Effect of pH on PAH Transport in Brush Border Basolateral Membrane Vesicles of Rabbit Proximal Tubule

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ABSTRACT

The effect of pH on the rate of PAH uptake was studied in rabbit renal basolateral membrane vesicles (BLMV) and brush border membrane vesicles (BBMV). In the absence of Na in incubation medium, a decrease in external pH (pH_o) led to an increase in probenecid-sensitive PAH uptake by BLMV. In the presence of Na, the probenecid-sensitive PAH uptake was unaltered when the pH_o decreased from 8.0 to 6.0 but further decrease in pH_o to 5.5 increased significanlty the uptake. The probenecid-sensitive PAH uptake was not affected by an alteration in pH *per se* in the absence of a pH gradient with or without the presence of Na. However, the presence of Na stimulated the probenecid-sensitive PAH uptake in all pH ranges tested over that measured in the absence of Na. A similar pattern of pH dependence on the PAH uptake was observed in BBMV but the presence of Na did not alter the probenecid-sensitive PAH uptake in the presence and absence of a pH gradient. Kinetic analysis for BLMV showed that Na or pH gradient increased Vmax of the probenecid-sensitive PAH uptake without a change in Km value. These results suggest that PAH is transported by OH^-/PAH exchange process in the luminal membrane, but the pH dependence in the BLMV is not unequivocally consistent with an anion exchange process. The PAH transport is dependent on Na in BLMV but not in BBMV.

Key Words: PAH transport; pH effect; rabbit kidney; membrane vesicles

INTRODUCTION

Organic anions such as *p*-aminohippurate(PAH) are actively secreted in the proximal tubule of the mammalian kidney.

Microperfusion studies strongly suggest, at least in the mammalian kidneys, that the step across the basolateral membrane involes the active process (Tune et al, 1969). Up to date, the mechanism for the active transport of PAH has been extensively investigated. However, neither the driving forces nor mechanisms for active transport are clearly understood at present. Studies with the renal cortical slices showed that PAH accumulation into the cell required an intact cellular metabolism(Cross & Taggart, 1950; Maxild & Møller, 1969) and is dependent on the presence of Na^+ in the bathing medium(Chung et al, 1970; Gerencser et al, 1973). In metabolically poisoned rabbit kidney slices, an inwardly directed Na^+ gradient caused a transient accumulation of PAH of up to twice the medium concentration ("overshoot") (Podevin et al, 1978). Since the effect appeared to be specific for Na, these data were considered as evidence for a Na^+/PAH cotransport system located in the basolateral membrane of proximal tubule cells.

Studies with the isolated basolateral membrane vesicles (BLMV) have confirmed that an inwardly

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directed Na^+ gradient stimulated PAH uptake (Kahn et al, 1985; Kasher et al, 1983; Sheikn & Mø ller, 1982). Unfortunately, however, most of studies with BLMV failed to observe Na^+-dependent PAH uptake against a concentration gradient, i.e., "overshoot" phenomenon, which may be considered as a strong evidence for Na^+/PAH cotransport. Only Sheikh & Møller (1982) in the rabbit renal BLMV obtained results consistent with Na/PAH cotransport, observing that imposition of an inwardly directed Na gradient produces an overshoot phenomenon. Thus, even experiments with membrane vesicles, which are extremely useful for studies on coupling between Na and solutes flux at the level of the specific membrane (Murer & Kinne, 1980), did not unequivocally demonstrate the presence of a Na^+/PAH cotransport system in the basolateral membrane of proximal tubule.

On the other hand, it is proposed that PAH accumulation may be driven by countertransport with anions being formed by metabolic processes(for review, see: Pritchard, 1987). Low et al (1984) found in the BLMV evidence for a common anion exchanger that accepts a variety of inorganic anions and organic anions including PAH. They also found that sulfate uptake could be stimulated by an inwardly directed H^+ gradient, suggesting the presence of OH^-/sulfate exchange or H^+/sulfate cotransport system which is shared by PAH. Recently, Eveloff (1987) has observed in experiment with rabbit renal BLMV that the simultaneous imposition of both OH^-(in to out) and Na (out to in) gradients produce a transient accumulation of PAH over the concentration gradient ("overshoot") but either the OH^- gradient or Na gradient alone did not produce the overshoot. These data indicate the existence of OH^-/PAH exchange system in the basolateral membrane. However, Ullrich et al., (1987a) found no interaction of PAH and inorganic anions such as OH^-, Cl^- and NO_3^-, but they observed the PAH transporter interacts with dicarboxylate of Krebs cycle. Several studies with BLMV on SO_4^- uptake suggest indirectly that PAH did not share the $SO_4^-/OH^-(HCO_3^-)$ exchange system (Pritchard & Renfro, 1983; Kuo & Aronson, 1986) and the SO_4^- transport system (Hagenbuch et al, 1985) by showing no *cis*-inhibition.

While there is general agreement concering the presence of an active transport process for PAH at the basolateral membrane, opinions differ on the mode of the PAH transport across the brush border membrane. The early study with isolated perfused tubule have proposed the existence of downhill PAH transport across the brush border membrane from cell into lumen (Tune et al, 1969). However, these investigators remained unsolved the question whether or not this step was one of carrier-mediated transport. Several investigators suggested that the flux of organic anions across the brush border membrane is by simple diffusion and does not involve a specific transport process (Cohen et al, 1975; Tune et al, 1977). On the other hand, the probenecid-sensitive PAH transport across the brush border membrane was suggested by Foulkes (1977). Studies with the membrane vesicles also provide evidence for probenecid-sensitive, carrier-mediated transport (Eveloff et al, 1979; Goldinger et al, 1984; Kinsella et al, 1979). Furthermore, the PAH uptake by BBMV of dog (Blømstedt & Aronson, 1980; Guggino et al, 1983) and rat (Kahn et al, 1983) shows OH^-/PAH exchange and their system is shared by uric acid and Cl^-. It is, however, unclear whether this exchange system is present in the brush border membrane of rabbit proximal tubule.

