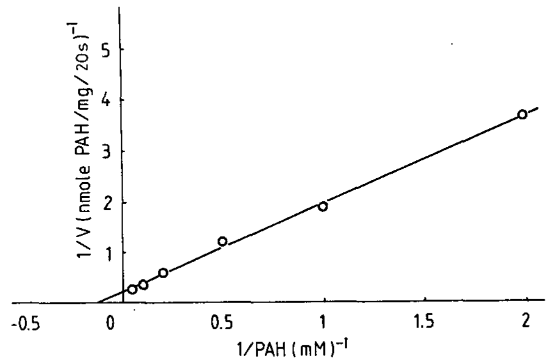

Fig. 6. Lineweaver-Burk plot of probenecid-sensitive PAH uptake by BBM. The experimental conditions were the same as in Fig. 5. Each point represents mean of four determinations.

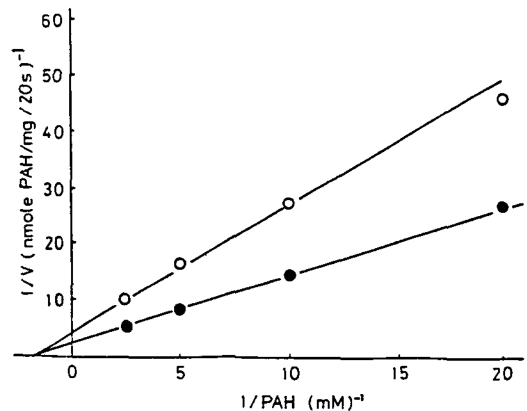

Fig. 7. Lineweaver-Burk plot of Na-dependent and independent PAH uptake by BLM. Vesicles prepared as described in Fig. 3. Uptake was determined after 20 s in a medium containing 100 mM mannitol, 20 mM KCl, 20 mM Hepes/Tris (pH 7.4), 10 μM valinomycin, with (●) or without (○) 100 mM NaCl and various concentrations of (^{3}H)—PAH. Data represent probenecid-sensitive uptake. Each point represents mean of four determinations.

22±0.07 nmol/mg protein/20s, respectively. To examine the effect of Na^+ on kinetic parameters of PAH transport in BLM, probenecid-sensitive PAH uptake was measured in the presence and absence of 100 mM NaCl in the incubation medium. As shown in Fig. 7, the imposition of an inwardly Na gradient increased apparent Vmax value 0.19±0.06 to 0.47±0.09 nmol/mg protein/20s without a significant change in apparent Km value (0.54 mM).

Kinetics TEA transport

Fig 8 shows the curves or concentraton dependence of TEA uptakeby BBM in the presence and absence of 1 mM Darstine, a potent competitive inhibitor of TEA transport (Schäli et al., 1983). Total uptake of TEA determined in the absence of Darstine (curve a) was increased curvilinearly as a function of TEA concentration at lower concentrations while it was linear at higher concentrations of TEA (>0.5 mM). The apparently linear nature of TEA uptake at higher concentrations suggests the presence of a diffusional component of uptake. In order to observe saturation of TEA uptake, it is necessary to correct for the diffusional component. In general, the diffusional component could be estimated by determining the amount of TEA uptake that could not be blocked by a maximum concentration of competitive inhibitor or by employing the straight line equation generated at higher TEA concentrations. As shown in Fig. 8, TEA uptake yielded a straight line in the presence of 1 mM Darstine (curve b) whose slope was 1.46 nmol/mg protein/20s per mmol, which was similar to that obtained from the straight line equation generated at higher TEA concentrations (>0.5 mM) in the total uptake curve (curve a) without Darstine (1.57 nmol/mg protein/20s per mmol). This result demonstrates that 1 mM Darstine was capable of completely suppressing carrier-mediated TEA

