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Role of chloride on carrier-mediated transport of *p*-aminohippurate in rat renal basolateral membrane vesicles

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Effect of inorganic anions on *p*-amino[^{3}H]hippurate transport in renal basolateral membranes has been studied using the vesicles preloaded with unlabeled *p*-aminohippurate (countertransport condition). The uptake of *p*-amino[^{3}H]hippurate was stimulated by the outward gradient of unlabeled *p*-aminohippurate and the labeled substrate was accumulated into the vesicles against its concentration gradient in the presence of Cl^-. The substitution of SCN^- and SO_4^{2-} for Cl^- in both sides of the vesicles depressed the initial rate and the overshoot magnitude of *p*-amino[^{3}H]hippurate uptake. These results suggest that Cl^- may play an important role for the carrier-mediated transport system of organic anion in renal basolateral membranes.

There are some studies concerning the existence of the carrier-mediated transport system for *p*-aminohippurate in renal basolateral membranes [1–3]. In recent years, further characteristics of this transport system have been reported by several groups. Sheikh and Møller [4] demonstrated that *p*-aminohippurate transport in rabbit basolateral membranes was driven by an Na^+ gradient via an electroneutral co-transport system. Kasher et al. [5] proposed that *p*-aminohippurate was transported by an Na^+ gradient-dependent anion exchange mechanism in rat basolateral membranes. Tse et al. [6] reported that *p*-aminohippurate transport in rabbit basolateral membranes was stimulated by divalent cations such as Mg^{2+}. Thus, the molecular mechanisms underlying the transport of *p*-aminohippurate across basolateral membranes are still controversial and remain unsolved. In the course of the studies on the transport of organic ions by rat renal brush-border and basolateral membranes [3,7–9], we have examined the effect of inorganic anions on *p*-amino[^{3}H]hippurate transport by basolateral membrane vesicles.

Basolateral membrane vesicles were isolated from the renal cortex of male Wistar albino rats (200–230 g) according to the methods of Percoll density gradient centrifugation described previously [10]. The purified membranes were suspended in a buffer comprising 100 mM mannitol and 20 mM Hepes-Tris (pH 7.5). The uptake of *p*-amino[^{3}H]hippurate (Amersham, 500 mCi/mmol) by the freshly isolated membrane vesicles was measured at 25°C by a rapid filtration technique [9]. In the regular assay, the reaction was initiated rapidly by adding 180 μl of buffer containing *p*-amino[^{3}H]hippurate to 20 μl of membrane vesicle suspension (1–3 mg of protein per ml), which was preloaded with unlabeled *p*-aminohippurate (countertransport condition). Protein was determined, after precipitation with ice-cold 10% (w/v) trichloroacetic acid, by the method of Lowry et al. [11] with bovine serum albumin as a standard.

As described previously [3], the uptake of *p*-

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Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid.

aminohippurate by basolateral membrane vesicles was stimulated by the countertransport effect, which is one of the criteria of carrier-mediated transport. Under the countertransport condition, the characteristics of the carrier-mediated transport system for *p*-aminohippurate can be more pronounced. Therefore, we have studied the effect of various inorganic anions on *p*-amino[^{3}H]hippurate transport using the vesicles preloaded with unlabeled *p*-aminohippurate.

In order to determine the most effective concentration of intravesicular *p*-aminohippurate for stimulating *p*-amino[^{3}H]hippurate uptake, the initial rate of *p*-amino[^{3}H]hippature uptake (0.05 mM) was measured by basolateral membrane vesicles preloaded with various concentrations of unlabeled *p*-aminohippurate (0.5–20 mM). As shown in Fig. 1, the initial rate of *p*-amino[^{3}H]hippurate uptake was most stimulated when membrane vesicles were preloaded with 5 mM of unlabeled *p*-aminohippurate.

Fig. 2 shows the effect of KCl and NaCl on *p*-amino[^{3}H]hippurate uptake. In the presence of either KCl or NaCl, the vesicles preloaded with unlabeled *p*-aminohippurate produced a marked stimulation and an 'overshoot' of *p*-amino[^{3}H]hippurate accumulation. The initial rate and overshoot magnitude of *p*-amino[^{3}H]hippurate uptake by the vesicles preloaded with unlabeled *p*-aminohippurate were the highest under the ionic condition of [K^+] inside and [Na^+] outside, although the uptake by unloaded vesicles was much lower. When mannitol was substituted isosmotically for KCl or NaCl, no stimulation of *p*-amino[^{3}H]hippurate uptake was observed even though the vesicles were preloaded with unlabeled *p*-aminohippurate. Therefore, the presence of salts could be necessary for the carrier-mediated transport of *p*-aminohippurate in basolateral membranes, and the following studies were carried out in the presence of [K^+] inside and [Na^+] outside using the vesicles preloaded with 5 mM *p*-aminohippurate.

