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Report

Decreased Transport of *p*-Aminohippurate in Renal Basolateral Membranes Isolated from Rats with Acute Renal Failure

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Transport of D-glucose, *p*-aminohippurate (organic anion), and tetraethylammonium (organic cation) was studied in the renal basolateral membrane vesicles isolated from rats with acute renal failure (ARF). ARF was induced by a single injection of uranyl nitrate. Carrier-mediated transport of *p*-aminohippurate, estimated under anion–anion exchange condition, was significantly decreased in basolateral membrane vesicles isolated from ARF rats. In contrast, there were no significant differences in D-glucose and tetraethylammonium uptake between normal and ARF rats. When normal basolateral membrane vesicles were incubated in vitro with uranyl nitrate, no significant inhibition in *p*-aminohippurate uptake was observed. These results suggest that organic anion transport is decreased in renal basolateral membranes from ARF rats, and this transport dysfunction cannot be explained by the direct interaction of uranyl nitrate with the organic anion carrier.

KEY WORDS: *p*-aminohippurate; tetraethylammonium; transport; basolateral membrane; kidney; acute renal failure.

INTRODUCTION

Acute renal failure (ARF) causes transport dysfunctions in renal tubules, as evidenced by glucosuria, aminoaciduria, and altered renal transport of organic ions (1–4). However, the molecular mechanisms underlying the dysfunctions of tubular transport in renal failure remain unsolved.

The epithelium of the proximal tubule is characterized by polar cells with two distinct membranes, luminal brush-border and contraluminal basolateral membranes. These two membranes differ in transport characteristics of solutes. Therefore, isolated membrane vesicles from the brush-border and basolateral surface of the epithelium are useful tools to study transport mechanisms of solutes in each membrane (5–7). We have reported transport characteristics of D-glucose and organic ions in the renal brush-border membranes isolated from ARF rats (8). In this study, we have examined the transport of these substrates in the renal basolateral membranes isolated from ARF rats, in order to estimate the alterations of transepithelial transport in disease states. For the induction of ARF in rats, uranyl nitrate was chosen, because this disease model is technically simple and well established (2,9). Present results indicate that the transport of *p*-aminohippurate is decreased in basolateral membrane vesicles isolated from ARF rats compared with normal rats, but the transport of D-glucose and tetraethylammonium is not altered. A preliminary report in abstract form has been published elsewhere (10).

MATERIALS AND METHODS

Membrane Preparation

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 (11,12). In general, the purified membranes were suspended in a buffer comprising 100 m*M* mannitol and 20 m*M* Hepes[3]–Tris (pH 7.5). For the induction of ARF, rats were injected s.c. with uranyl nitrate (10 mg/kg body weight) and sacrificed 3 days later. The development of ARF was confirmed by the increased levels of creatinine and urea nitrogen in serum and of alkaline phosphatase and glucose in urine (8).

Transport Studies

Uptake of labeled D-glucose, *p*-aminohippurate, and tetraethylammonium by the freshly isolated basolateral membrane vesicles was measured by a rapid filtration technique (11,12). The reaction was initiated rapidly by adding buffer containing labeled substrate to 20 μl of membrane suspension (2–5 mg of protein per ml) at 25°C. At the stated times, the incubation was stopped by diluting a reaction sample

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[3] Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ATPase, adenosine triphosphatase.

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with 1 ml of ice-cold stop solution comprising 20 m*M* Hepes–Tris (pH 7.5) and either 150 m*M* KCl (for D-glucose), 150 m*M* NaCl (for *p*-aminohippurate), or 150 m*M* LiCl (for tetraethylammonium). The tube contents were immediately poured onto Millipore filters (HAWP, 0.45 μm, 2.5-cm diameter) and washed once with 5 ml of ice-cold stop solution. The radioactivity of dried filters was determined by 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 of membrane vesicles. This value was subtracted from uptake data. To test the *in vitro* effect of uranyl nitrate on *p*-aminohippurate transport, basolateral membranes isolated from normal rats were preincubated with uranyl nitrate (1 m*M*) before transport studies. We have previously found that this concentration of uranyl nitrate was high enough to inhibit the activities of enzymes such as $(Na^+ + K^+)$-ATPase in the cortical homogenates from normal rats (8).

Analytical Methods

Protein was measured, after precipitation with ice-cold 10% (w/v) trichloroacetic acid, by the method of Lowry *et al.* (13) with bovine serum albumin as a standard. $(Na^+ + K^+)$-ATPase[3] (EC 3.6.1.3) and alkaline phosphatase (EC 3.1.3.1) were assayed as described previously (11).