The present study was designed to observe the effect of pH on PAH transport in BLMV, and to determine whether its effect could arise from OH^-/PAH exchange system in rabbit renal proximal tubule.

METHODS

Preparation of membrane vesicles

Basolateral and brush border membranes were

prepared by a Percoll density gradient centrifugation method according to Scalera et al (1981) with some modifications (Kim et al, 1987). New Zealand White rabbits were killed by a blow to the neck. The kidneys were perfused with an ice-cold solution containing 140 mM NaCl, 10 mM KCl, and 1.5 mM $CaCl_2$. The cortices were dissected, minced, and homogenized in 10%(wt/vol) sucrose buffer (250 mM sucrose and 10 mM triethanolamine, pH 7.6) with a tissue homogenizer for 10 strokes. The homogenate was centrifuged at 1,200 g for 100 min. The supernatant was collected and recentrifuged at 9,500 g for 15min. The supernatant was combined with the upper layer of the pellet and centrifuged at 25,000 g for 30 min. The white, upper fluffy layer was gently separated from underlying, dark lower layer and resuspended in 26.5 ml of sucrose buffer by homogenizing with a tissue homogenizer for 10 strokes. Percoll (3.5 ml) was added, mixed vigorously and the mixture was centrifuged at 31,000 g for 45 min. The top 5 ml of the resulting gradient were discarded. The next 8 ml, having a very high Na-K-ATPase activity, was collected as basolateral membrane vesicles (BLMV) and the last 17 ml as brush border membrane vesicles (BBMV). The BBMV were further purified by the Mg^{2+}-precipitation procedure by Kinsella et al(1979). Each membrane fraction was diluted with an equal volume of sucrose buffer and centrifuged at 65,000 g for 1 h to remove the Percoll. After centrifugation, the Percoll formed a very dense glassy pellet, and the membranes formed a loose fluffy layer above the Percoll. The membranes were resuspended in vesicle buffer containing usually 100 mM mannitol, 50 mM KCl, 20 mM N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (Hepes)/Tris (pH 7.4) by passing it several times through a 25-gauge needle and centrifuged again at 65,000 g for 1 h to remove the remaining Percoll. The final membrane pellet was resuspended in intravesicular buffer and frozen in liquid nitrogen until used. Before the experiments the membrane vesicles were preincubated at 37℃ for 30 min.

Marker enzyme assays

Na-K-ATPase activity was assayed according to Jørgensen and Skou(1971). Alkaline phosphatase activity was determined as described by Linhardt and Walter(1963). Protein concentration was determined according to Bradford(1976) using bovine serum albumin as a standard.

Measurement of transport

Uptakes of [^{3}H]PAH and D-[^{3}H]glucose were determined by a rapid filtration techique similar to that described by Goldinger et al(1984). The reaction was initiated by adding 50 μl vesicle suspensoin (8 mg/ml) to 500 μl of the uptake medium maintained at 25℃. The composition of the experimental solutions are given in the appropriate figure legends. At appropriate times, 100 μl of reaction mixture were removed and quickly filtered through a 0.45 μm HAWP nitrocellulose filter (Millipore). The filters used were soaked overnight in solution containing 100 mM NaCl, 1 mM PAH, and 1mM Hepes/Tris (pH 7.4) before use. The filters were washed with 5 ml of ice-cold stop solution containing 100 mM mannitol, 100 mM NaCl, and 20 mM Hepes/Tris (pH 7.4), and dissolved in 1 ml of 2-methoxyethanol (Eastman).

The radioactivity remaining on the filters was counted by standard liquid scintillation techniques. All uptakes were corrected for nonspecific fillter binding which was determined by incubating vesicles in distilled water containing 0.1% deoxycholic acid and [^{3}H]PAH or D-[^{3}H]glucose filtering as described above, and washing with 5 ml of distilled water. The rate of probenecid-sensitive PAH uptake was evaluated by subtracting the uptake in the presence of 2 mM probenecid from the total uptake measured in the absence of probenecid. In experiments altering the pH, the PAH uptake was measured in the presence of valinomycin and K (50 mM

in and out) to minimize a possible proton diffusion potental. All experiments were performed in at least three separate membrane preparations. Statistical significance was determined with Student's t test for unpaired data and a one-way analysis of variance.

Chemicals

[^{3}H]PAH and [^{3}H] glucose were obtained from New England Nuclear (Boston, MA). Valinomycin, PAH, probencid and ouabain were obtained from Sigma Chemical Co (St.Louis, MO). Percoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Other chemicals were of the highest purity available from chemical sources.

RESULTS

Purity of membrane fractions

The degree of purification was determined by assaying the activity of Na-K-ATPase, the marker enzyme for BLMV, and of alkaline phosphatase, the marker enzyme for BBMV. BLMV fraction was enriched in Na-K-ATPase specific activity 22.97-fold over crude homogenate and in alkaline phosphatase activity 1.13-fold. BBMV were enriched in alkaline phosphatase activity 24.20-fold over crude homogenate, while the Na-K-ATPase was decreased to 0.70-fold (Table 1).

Glucose uptake

The purity of membrane preparation was evaluated functionally by measuring the Na^+-dependent D-glucose uptake. In BBMV D-glucose uptake is stimulated by a Na gradient, showing the "overshoot" (Aronson & Sactor, 1975). As shown in Fig. 1, the Na^+-dependent uptake of D-glucose by BBMV was increased about 10-fold compared to the Na^+-independent uptake at 30 s, showing the overshoot phenomenon over the equilibrium value. On the other hand, the inital rate of D-glucose uptake by BLMV was virtually the same in the presence and absence of a Na^+ gradient. Moreover, the uptake showed no overshoot. These result, together with the enzyme studies in Table 1, provided strong evidence that BBMV and BLMV used in this study were highly purified.

