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MECHANISMS OF *p*-AMINOHIPPURATE TRANSPORT BY BRUSH-BORDER AND BASOLATERAL MEMBRANE VESICLES ISOLATED FROM RAT KIDNEY CORTEX

RYOHEI HORI, MIKIHISA TAKANO, TOMONOBU OKANO, SHIKIFUMI KITAZAWA and KEN-ICHI INUI

Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto, 606 (Japan)

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The uptake of [^{3}H]-labeled *p*-aminohippurate by brush-border and basolateral membrane vesicles isolated from rat renal cortex has been studied by a rapid filtration technique. Some characteristics of carrier-mediated transport for *p*-aminohippurate were demonstrated in basolateral membrane vesicles: the uptake was inhibited by probenecid or 4,4′-diisothiocyano-2,2′-disulfonic stilbene (DIDS), was saturable, was stimulated by the countertransport effect, and showed discontinuity in the Arrhenius plot. In contrast, brush-border membrane vesicles failed to display saturability of *p*-aminohippurate uptake and stimulation by the countertransport effect, although probenecid and DIDS reduced the uptake. Furthermore, *p*-aminohippurate uptake by brush-border membrane vesicles was influenced more sensitively by alteration in the membrane potential compared with that by basolateral membrane vesicles.

Introduction

The recent development of the techniques for the isolation of plasma membrane vesicles that retain transport activity has permitted more detailed studies of the mechanisms involved in the solute transport across tubular cells [1,2]. However, there are only a few reports [3–7] concerning the plasma membrane transport of organic anion, which is responsible for the active secretion or organic anion in the proximal tubules [8]. The molecular details such as transport systems and driving forces for organic anion secretion still remain poorly characterized. The present studies were designed to compare the characteristics of *p*-aminohippurate uptake by brush border and basolateral membrane vesicles isolated from rat renal cortex. A preliminary report in an abstract form has been published elsewhere [9].

Experimental procedures

Brush border and basolateral membrane vesicles were isolated from the renal cortex of male Wistar albino rats, 190–230 g, according to our previous report [10]. The purified membranes were suspended in a buffer comprising 100 mM mannitol/20 mM Hepes-Tris (pH 7.5) (buffer A). Alkaline phosphatase (EC 3.1.3.1) and aminopeptidase (EC 3.4.11.2) were enriched 10-fold in brush-border membrane preparation compared with those found in the homogenate, and $(Na^+ + K^+)$-ATPase (EC 3.6.1.3) was enriched 22-fold in the basolateral membrane preparation. The uptake of *p*-aminohippurate by the freshly isolated membrane vesicles was measured by a rapid filtration technique [10,11]. In most experiments the reaction was initiated rapidly by adding 20 μl buffer A, containing 0.25 mM *p*-amino[^{3}H]hippurate

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; DIDS, 4,4′-diisothiocyano-2,2′-disulfonic stilbene.

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(Amersham, 374 mCi/mmol) plus 200 mM NaCl or other salts, to 20 μl (20–60 μg protein) of membrane vesicle suspension at 25°C. At the stated times, the incubation was stopped by diluting a reaction sample with 1 ml ice-cold buffer A. The tube contents were immediately poured onto Millipore filters (HAWP, 0.45 μm, 2.5 cm diameter) and washed with 5 ml ice-cold buffer A. The radioactivity of dried filters was determined by a liquid scintillation counting. Protein and marker enzymes were assayed as previously described [10].

Results

Fig. 1 shows the inhibitory effect of probenecid and DIDS at various concentrations on *p*-aminohippurate uptake by brush-border and basolateral membrane vesicles. DIDS, a specific inhibitor for anion transport into red blood cells [12], inhibited more strongly the uptake of *p*-aminohippurate than did probenecid. The degree of the inhibition in brush-border and basolateral membrane vesicles was almost similar. These data differ from those of Berner and Kinne [3] who found a much greater effect of probenecid on *p*-aminohippurate transport in basolateral membranes as compared with brush-border membranes.

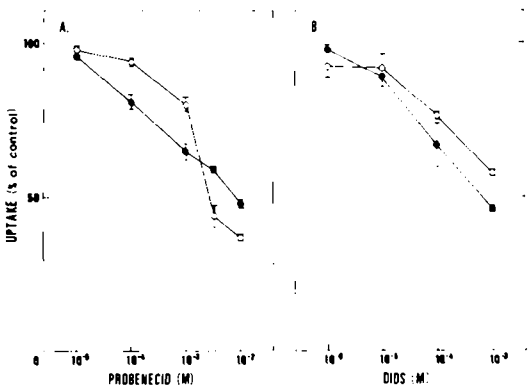

Fig. 1. Concentration dependence of the inhibition of *p*-aminohippurate uptake by probenecid (A) and DIDS (B) in brush border (○) and basolateral membrane vesicles (●). The uptake for 1 min was determined in the presence of probenecid (A) or DIDS (B) as described in the text. In the case of DIDS, membrane vesicles were pretreated with DIDS for 10 min at 25°C prior to beginning the uptake. Incubation medium contained 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 0.125 mM *p*-amino[^{3}H]hippurate and 100 mM NaCl in the presence of probenecid or DIDS shown in the abscissa. Each point represents mean ± S.E. of 3–5 determinations from a typical experiment.