Materials

D-[^{3}H]Glucose (5.7 Ci/mmol) and *p*-amino[^{3}H]hippurate (365 mCi/mmol) were obtained from Amersham International, Ltd. (Buckinghamshire, UK), and [^{14}C]tetraethylammonium (3.7 mCi/mmol) from Du Pont–New England Nuclear (Boston, MA). Uranyl nitrate was obtained from Nakarai Chemicals (Kyoto, Japan). All other chemicals used for the experiments were of the highest purity available.

RESULTS

The quality of the basolateral membrane preparation from normal and ARF rats was evaluated by measuring marker enzyme activities. $(Na^+ + K^+)$-ATPase, the marker enzyme for basolateral membranes, was enriched more than 20-fold in the isolated membranes compared with the level in homogenate, and there was no significant difference between the enrichment for both groups (normal, 27.8 ± 8.2; ARF, 22.4 ± 8.8; mean ± SE of five experiments). In addition, contamination by brush-border membranes was low in both membrane preparations as evidenced by low enrichment of alkaline phosphatase (normal, 3.0 ± 0.8; ARF, 2.2 ± 0.2; mean ± SE of five experiments). Thus, the extent of purification for basolateral membranes isolated from ARF rats was similar to that from normal rats.

Figure 1 shows *p*-aminohippurate uptake by basolateral membrane vesicles isolated from normal and ARF rats. *p*-Aminohippurate is transported by a carrier-mediated process for organic anion in the renal basolateral membranes (14–16), and several reports indicated that the transport is driven by an anion–anion exchange system (17–21). Therefore, we examined *p*-aminohippurate uptake under the countertransport conditions as reported previously (18). That is, membrane vesicles preloaded with a high concentration of unlabeled *p*-aminohippurate were diluted into the buffer containing labeled substrate. Under the present conditions, apparent overshoot uptake of *p*-aminohippurate was observed. In the vesicles isolated from ARF rats, the initial rate and overshoot magnitude of *p*-aminohippurate uptake were significantly decreased. In contrast to *p*-aminohippurate transport, no significant differences between normal and ARF rats were observed in the uptake of D-glucose and tetraethylammonium by basolateral membrane vesicles (Fig. 2).

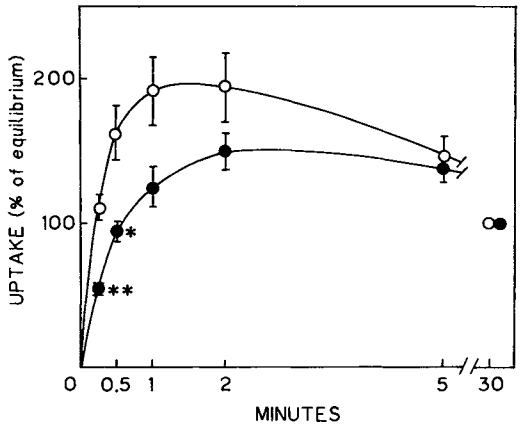

Fig. 1. *p*-Aminohippurate uptake by basolateral membrane vesicles. Membrane vesicles, isolated from normal (○) or ARF (●) rats, were preincubated in 100 m*M* mannitol, 20 m*M* Hepes–Tris (pH 7.5), and 5 m*M* *p*-aminohippurate for 60 min. Then the aliquots (20 μl) were incubated with the substrate mixture (180 μl) comprising 100 m*M* mannitol, 20 m*M* Hepes–Tris (pH 7.5), 100 m*M* NaCl, and 0.05 m*M* *p*-amino[^{3}H]hippurate. Each point represents the mean ± SE of three separate experiments performed in three determinations. (*) $P < 0.05$ and (**) $P < 0.01$; significant difference from normal using Student's *t* test.

It is important to test whether the decreased transport of *p*-aminohippurate in ARF is due to the direct effect of uranyl nitrate or not. Therefore we examined the *in vitro* effect of uranyl nitrate on the transport of *p*-aminohippurate by basolateral membrane vesicles isolated from normal rats. As shown in Fig. 3, no significant inhibition on *p*-aminohippurate uptake was observed by uranyl nitrate, suggesting that the decreased transport of *p*-aminohippurate in basolateral membranes in ARF rats is not due to the direct interaction of uranyl nitrate with the organic anion carrier, but could be secondarily induced after the impairment of the integrity for tubular cells.

DISCUSSION

Carrier-mediated transport of *p*-aminohippurate in basolateral membrane vesicles isolated from ARF rats was significantly decreased compared with that from normal rats. On the other hand, there was no change in D-glucose and tetraethylammonium transport in basolateral membranes from ARF rats, indicating that the effects of ARF on the transport activities are different in each transport system.