PAH uptake by BLMV and BBMV

The osmotic sensitivity of PAH uptake by the membrane vesicles was determined in order to distinguish between membrane binding and transport. Intravesicular volume may be modifed by the addition of various concentrations of impermeant solute to the incubation medium. Under these conditions, varying PAH uptake into an internal space directly reflects changes in intravesicular volume at equilibrium. Fig. 2 shows the effect of varying the extravesicular osmolarty by the addition of sucrose on PAH equilibrium uptake at 60 min. An increase in the osmotic gradient across the membrane decreased PAH uptake by both membrane vesicles, indicating

Table 1. Specific Activity for Marker Enzymes in BLMV and BBMV

	Na-K-ATPase (μ molPi/mg/hr.)	Enrichment (fold)	Alkaline phosphatase (μ mol/mg/hr.)	Enrichment (fold)
Homogenate	4.80±0.89 (4)		1.25±0.12 (6)	
Basolateral membrane	110.50±5.13 (4)	22.97	0.88±0.09 (6)	0.70
Brush border	5.44±0.25 (4)	1.13	30.25±2.14 (4)	24.20

Enrichment indicates the ratio of specific activities relative to the homogenate. Data repersent mean±SEM. Number of experiments is given in parenthesis.

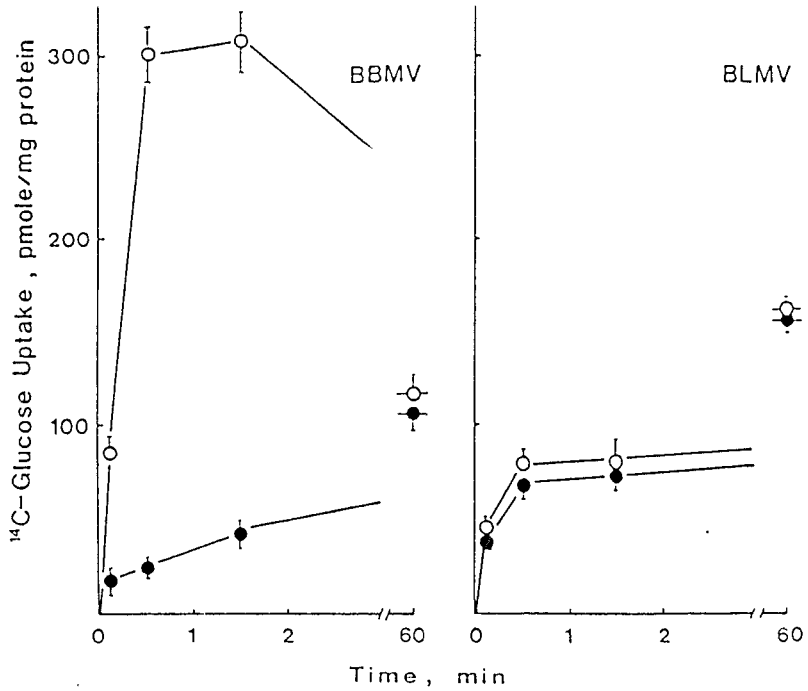

Fig. 1. Time course of D-[14]glucose uptake in rabbit renal brush border membrane vesicles (BBMV) and basolateral membrane vesicles (BBMV). Membrane vesicles were preloaded with 100 mM mannitol, 100 mM KCl, and 20 mM Hepes/Tris (pH 7.4). Uptake of 50 μM D-glucose was mesaured in buffers containing 100 mM mannitol, 100 mM NaCl (○) or 100 mM KCl(●) and 20 mM Hepes/Tris (pH 7.4). Each point represents mean±SEM of three determinations.

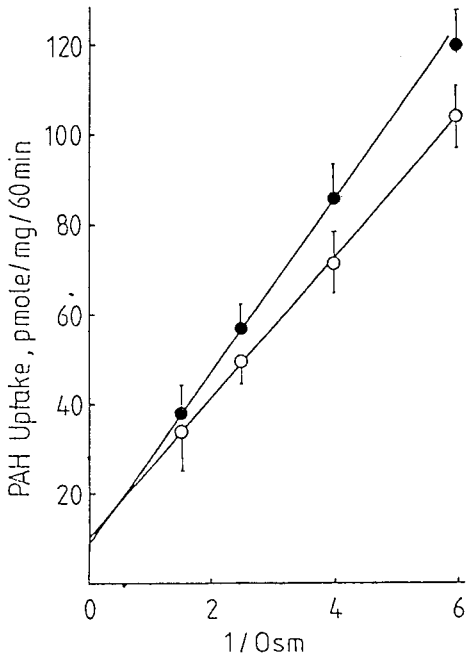

Fig. 2. Effects of increasing osmolarity on PAH uptake into BLMV (○) and BBMV(●). Membrane vesicles were preloaded with 100 mM mannitol, 50 mM KCl, 20 mM Hepes/Tris (pH 7.5). Uptake of PAH was measured after 60 min in buffers containing 100 mM NaCl, 50 mM KCl, 20 mM Hepes/Tris (pH 7.5), 50 mM ^{3}H-PAH and various concentrations of sucrose. Each point represents mean±SEM of three determinations.