Fig. 2 shows the curves for the concentration dependence of *p*-aminohippurate uptake by brush-border and basolateral membrane vesicles. The relationship between concentration and rate of uptake was nonlinear in basolateral membranes, providing evidence for saturability, although no evidence for saturation of the uptake by brush-border membrane vesicles was observed in the concentration range used. Concerning the concentration dependence for *p*-aminohippurate uptake by brush-border membrane vesicles, present results are different in part from those of Kinsella et al [4], who demonstrated the kinetic parameters not only in basolateral membranes but also in brush-border membranes isolated from dog kidney. The species difference may be one of the reason for this discrepancy.

Furthermore, in order to confirm the differences in *p*-aminohippurate transport by brush-border and basolateral membrane vesicles, we studied the effect of countertransport on *p*-aminohippurate uptake (Fig. 3). Vesicles preloaded with high concentration of unlabeled *p*-aminohippurate showed enhancement of *p*-amino[^{3}H]hip-

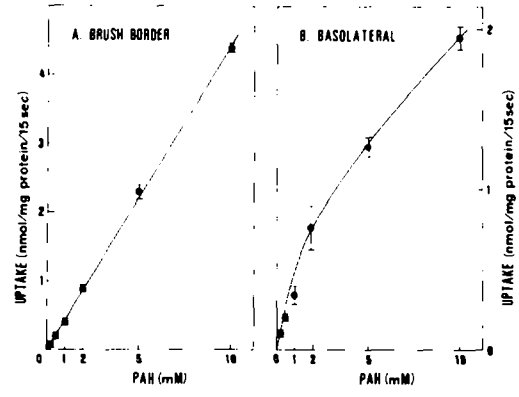

Fig. 2. Concentration dependence of *p*-aminohippurate (PAH) uptake by brush-border (A) and basolateral membrane vesicles (B). The uptake for 15 s at concentrations between 0.125 and 10 mM was determined as described in the text. Incubation medium comprised 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/*p*-amino[^{3}H]hippurate/100 mM NaCl. Each point represents mean ± S.E. of three determinations from a typical experiment.

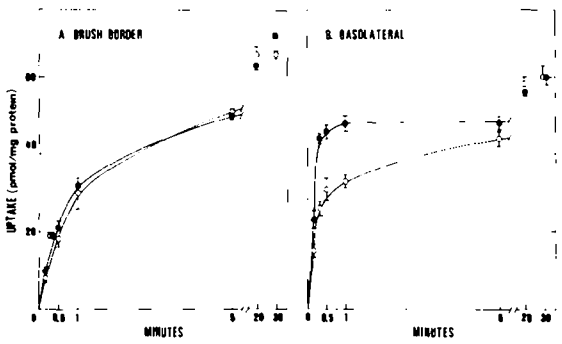

Fig. 3. Countertransport effects on *p*-aminohippurate uptake by brush-border (A) and basolateral membrane vesicles (B). Membrane vesicles were preincubated in 100 mM mannitol/20 mM Hepes-Tris (pH 7.5), with (●) or without (○) 0.5 mM unlabeled *p*-aminohippurate for 30 min, and then the aliquots (20 μl) were incubated with the substrate mixture (200 μl) comprising 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/0.05 mM *p*-amino[^{3}H]hippurate/100 mM NaCl during the indicated periods. Each point represents mean ± S.E. of two experiments performed in duplicate determinations.

purate accumulation by countertransport only in basolateral membranes, while no change of the uptake was observed in brush-border membranes.

Fig. 4 shows the temperature-dependence of *p*-aminohippurate uptake by brush-border and basolateral membrane vesicles. The Arrhenius plot for the uptake by brush-border membrane vesicles was linear over the temperature range studied (5–37°C), and gave the activation energy of 8.5 kcal/mol. In contrast, the Arrhenius plot for the uptake by basolateral membrane vesicles was biphasic, with the activation energies of 2.8 and 6.5 kcal/mol. As Smedt and Kinne [13] reported a biphasic Arrhenius plot with respect to Na^+-dependent D-glucose transport by brush-border membrane vesicles, the present data suggest the contribution of a carrier-mediated transport system for *p*-aminohippurate in basolateral membranes.