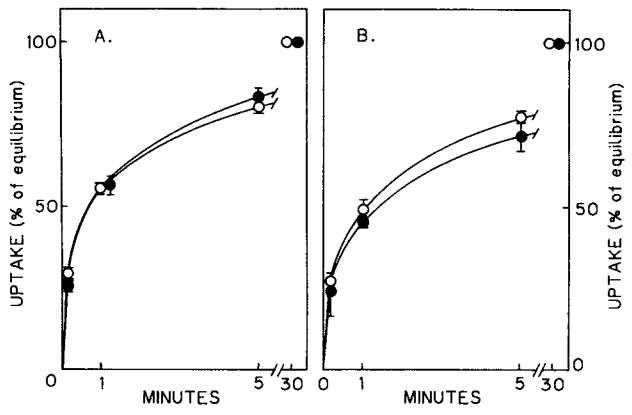

Fig. 2. Uptake of D-glucose (A) and tetraethylammonium (B) by basolateral membrane vesicles. (A) Membrane vesicles (20 μl), suspended in 100 m*M* mannitol, 20 m*M* Hepes–Tris (pH 7.5), and 100 m*M* KCl, were incubated with the substrate mixture (20 μl) comprising 100 m*M* mannitol, 20 m*M* Hepes–Tris (pH 7.5), 100 m*M* KCl, and 0.1 m*M* D-[^{3}H]glucose. (B) Membrane vesicles (20 μl), suspended in 100 m*M* mannitol, 20 m*M* Hepes–Tris (pH 7.5), and 100 m*M* KCl, were incubated with the substrate mixture (20 μl) comprising 100 m*M* mannitol, 20 m*M* Hepes–Tris (pH 7.5), 100 m*M* KCl, and 2.5 m*M* [^{14}C]tetraethylammonium. Membrane vesicles were isolated from normal (○) or ARF (●) rats. Each point represents the mean ± SE of three separate experiments performed in two determinations.

The mechanism for the decreased transport of *p*-aminohippurate in basolateral membranes from ARF rats is not clear. It may be due to the decreased number (availability) of the carrier, the decreased affinity of the substrate for the carrier, or the increased rate of dissipation of the driving force; any of these factors should decrease the *p*-aminohippurate transport in basolateral membranes *in vitro* and *in vivo*.

We have previously reported the transport functions of the renal brush-border membranes isolated from rats with ARF induced by uranyl nitrate (8). If we combine the former results and those reported here, predictable dysfunctions for

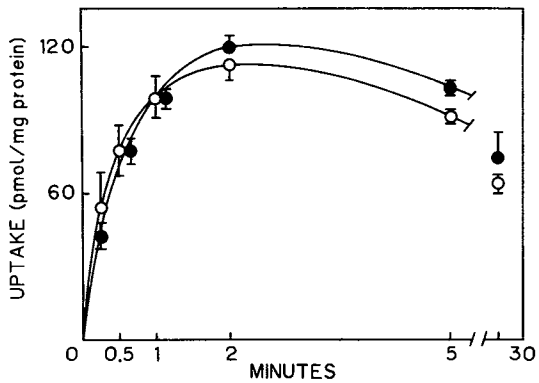

Fig. 3. Effect of uranyl nitrate on *p*-aminohippurate uptake by basolateral membrane vesicles. Membrane vesicles isolated from normal rats were preincubated in 100 m*M* mannitol, 20 m*M* Hepes–Tris (pH 7.5), with (●) or without (○) 1 m*M* uranyl nitrate at 25°C for 10 min. The uptake of *p*-amino[^{3}H]hippurate was measured as described in the legend to Fig. 1. Each point represents the mean ± SE of three determinations from a typical experiment.

renal transepithelial transport of D-glucose, organic anion (*p*-aminohippurate), and organic cation (tetraethylammonium) in ARF rats could be as follows. (i) The reabsorption of D-glucose is decreased due to the reduced transport in brush-border membranes, and this dysfunction is explained by enhanced dissipation of Na^+ gradient (driving force for D-glucose transport in brush-border membranes). (ii) Secretion of organic anion is decreased due to the reduced transport in basolateral membranes. (iii) Secretion of organic cation is decreased due to the reduced transport in brush-border membranes, and this dysfunction is not attributable to the changes of driving force (H^+ gradient). Apparently, dysfunctions occur in the membranes where each substrate is assumed to be transported actively, namely, D-glucose (5,11,22) and tetraethylammonium (7,8,12,23,24) in brush-border membranes and *p*-aminohippurate (17–21) in basolateral membranes. Therefore, active transport processes may be more sensitive to the renal failure than facilitated transport processes. However, further studies such as different time schedules after uranyl nitrate injection, different doses of uranyl nitrate, and different methods for the induction of ARF are needed to support this hypothesis.

In conclusion, by using isolated membrane vesicles, transport functions of the renal epithelial membranes were analyzed independent of other factors such as glomerular filtration, membrane potential, and renal blood flow. These findings will provide useful information for further understanding of tubular transport in ARF.

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