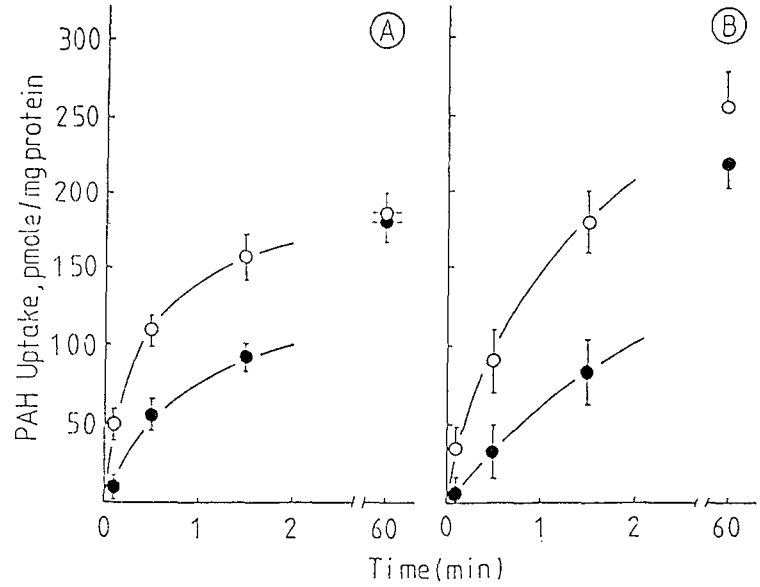

Fig. 3. Time course of PAH uptake into BLMV (A) and BBMV (B). Membrane vesicles were prepared as in Fig. 2. Uptake of PAH was measured in buffers containing 100 mM mannitol, 50 mM KCl, 100 mM NaCl, 20 mM Hepes/Tris (pH 7.5), and 50 μM ^{3}H-PAH in the presence (●) and absence (○) of 2 mM probenecid. Each point represents mean±SEM of three determinations.

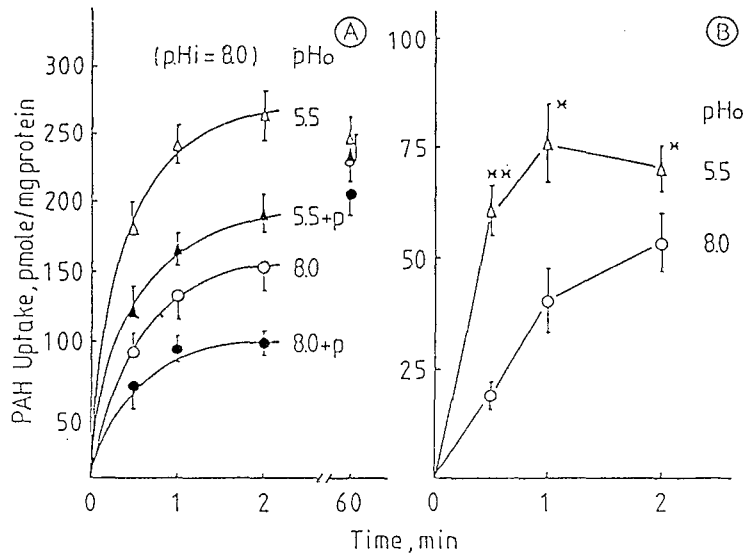

Fig. 4. Time course of PAH uptake into BLMV. Membrane vesicles were prepared as in Fig. 2 but in the pH 8.0 buffer. (A) Uptake of PAH was measured in buffers containg 100 mM mannitol, 50 mM KCl, 20 mM N-morpholinoethanesulfonic acid (Mes)/Tris (pH 5.5-6.0) or 20 mM Hepes/Tris (pH 7.0-8.0), 2 μM valinomycin, and 50 μM ^{3}H-PAH in the presence and absence of 2 mM probencid (P). (B) The probenecid-sensitive PAH uptake was evaluated from (A) by subtracting the uptake in the presence of probencid from total uptake in the absence of probenecid. Each point represents mean±SEM of four determinations. * $P<0.05$, ** $P<0.01$ compared to $pH_o=8.0$

the uptake into an osmotically reactive intravesicular space. Extrapolating PAH uptake to infinite osmolarity suggests that binding in both membrane vesicles was less than 18% of the equilibrium uptake at normal external buffer osmolarity. Therefore, the uptake data were not corrected for binding.

Fig. 3 shows the time course of PAH uptake by the BLMV and BBMV in the presence and absence of 2 mM probenecid. Inhibition of PAH uptake by probenecid distinguishes carrier-mediated transport from non-specific uptake through a leaky pathway or simple diffusion. The presence of 2 mM probenecid inhibited PAH uptake by BLMV and BBMV 50 and 60%, respectively, at 30 sec of incubation.

Effect of pH on PAH uptake by BLMV

The time courses of a Na^+-independent PAH uptake by BLMV in the presence and absence of 2 mM probenecid are given in Fig. 4A. When the external pH(pH_o) was decreased from 8.0 to 5.5, there was a marked increase in PAH uptake in the presence and absence of probencid while the equilibrium values at 60 min showed no significant difference. However, the uptake in the absence of probenecid was more increased than in the presence of probenecid. Thus, as shown in Fig. 4B, lowering pH_o from 8.0 to 5.5 stimulated probenecid-sensitive PAH uptake 200, 90 and 23% at 30, 60 and 90 sec of incubation time, respectively, indicating the stimulation of a carrier-mediated PAH transport with decreased pH_o. The finding that decreasing pH_o caused the increase in PAH uptake even in the presence of probenecid could be to a increase in the passive diffusion because undissociated from of