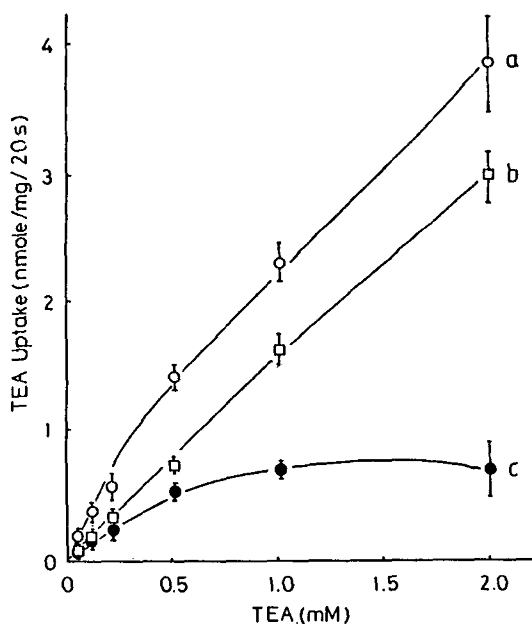

Fig. 8. Concentration dependence of TEA uptake by BBM in the absence (curve a) and presence (curve b) of 1 mM Darstine. Vesicles were preloaded with 100 mM mannitol, 20 mM KCl and 20 mM Hepes/Tris (pH 7.4). Uptake was determined after 20 s in a medium containing 100 mM mannitol, 20 mM KCl, 20 mM Hepes/Tris (pH 7.4), 10 μM valinomycin and various concentrations of (^{14}C)-TEA. The saturable uptake (curve c) was obtained by correcting for the nonsaturable component, which was not inhibitable by 1 mM Darstine, from total uptake. Each point represents mean ± S.E. of four determinations.

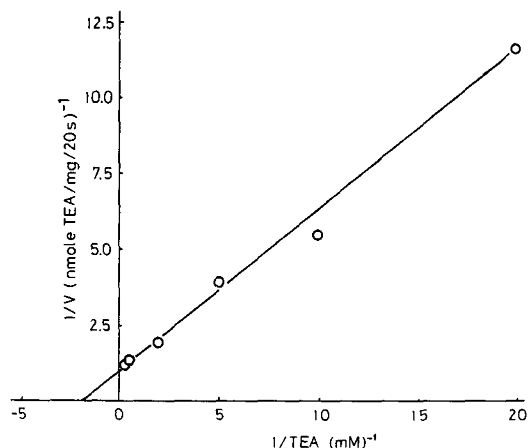

Fig. 9. Lineweaver-Burk plot of saturable component of TEA uptake obtained in Fig. 8.

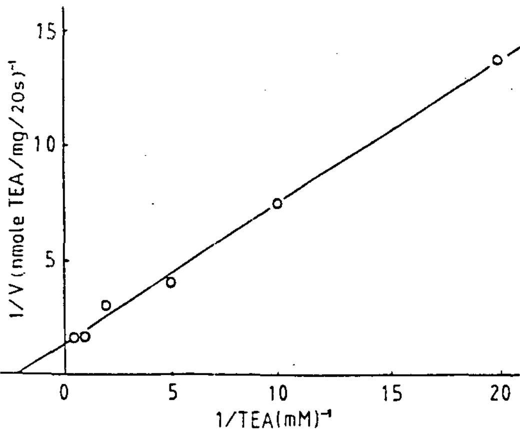

Fig. 10. Lineweaver-Burk plot of TEA uptake by BLM. Experimental conditions were the same as in Fig. 8. Each point represents mean of four determinations.

uptake. Thus, when uptake was corrected for non-saturable component which was determined in the presence of 1 mM Darstine, the phenomenon of saturation become apparent (curve c). In BLM similar method were employed to correct the non-saturable component of TEA uptake. The data obtained from saturation curves were used to evaluate the kinetic parameters for both membrane vesicles (Figs. 9 and 10). Lineweaver-Burk plots for TEA transport indicate that the apparent values of Km and Vmax in BBM were 0.55 ± 0.15 mM and 1.04 ± 0.23 nmol/mg protein/20s, and in BLM were 0.46 ± 0.04 mM and 0.61 ± 0.06 nmol/mg protein/20s, respectively.