Fig. 3 shows the effect of substitution of SCN^- or SO_4^{2-} for Cl^- on *p*-amino[^{3}H]hippurate uptake by basolateral membrane vesicles. The substitution of SCN^- and SO_4^{2-} in both sides of the vesicles depressed the initial rate of *p*-amino[^{3}H]hippurate uptake by 57 and 77%, respectively. When *p*-amino[^{3}H]hippurate uptake was measured in the presence of SCN^-, accumulation of *p*-

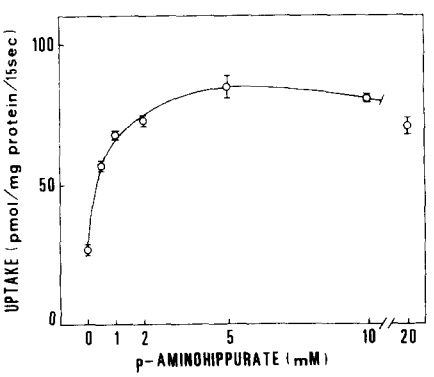

Fig. 1. Effect of the preloaded *p*-aminohippurate on *p*-amino[^{3}H]hippurate uptake by basolateral membrane vesicles. Membrane vesicles were preincubated in 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5) and 100 mM KCl with various concentrations of unlabeled *p*-aminohippurate for 60 min, and then the aliquots (20 μl) were incubated with the substrate mixture (180 μl) comprising 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 100 mM NaCl and 0.05 mM *p*-amino[^{3}H]hippurate for 15 s. Each point represents the mean ± S.E. ($n = 4$).

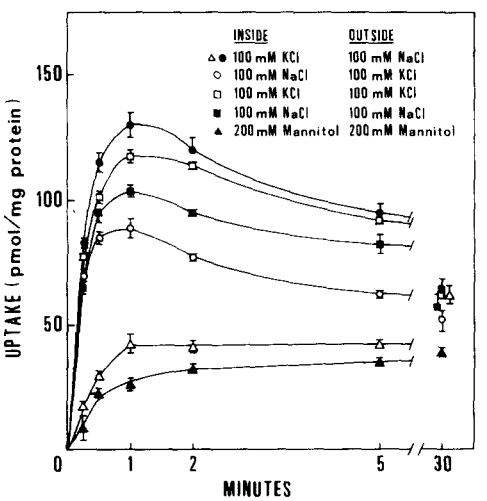

Fig. 2. Effect of inorganic ions on *p*-amino[^{3}H]hippurate uptake by basolateral membrane vesicles. Membrane vesicles were preincubated in 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), with (□, ■, ○, ●, ▲) or without (△) 5 mM *p*-aminohippurate for 60 min, and then the aliquots (20 μl) were incubated with the substrate mixture (180 μl) comprising 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5) and 0.05 mM *p*-amino[^{3}H]hippurate. Ionic conditions were shown in the figure. Each point represents the mean ± S.E. ($n = 3$–9).

amino[^{3}H]hippurate against its concentration gradient was still observed, but the magnitude of overshoot was decreased. In the presence of SO_4^{2-}, the transient accumulation of *p*-amino[^{3}H]hippurate against its concentration gradient was completely abolished. These results suggest that Cl^- may be the most effective inorganic anion for the transport of *p*-aminohippurate in basolateral membranes compared with SCN^- and SO_4^{2-}. It is reasonable to assume that Cl^- may act as a modulator for *p*-aminohippurate transport rather than as a driving force, because the vesicles were equilibrated with Cl^- in the present experiments. Furthermore, it should be examined whether the amount of unlabeled *p*-aminohippurate preloaded in the vesicles was the same in each ionic condition of Fig. 3. However, the equilibrium values of 5 mM *p*-amino[^{3}H]hippurate uptake by basolateral membrane vesicles were essentially the same in the presence of KCl, KSCN and K_2SO_4 gradient (Cl^-, 10.2 ± 0.1; SCN^-, 9.3 ± 0.1; SO_4^{2-}, 9.9 ± 0.2 nmol/mg protein per 60 min, mean ± S.E. of four determinations).

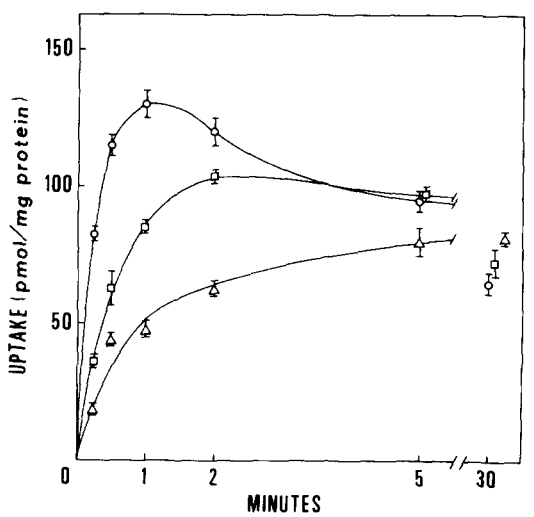

Fig. 3. Effect of inorganic anions on *p*-amino[^{3}H]hippurate uptake by basolateral membrane vesicles. Membrane vesicles were preincubated in 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 5 mM *p*-aminohippurate and either 100 mM KCl (○), 100 mM KSCN (□) or 50 mM K_2SO_4 (△) for 60 min, and then the aliquots (20 μl) were incubated with the substrate mixture (180 μl) comprising 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 0.05 mM *p*-amino[^{3}H]hippurate and either 100 mM NaCl (○), 100 mM NaSCN (□) or 50 mM Na_2SO_4 (△). Each point represents the mean ± S.E. (n = 4–9).