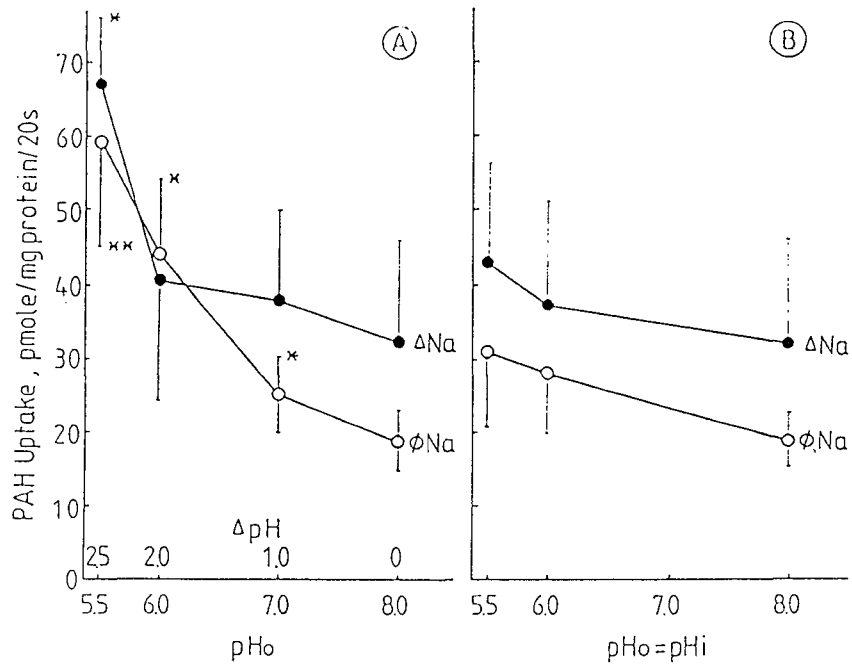

Fig. 5. pH dependence of probenecid-sensitive PAH uptake into BLMV. (A) Membrane vesicles were prepared as in Fig. 4. Uptake of PAH was measured in buffers containing 100 mM mannitol, 50 mM Hepes/Tris (pH 7.0-8.0), and 50 μM ^{3}H-PAH in the presence (ΔNa) and absence (ϕNa) of 100 mM NaCl. (B) Membrane vesicles were prepared as in Fig. 4 but in the same buffers as in internal pH (pH_i): in this case, pH_i and pH_o were equal. The probenecid-sensitive PAH uptake was evaluated as in Fig. 4. Each point represents mean $\pm$ SEM of five determinations. $*p<0.05$, $**p<0.01$ compared to $pH_o=8.0$.

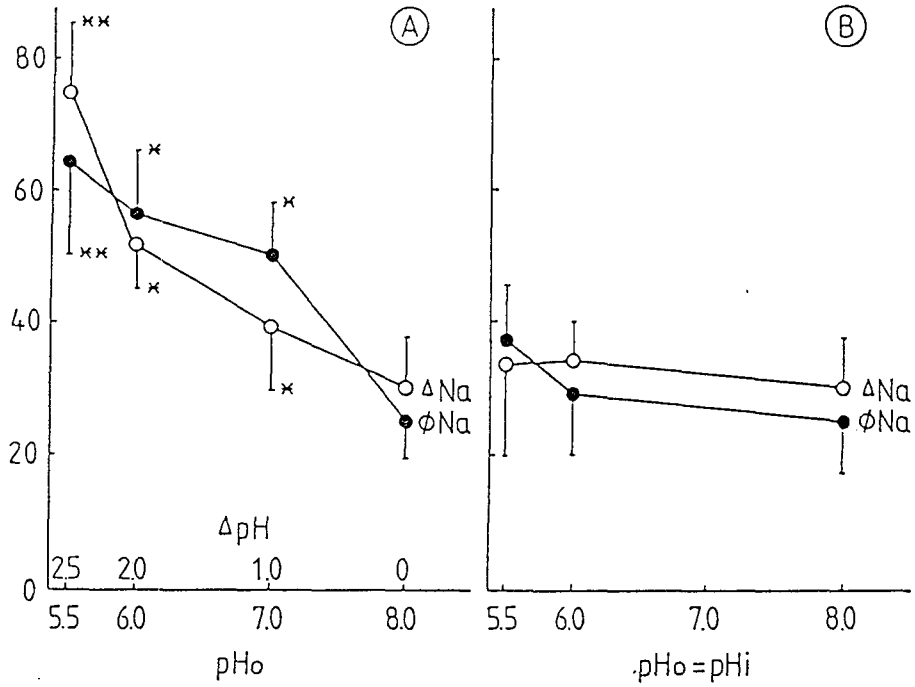

Fig. 6. pH dependence of PAH uptake into BBMV. The experimental conditions were the same as in Fig. 5.

PAH, which is more permeable than the anionc form(Kippen et al, 1979), was increased as the pH was decreased.

Fig. 5A shows the effect of varying the pH_o at a constant internal pH(pH_i) 8.0 on the probenecid-sensitive PAH uptake by BLMV after 20-sec incubation. In the absence of Na^+, the rate of PAH uptake increased significantly as the pH_o decreased between 8.0 and 5.5. At pH_o 5.5 the PAH uptake was 3-fold stimulated compared to pH_o of 8.0. However, in the presence of an inwardly directed Na^+ gradient, the rate of PAH uptake showed no significant change in a range of pH_o between 8.0 and 6.0. But a further decrease in pH_o to 5.5 stimulated significantly the PAH uptake. In contrast, in the absence of a pH gradient, varying the pH_o showed no significant change in the PAH uptake in the presence and absence of Na^+ (Fig. 5B). Thus, a pH gradient but not pH *per se.* increased the PAH uptake. Fig. 5B also showed that the presence of Na^+ in the absence of a pH gradient stimulated significantly the probenecid-sensitive PAH uptake compared to that measured in the absence of Na^+ at all pH ranges tested ($p<0.05$).

A similar pH dependence of the probenecid-sensitive PAH uptake was observed in BBMV (Fig. 6A and B). However, in contrast to BLMV, there was no significant difference between uptakes in the presence and absence of Na^+ in all pH values, indicating that the PAH uptake by BBMV was not dependent on the external Na^+, in agreement with the result by Kahn and Aronson (1983) in dog renal BBMV.

Fig. 7 shows the effect of pH_o on kinetics of the PAH transport by BLMV. The rates of probenecid-sensitive PAH uptake (20sec) were measured in the presence and absence of Na^+ at various substrate concentrations with ($pH_i=8.0$, $pH_o=5.5$) or without a pH gradient ($pH_i=pH_o=8.0$). Data were fitted to saturable, single-site binding model: $V = Vmax\,[S]/(Km+[S])$, where V is the

uptake of PAH (S); Vmax is the maximal rate of uptake; and Km is the apparent Michaelis constant. The kinetic parameters obtained from this plot are given in Table 2. When the pH_o was varied from 8.0 to 5.5 in the absence of Na, the Vmax value was significantly increased from 0.179±0.048 to 0.421±0.012 n mole/mg protein/ 20 sec without a significant change in Km value. A similar result was also obtained in the presence of 100 mM Na, showing that decreasing pH_o from 8.0 to 5.5 increased Vmax from 0.281±0.013 to 0.344±0.024 n mole/mg protein/20sec. These fondings that Vmax is altered without a change in Km with varying the pH suggest a pH dependent alteration of the transport system itself. Table 2 shows also at pH_o 8.0 (the absence of a pH gradient) the presence of Na^+ increased Vmax of the PAH transport compared to that obtained in the absence of Na^+ while at pH_o 5.5 (the presence of a pH gradient) Na^+ decreased rather Vmax.