DISCUSSION

Kinetic parameters of PAH transport

The apparent values of Km and Vmax for PAH transport in BBM vesicles, the transport in BLM vesicles reflects a transport system with higher affinity and lower capacity.

The existence of a carrier-mediated transport system for PAH has been identified in renal BBM vesicles (Kinsella et al., 1979b; Blomstedt & Aronson, 1980). On the other hand, several studies suggested that PAH is transported by only simple diffusion across the renal BBM (Berner & Kinne, 1976; Kippen et al., 1979). In the present study, a saturable, probenecid-sensitive component of PAH transport with BBM vesicles could be clearly detected which indicates the existence of a carrier-mediated process. The presence of probenecid-sensitive PAH uptake across the BBM was suggested by in vivo experiments (Foulkes, 1977).

The reason of this discrepancy may be due to the characteristics of BBM with a higher Km value (8.46 mM) for PAH transport compared to BLM (0.35 mM).

The values of Km and Vmax obtained from the present study for both membranes are in good agreement with findings of Kinsella et al., (1979b), who observed the apparent Km values of 0.56 and 3.8 mM, and Vmax values of 0.87 and 5.0 nmol/mg protein/min in BLM and BBM, respectively. A Km value for BLM from the present study was comparable also with 0.54 mM reported by Berner & Kinne (1976). Interestingly, the Km value obtained from this study with BLM was in excellent agreement with 0.987 mM in rat kidney slices (Pritchard, 1978), 0. 236 to 0.59 mM in rabbit kidney slices (Park et al., 1971; Gerencser et al., 1973; Ecker & Hook, 1974; Podevin & Boumendil-Podevin, 1975; Kikuta & Hoshi, 1979; Kim et al., 1986), 0.17 mM in cat kidney slice (Kim, 1982) and 0.54 mM in rabbit kidney isolated tubules (Huang & Lin, 1965). The result obtained from this study also showed that the imposition of a Na gradient increased Vmax without a change in Km value (Fig. 7). This finding agreed with that reported in rabbit kidney slices (Gerencser et al., 1973; Podevin & Boumendil-Podevin, 1977) but differed from that of Kasher et al., (1983) who found a mixed type by Na^+ in rat BLM.

Kinetic parameters of TEA transport

The data obtained from the present study indicate

that the apparent Km values were similar in BBM and BLM, but the apparent Vmax value for BBM was 1.7 times higher than that for BLM.

A Km value of 0.55 mM for BBM in this study was of the same order of magnitude as 0.8 mM for TEA in rat renal BBM (Takano et al., 1984). This value also was in agreement with 0.54 mM for procainamide (McKinney & Kunnemann, 1985) and 0.63 mM for N-methylnicotinamide (Wright, 1985) in rabbit renal BBM. It is of interest that in BLM the apparent Km value of 0.46 mM for TEA determined in the present study was comparable with 0.233 mM in rabbit kidney cortical slices (Kim & Jung, 1985) and was 0.14 mM for TEA in mouse kidney cortical slices (Holm, 1972) and 0.15 mM for NMN in dog kidney slices (Ross & Farah, 1966).

The similar Km values for PAH or TEA observed in the BLM vesicles and in the intact cell preparations suggest that the properties of transport of organic ions in isolated membrane vesicles were unaltered during the isolation procedure of preparation.

Much data on organic ion transport across the BLM have been reported with the intact cell preparations, especially with the kidney cortical slices. On the other hand, there are no data available for brush-border membranes from the intact tissues for comparision with the results obtained with vesicles, because neither in vitro nor in vivo techniques are useful for determining kinetic parameters for a specific BBM transport pathway.

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