In order to examine the effect of inorganic anions on organic cation transport, we measured the uptake of [^{3}H]tetraethylammonium (New England Nuclear, 99 mCi/mmol) by basolateral membrane vesicles preloaded with unlabeled tetraethylammonium under the same experimental conditions in Fig. 3. However, substitution of inorganic anion had no effect on the uptake of 0.1 mM [^{3}H]tetraethylammonium (Cl^-, 100; SCN^-, 102; SO_4^{2-}, 99 pmol/mg of protein per 15 s, mean value of two determinations). These results suggest that inorganic anions may affect specifically the carrier-mediated transport of organic anion in basolateral membranes.

Fig. 4 shows the effect of Cl^- concentrations on the initial rate of *p*-amino[^{3}H]hippurate uptake. The increasing concentrations of Cl^- in both sides of the vesicles from 0 to 100 mM produced a sigmoidal stimulation on the rate of *p*-amino[^{3}H]hippurate uptake, and the maximal stimulation was observed at the concentration of 100 mM.

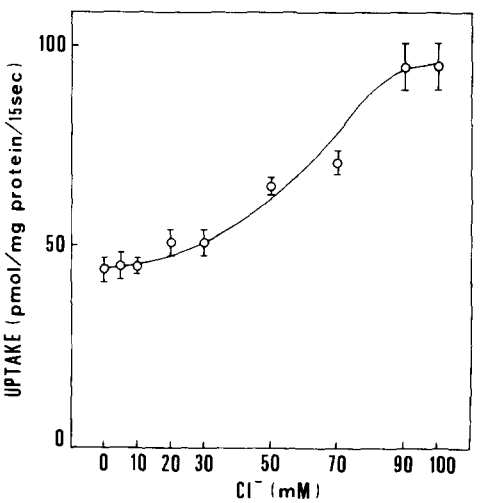

Fig. 4. Effect of Cl^- concentrations on *p*-amino[^{3}H]hippurate uptake by basolateral membrane vesicles. Membrane vesicles were preincubated in 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5) and 5 mM *p*-aminohippurate with various concentrations of KCl for 60 min, and then the aliquots (20 μl) were incubated for 15 s with the substrate mixture (180 μl) comprising 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5) and 0.05 mM *p*-amino[^{3}H]hippurate with various concentrations of NaCl. The concentration of Cl^- was the same in the inside and outside of the vesicles. Total salt concentration of 100 mM was maintained with KSCN (inside) and NaSCN (outside). Each point represents the mean ± S.E. (n = 4).

Several groups reported that *p*-aminohippurate uptake by cortical slices in the presence of Cl^- was higher than that in the presence of other inorganic anions [12,13]. In the present study the depression of *p*-aminohippurate uptake by basolateral membrane vesicles, observed when Cl^- was replaced by other inorganic anions, is compatible to the results of them. Podevin et al. [12] discussed that the inhibitory effect of SO_4^{2-} on Na^+-dependent transport of *p*-aminohippurate resulted from the change in membrane potential, which was more interior-positive when Na_2SO_4 was substituted for NaCl. In our experiments, however, inorganic anions were equilibrated across the membranes and thereby the effect of membrane potential should be negligible. Therefore, it seems reasonable to conclude that inorganic anion itself could be important for the regulation of carrier-mediated transport system of *p*-aminohippurate in basolateral membranes.

Goldinger et al. [13] suggested that the inhibition of *p*-aminohippurate transport by SCN^- and NO_3^- was competitive in nature and that it might involve electrostatic binding to protein at the transport site. More recently, Löw et al. [14] reported the existence of an anion exchange system in rat renal basolateral membranes, showing that the exchanger accepts various inorganic and organic anions such as SO_4^{2-}, Cl^- and *p*-aminohippurate. If these inorganic anions had the affinity for *p*-aminohippurate transport site, *p*-amino[^{3}H]hippurate uptake in the presence of high concentration of anions such as Cl^-, SCN^- and SO_4^{2-} in both sides of the vesicles should be lower than that in the presence of isosmotically substituted mannitol, but this is not the case in our study. Therefore, in our experimental conditions, there seems to be little possibility that these inorganic anions competitively inhibited *p*-aminohippurate uptake.

The stimulative effect of Cl^- on *p*-amino[^{3}H]hippurate uptake was maximal at the concentration of about 100 mM. This concentration of Cl^- is compatible with the concentration of Cl^- in the peritubular fluid [15]. It is conceivable that the physiological concentration of Cl^- in the peritubular fluid may be important for the normal function of *p*-aminohippurate transport system in basolateral membranes.

In conclusion, the present data indicate that the carrier-mediated transport of *p*-aminohippurate in renal basolateral membranes can be stimulated in the presence of Cl^-, and Cl^- may play an important role for the active secretion of organic anion in renal tubules.

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