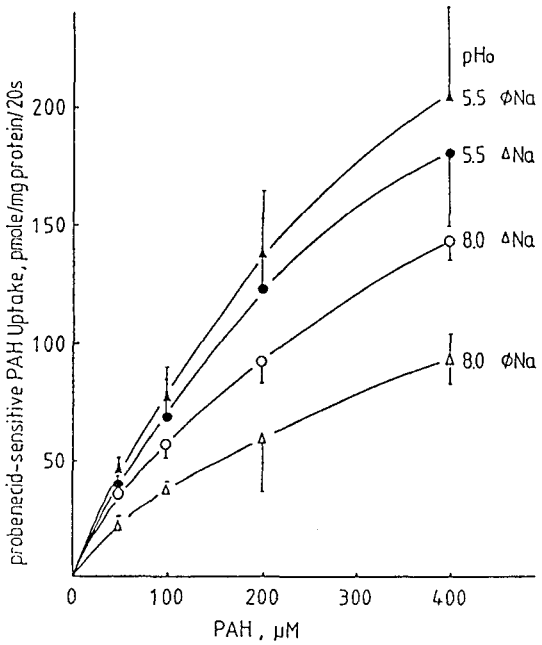

Fig. 7. Effect of concentration on pH dependence of probencid-sensitive PAH uptake into BBMV. Membrane vesicles were prepared as in Fig. 4. Uptake of PAH was measured as in Fig. 4 after 20 sec of incubation but in various concentrations of ^{3}H-PAH. Lines depict the best fit of the data points to an equation with a saturable, single-site binding model (see text). Each point represents mean±SEM of five determinations.

DISCUSSION

pH dependence of PAH uptake in BLMV

The effect of pH on the PAH transport was studied with rabbit renal BLMV by Eveloff (1987) who found that an outwardly directed hydroxyl gradient stimulated the PAH uptake, led to conclude that the effect of pH resulted from the anion exchange mechanism, i.e., OH^-/PAH exchange. It has also been proposed by others that PAH may be transported across the basolateral membrane of the proximal tubules by an anion exchange process. Low et al. (1984) demonstrated that sulfate uptake into rat renal BLMV can be stimulated by anions such as PAH,

Table 2. Kinetic parameters for PAH uptake in BLMV

Na_o (mM)	pH_i	pH_o	Vmax (n mole/mg protin/20s)	Km (μM)
0	8.0	8.0	0.179 ± 0.048^{ac}	377±17
0	8.0	5.5	0.421 ± 0.012^{ad}	401±18
100	8.0	8.0	0.281 ± 0.013^{bc}	363±29
100	8.0	5.5	0.344 ± 0.024^{bd}	376±44

Values are mean ± SEM of five deferminations

Values with superscripts a, b, c and d were significantly different from value showing the same superscript ($P<0.05$)

acetate, pyruvate and hydroxylion. They proposed the presence of a common anion exchange system for inorganic and organic anions which can additionally be driven by a Na^+ gradient.

On the other hand, many investigators failed to obtain evidence that the anion exchange system for $SO_4^-/OH^-(HCO_3^-)$ in renal basolateral membrane accepts directly PAH as substrate (Pritchard & Renfro, 1983; Hagenbuch et al, 1985; Kuo & Aronson, 1986). Similar results were reported in studies with the microperfused rat proximal tubules by Ullrich et al, (1987a, 1987b) who observed no interaction of PAH and inorganic anions. They demonstrated that PAH transport is inhibited only by dicarboxylates such as succinate and glutarate with high potency, led to conclude that the PAH transport system accepts several dicarboxylates. These studies also showed no difference in contraluminal PAH uptake between pH 6.0 and 8.0, inconsistent results with regard to the presence of OH^-/PAH exchange process. Thus, although a PAH/anion exchange system is present in the basolateral membrane, more likely candidates of counterion for PAH may include several Krebs cycle intermediates, a conclusion supported by Shimada et al (1987), rather than inorganic anion such as hydroxyl. Such assumption was previously suggested by Sheikh & Møller (1983) in renal cortical slices.

The results obtained from this study in BLMV indicate that an outwardly directed pH gradient stimulates the probenecid-sensitive PAH uptake in the absence of Na. The probenecid-sensitive PAH uptake, however, is not affected by a change in pH *per se* in the absence of a pH gradient (Fig. 5). The possible explanation for the results could be that the pH dependence of PAH uptake reflects H^+/PAH cotransport or OH^-/PAH exchange. However, the finding that a pH gradient did not stimulate the PAH uptake between pH_o 6.0 and 8.0 in the presence of Na^+ in incubation medium is inconsistent with the presence of OH^-/PAH exchange system. If the effects of pH were due to anion exchange, when pH and Na^+ gradients were simultaneously imposed, the PAH uptake should be additionally stimulated, which did not occur, compared to the presence of a Na^+ gradient alone. These results rather would be expected if proton ions compete with Na^+ at Na^+ acting site.