The role of membrane potential as a driving force for *p*-aminohippurate uptake by brush-border and basolateral membrane vesicles was studied by applying different anion gradients with sodium directed into the vesicles (Fig. 5). The more permeant lipophilic anion, SCN^-, thought to facilitate a more rapid development of interior negative membrane potential, was compared with less permeant anions, such as Cl^- and SO_4^{2-}. Anion permeability to biological membrane generally follows in the order of $SCN^- > Cl^- > SO_4^{2-}$ [14]. As shown in Fig. 5, *p*-aminohippurate uptake by brush-border membrane vesicles was higher when chloride was replaced by sulfate, and lower when chloride was replaced by thiocyanate. On the other hand, this effect for the uptake induced by anion gradients was small in extent in basolateral mem-

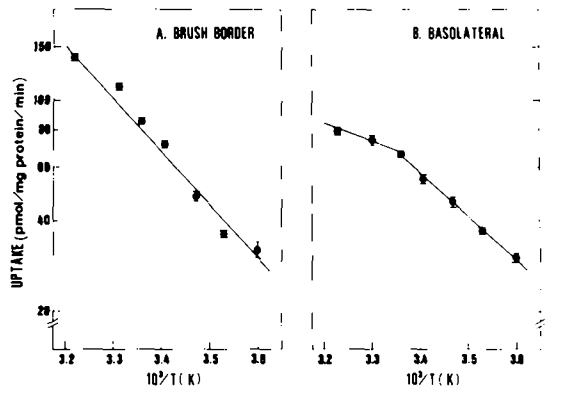

Fig. 4. Temperature dependence of *p*-aminohippurate uptake by brush-border (A) and basolateral membrane vesicles (B). The uptake for 1 min was determined at 5, 10, 20, 25, 30 and 37°C as described in the text. Incubation medium comprised 100 mM mannitol/20 mM Hepes-Tris (pH 7.5)/0.125 mM *p*-amino[^{3}H]hippurate and 100 mM NaCl. Each point represents mean ± S.E. of four determinations from a typical experiment.

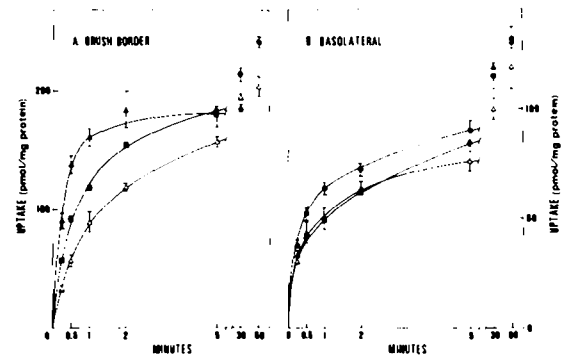

Fig. 5. Effect of anion gradients on *p*-aminohippurate uptake by brush-border (A) and basolateral membrane vesicles (B). Incubation medium comprised 100 mM mannitol/20 mM Hepes-Tris, (pH 7.5)/0.125 mM *p*-amino[^{3}H]hippurate with either 100 mM NaCl (●), 100 mM NaSCN (△) or 50 mM Na_2SO_4 (▲). Each point represents mean ± S.E. of 1–3 experiments performed in duplicate determinations.

brane vesicles. These results suggest that an increase of the inside-negative membrane potential decreases *p*-aminohippurate uptake, and this effect is more evident in brush-border membranes compared with basolateral membranes.

Discussion

The above results show that the uptake of *p*-aminohippurate by basolateral membrane vesicles satisfies some of the criteria for carrier-mediated process; namely, the process is saturable, temperature dependent, inhibited by anion transport inhibitors, and undergoes a countertransport effect. In contrast, brush-border membrane vesicles failed to display the capacity to accelerate the exchange of *p*-aminohippurate and saturability of the uptake, although probenecid and DIDS reduced *p*-aminohippurate transport. Therefore, it may be reasonable to assume that *p*-aminohippurate is transported across brush-border membranes by a gated channel, which responds to anionic charge, rather than by a simple diffusion.

Berner and Kinne [3] demonstrated that the inside-positive membrane potential can act as a driving force for *p*-aminohippurate uptake in basolateral membrane vesicles. In this study, however, *p*-aminohippurate uptake by brush border membrane vesicles was influenced more sensitively by the alteration of the membrane potential compared with that by basolateral membrane vesicles, and it was significantly stimulated by the membrane potential induced with various anion gradients, which renders the intravesicular space more positive. This result is compatible with the secretion of *p*-aminohippurate at the luminal side in vivo, because the intracellular compartment has more negative electrical potential than the luminal fluid compartment (approx. −60 mV) [15].

Acknowledgements

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