In kinetic analysis of PAH uptake, a pH gradient as well as a Na gradient increased Vmax without a change in Km value. Thus, Na^+ and proton may influence the PAH transporter with the same behavior. Interestingly, a similar pH dependence was observed in Na^+-dependent phosphate transport across the brush border membrane of rat proximal tubule (Hoffman et al, 1976; Burchardt et al, 1981), although it showed an opposite pH dependence which increases its uptake with increasing pH_o. According to these investigator, the phosphate uptake is not affected by the pH_i or by a pH difference across the membrane, suggesting a pH gradient provides no additional driving force or phosphate uptake. Furthermore, their data indicate that a change in pH_o affects Vmax of the phosphate transport system while remains unalterd Km value, and a strong pH dependence can be abolished by increasing Na concentrations. Thereby they have proposed that the pH changes primarily affect Vmax of the phosphate transport system possible by altering pH-dependent affinity of the system for Na. In renal brush border membrane the phosphate transport is coupled to Na^+ (for review, see: Gmaj & Murer, 1986). However, it is unclear whether the PAH transport across the basolateral membrane is directly coupled to Na^+ although its uptake is stimulated by Na^+ (for review, see: Pritchard, 1987). Thus, the exact mechanism of the lack of pH dependece on the PAH uptake in the presence of Na cannot be elucidated until we fully understand the underlying mechanism of Na^+ effect on the PAH transport across the basolateral membrane. Also further studies are required to determine whether the pH

dependence of the PAH uptake observed in the absence of Na^+ reflects the existence of a OH^-/PAH exchange process.

pH dependence of PAH uptake in BBMV

In BBMV, an outwardly directed pH gradient stimulated probenecid-sensitive PAH uptake but altering pH *per se* in the absence of a pH gradient did not, a similar result to that observed in BLMV. In contrast to BLMV, however, the presence of Na^+ did not affect the PAH uptake in the presence or absence of a pH gradient. The difference in the Na^+-dependence indicates different transport systems in the brush border and basolateral membrane of proximal tubule. This also may provide evidence that the pH dependence of the PAH uptake present in BLMV did not result from the contamination of BBMV.

The pH dependence of probenecid-sensitive PAH uptake by BBMV is considered as evidence for a OH^-/PAH exchange system which is insensitive to the presence of Na^+. Blømstedt & Aronson (1980) demonstrated that the PAH transport in dog renal BBMV occurs via a anion exchange process which is shared by uric acid and OH^-. Subsequent studies by Aronson and colleagues (Kahn & Aronson, 1983; Guggino et al, 1983) with dog BBMV, and Kahn et al (1983) with rat BBMV confirmed these initial findings. According to their data, the anion exchange process present in the brush border membrane has the affinity for organic anions such as uric acid, PAH, pyrazinoate, and lactate as well as inorganic anions HCO_3^-, OH^-, and Cl^-. Thus, the luminal anion exchange system seems to be relatively broad in substrate specificity. Weinman et al(1983) found in perfused rat proximal tubule a process of anion exchange for uric acid and PAH, a result consistent with the vesicle data.

In conclusion, probenecid-sensitive PAH uptakes by BLMV and BBMV are stimulated by an outwardly directed pH gradient but do not by a change in the pH_o *per se* in the absence of a pH gradient. The pH dependence of probenecid-sensitive PAH uptake by BBMV may result from the OH^-/PAH exchange or H^+/PAH cotransport, but it is unclear whether the pH dependence in BLMV results from the anion exchange mechanism.

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REFERENCES

Aronson PS & Sactor B (1975). The Na^+ gradient-dependent transport of D-glucose in renal brush border membranes. *J Biol Chem* 250, 6032-6039

Blømstedt JW & Aronson PS (1980). pH-gradient-stimulated transport of urate and p-aminohippurate in dog renal micorvillus membrane vesicles. *J Clin Invest* 65, 931-934

Bradford MM (1976). A rapid and sensitve method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ana Biochem* 72, 248-254

Burckhardt G, Stern H & Murer H (1981). The influence of pH on phosphate transport into rat renal brush border membrane vesicles. *Pflügers Arch*390, 190-197

Chung ST, Park YS & Hong SK (1970). Effect of cations on transport of weak organic acids in rabbit kidney slices. *Am J Physiol* 219, 30-33

Cohen L, Lapkin R & Kaloyanide GJ (1975). Effect of gentamicin on renal function in the rat. *J Pharmacol Exp Ther* 193, 264-273

Cross RJ & Taggart JV (1950). Renal tubular transport: Accumulation of p-aminohippurate by rabbit kidney slices. *Am J Physiol* 161, 181-190

Eveloff J, Kinne R & Kinter WB (1979). p-Aminohippuric acid transport into brush border vesicles isolated from flounder kidney. *Am J Physiol* 237, F291-F298

Foulkes EC (1977). Movement of p-aminohippurate between lumen and cells of renal tubule. *Am J Physiol* 232, F424-F428

Gerencser GA, Park YS & Hong SK (1973). Sodium influence upon the transport kinetice of p-aminohippurate in rabbit kidney slices. *Proc Soc Exp Biol Med* 144, 440-444

Gmaj P & Murer H (1986). Cellular mechanisms of inorganic phosphate transport in kidneny. *Physiol Rev* 66, 36-70

Goldinger JM, Khalsa BDS & Hong SK (1984). Photoaffinity labeling of organic anion transport system in proximal tubule. *Am J Physiol* 247, C217-C227

Guggino SE, Martin GJ & Aronson PS (1983). Specificity and modes of the anion exchanger in dog renal microvillus membranes. *Am J Physiol* 244, F612-F621

Hagenbuch B, Stange G & Murer H (1985). Transport of sulphate in rat jejunal and rat proximal tubular basolateral membrane vesicles. *Pflügers Arch* 405, 202-208

Hoffman N, Thees M & Kinne R (1976). Phosphate transport by isolated renal brush border vesicles. *Pflügers Arch* 362, 147-156

Jørgensen PL & Skou JC (1971). Purification and characterization of (Na^+-K^+)-ATPase in preparations from the outer medulla of rabbit kidney. *Biochim Biophys Acta* 233, 366-380

Kahn AM, Branham S & Weinman EJ (1983). Mechanism of urate and p-aminohippurate transport in rat renal microvillus membrane vesicles. *Am J Physiol* 245, F151-F158

Kahn AM & Aronson PS (1983). Urate transport via anion exchange in dog renal microvillus membrane vesicles. *Am J Physiol* 244, F56-F63

Kahn AM, Shelat H & Weinman EJ (1985). Urate and p-aminohippurate transport in rat renal basolateral membrane vesicles. *Am J Physiol* 249, F654-F661

Kasher JS, Holohan PD & Ross CR (1983). Na^+ gradient-dependent p-aminohippurate (PAH) transport in rat basolateral membrane vesicles. *J Pharmacol Exp Ther* 227, 122-129

Kim YK, Woo JS & Lee SH (1987). Kinetics of organic ion transport across rabbit renal brush border and basolateral membrane vesicles. *Korean J Physiol* 21, 273-282

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

Kippen I, Hirayama B, Klinenberg JR & Wright EM (1979). Transport of p-aminohippuric acid, uric acid and glucose in highly purified rabbit renal brush border membranes. *Biochim Biophys Acta* 556, 161-174

Kuo S-M & Aronson PS (1986). Anion specificity of the SO_4-HCO_3 exchange in renal basolateral membrane vesicles (BLMV). *Fed Proc (abstract)* 45, 178

Linhardt K & Walter K (1963). Phosphatases. In: Bergeyer HU (ed) Methods in Enzymatic Analysis. *New York, Academic* p 783-785

Low I, Friedrich T & Burckhardt G (1984). Properties of an anion exchanger in rat renal basolateral membrane vesicles. *Am, J Physiol* 246, F334-F342

Maxild J & Møller JV (1969). Metabolic studies on renal transport of p-aminohippurate in vitro. *Biochim Biophys Acta* 184, 614-624

Murer H & Kinne R (1980). The use of isolated membrane vesicles to study epithelial transport processes. *J Membr Biol* 55, 81-95

Pritchard JB(1987). Luminal and peritubrlar steps in renal transport of p-aminohippurate. *Biochim Biophys Acta* 906, 295-308

Podevin RA, Boumendil-Podevin EF & Priol C (1978). Concentrative PAH transport by rabbit kidney slices in the absence of metabolic energy. *Am J Physiol* 235, F278-F285

Pritchard JB & Renfro JL (1983). Renal sulfate transport at the basolateral membrane is mediated by anion exchange. *Proc Natl Acad Sci USA* 80, 2603-2607

Scalera V, Huang YK, Hildemann B & Murer H (1981). A simple isolation method for basal-lateral plasma membranes from rat kidney cortex. *Member Biochem* 4, 49-61

Sheikh MI & Møller JV (1982). Na^+-gradient dependent stimulation of renal transport of p-aminohippurate. *Biochem J* 208, 243-246

Sheikh MI & Møller JV(1983). Natureof Na^+-independent stimulation of renal transport of p-aminohippurate by exogenous metabolites. *Biochem Pharmacol* 32, 2745-2749

Shimada H, Moewes B & Burckhardt G (1987). Indirect coupling to Na^+ of p-aminohppuric acid utake into rat renal basolateral membrane vesicle. *Am J Physiol* 253, F795-F801

Tune BM, Burg MB & Patlak CS (1969). Characteristics of p-aminohippurate transport in proximal renal tubules. *Am J Physiol* 217, 1057-1063

Tune BM, Wu KY & Kempson R (1977). Inhibition of transport and prevention of toxicity of cephaloridine in the kidney. Dose-responsiveness of the rabbit and guinea pig to probenecid. *J Pharmacol Exp Ther* 202, 466-471

Ullrich KJ, Rumrich G, Fritzsch G & Kloss S (1987a). Contraluminal para-aminohippurate (PAH) transport in the proximal tubule of the rat kidney. I. Kinetics, influence of cations, anions, and capillary preperfusion. *Pflügers Arch* 409, 229-235

Ullrich KJ, Rumrich G, Fritzsch G & Kloss S (1987b). Contraluminal para-aminohippurate (PAH) transport in the proximal tubule of the rat kidney. II. Specificity: aliphatic dicarboxylic acids. *Pflügers Arch* 408, 38-45

Weinman EJ, Sanson SC, Bennett S & Kahn AM (1983). Effect of anion exchange inhibitors and para-aminohippurate on the transport of urate in the rat proximal tubule. *Kid Int* 23, 832-837

= 국문초록 =

가토 신장 근위세뇨관의 Brush Border 및 Basolateral Membrane Vesicle에서 PAH 이동에 미치는 pH의 영향

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가토 신장 근위세뇨관에서 분리한 brush border membrane vesicle(BBMV)과 basolateral membrane vesicle(BBMV)에서 rapid filtration 방법으로 PAH 이동에 대한 pH의 영향을 관찰하였다. BLMV에서 용액내 Na이 없을 때 외부 pH(pH_o)를 8.0에서 5.5까지 감소시켰을 때 probenecid-sensitive PAH 이동은 유의하게 증가되었다. 용액내 Na이 있을 때 pH_o가 8.0에서 6.0까지 변화하여도 PAH 이동에는 영향이 없었으나 5.5까지 더욱 감소시켰을 때 PAH 이동이 증가하였다. 그러나 vesicle 내 · 외에 pH gradient없이 pH_o를 내부 pH(pH_i)와 동일하게 변화시켰을 때 PAH 이동은 영향을 받지 않았다. pH gradient가 없을 때 시험된 pH범위에서 Na은 PAH 이동을 증가시켰다.

BBMV에서도 BLMV에서와 유사한 pH 의존성을 보였으나 Na의 존재는 PAH 이동에 영향을 미치지 못하였다. BLMV에서 동력학적 분석 결과 일정한 pH_i에서 pH_o 감소는 Km에 변화없이 PAH 이동에 대한 Vmax를 유의하게 증가시켰다.

이러한 결과로 BBMV에서 PAH 이동에 대한 pH의 영향은 OH^-/PAH 교환기전에 기인하는 것으로 추측되나 BLMV에서 pH 의존성은 음이온 교환기전만으로 설명될 수 없다. 또한 BLMV에서는 PAH 이동이 Na에 의존하나 BBMV에서는 Na에 의존하지 않음을 가르킨다.