Biochimica et Biophysica Acta, 1150 (1993) 103–110

BBAMEM 76041

Transport of organic cation in renal brush-border membrane from rats with renal ischemic injury

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(Received 23 February 1993)

Key words: Renal transport; Organic cation; Tetraethylammonium; Ischemic injury; Reperfusion; Brush-border membrane

Transport of tetraethylammonium, an organic cation, has been studied using renal brush-border membrane vesicles isolated from rats with ischemic and ischemia-reperfusion injury. H^+ gradient-dependent uptake of tetraethylammonium slightly, but significantly, decreased in brush-border membrane vesicles from ischemic kidneys. When the kidney was reperfused after ischemia, the extent of the decrease of tetraethylammonium uptake was much greater than that after ischemia alone. The V_{max} value of tetraethylammonium uptake by brush-border membrane vesicles from reperfused kidneys was decreased compared with control, without any change in the K_m value. The tetraethylammonium uptake by the vesicles from reperfused kidneys was decreased both in the presence and absence of the outward H^+ gradient (driving force). Uptake of D-glucose in renal brush-border membrane vesicles was also decreased by ischemia and again, reperfusion caused a further decrease of the uptake. Reperfusion also induced marked changes in the enrichment and recovery of marker enzymes in the isolated brush-border membrane fraction compared with ischemia. These findings suggest that renal ischemic injury altered the transport properties of tetraethylammonium as well as D-glucose, and that reperfusion after ischemia induced further damages on these functions in the brush-border membrane.

Introduction

Renal ischemia stands as a major cause of acute renal failure [1]. Morphological studies have shown that the proximal tubule, particularly brush-border membrane, is a major target for ischemic injury [2–4]. Ischemic renal failure occurs following an episode of severe hemorrhagic shock, endotoxic sepsis, thermal burns, and transplantation surgery, to which various therapeutic drugs are administered. Although renal ischemia is reported to cause functional impairment such as reductions in proximal tubule fluid, sodium and glucose reabsorption [5,6], little is known about the transport of drugs in the kidney with ischemic injury.

Cationic drugs such as cimetidine and procainamide are actively secreted by an organic cation transport system in the renal proximal tubules [7]. Recently, the isolated plasma membrane vesicles have been widely used as an in vitro model system for studying the transport of nutrients, electrolytes, and organic ions in the renal tubule, and have helped elucidate the mechanisms of the absorptive and the secretory processes [8]. In this [9–12] and other laboratories [13,14], brush-border and basolateral membranes have been used for the analysis of transport systems for organic cations in the renal proximal tubules. These findings suggest that tetraethylammonium, a prototype organic cation, is transported across the basolateral membrane via a carrier-mediated system and that this process is stimulated by a cell-interior negative membrane potential. In the brush-border membrane, tetraethylammonium transport is driven by an H^+ gradient via an electroneutral H^+/organic cation antiport system, and various compounds such as aminocephalosporins [15], cimetidine [16], procainamide [17] and morphine [18] are transported by the antiport system.

In the present study, we examined the effect of ischemia on the transport of tetraethylammonium in the renal brush-border membrane vesicles. As ischemic

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Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino) ethanesulfonic acid; ATPase, adenosine triphosphatase.

renal injury should occur during ischemia and/or the reperfusion period, we evaluated the transport of tetraethylammonium in the brush-border membrane isolated from either ischemic or ischemia-reperfusion kidneys. The effect of ischemia and ischemia-reperfusion on D-glucose transport was also studied. The present findings indicate that tetraethylammonium transport in the renal brush-border membrane is slightly dysfunctioned by ischemia and that the transport is further decreased by reperfusion after ischemia. Ischemia-reperfusion also induced severe damages on D-glucose transport and enzyme activities in the brush-border membrane.

Materials and Methods

Preparation of brush-border membrane vesicles

Brush-border membrane vesicles were isolated from the renal cortex of male Wistar albino rats (200–230 g). Unilateral ischemia was induced under anesthesia with sodium pentobarbital (50 mg per kg body weight) by clamping the left renal pedicle for 50 min [19]. In some experiments, blood was allowed to reflow for 15 min after ischemia into the left renal artery. In each case, the right kidney served as the control.

The isolation procedure was based on the Mg/EGTA precipitation method as described previously [20]. All steps were performed on ice or at 4°C. Briefly, the cortex was homogenized with a Universal Homogenizer (Nihonseiki, Tokyo, Japan) at full speed for 2 min in 300 mM mannitol, 12 mM Tris-HCl (pH 7.1) and 5 mM EGTA to make a 10% homogenate. After dilution with distilled water (1:1), $MgCl_2$ was added to a final concentration of 10 mM, and the homogenate was allowed to stand for 15 min. The homogenate was centrifuged at $1900 \times g$ for 15 min in a Hitachi High Speed Refrigerated Centrifuge SCR 20B (rotor RPR20-2). The supernatant was centrifuged at $24000 \times g$ for 30 min. The pellet was resuspended in the buffer (10 ml/g cortex) comprised of 150 mM mannitol, 6 mM Tris-HCl (pH 7.1) and 2.5 mM EGTA, and homogenized in a glass/Teflon Potter homogenizer with 10 strokes at 1000 rpm. $MgCl_2$ was added to a final concentration of 10 mM, and the suspension was allowed to stand for 15 min, then centrifuged at $1900 \times g$ for 15 min. The supernatant was centrifuged at $24000 \times g$ for 30 min. The pellet was resuspended in an experimental buffer (20 ml/g cortex), and centrifuged at $24000 \times g$ for 30 min. The final pellet (purified brush-border membrane) was resuspended in the same experimental buffer 10 times through a fine needle (0.4×19 mm) with a plastic syringe. Usually, the experimental buffer contained 100 mM mannitol and 10 mM Mes (pH 6.0) unless otherwise stated. The membrane vesicles were used for transport studies on the day they were prepared.

Protein determination

Protein was determined, after precipitation with ice-cold 10% (w/v) trichloroacetic acid, by the method of Lowry et al. [21] with bovine serum albumin as a standard.

ATP assay

ATP content in the renal cortex during 50 min of ischemia was measured by the method of Williamson and Corkey [22]. Briefly, the kidneys were decapsulated, and a thin slice of the renal cortex was prepared with a Stadie-Riggs microtome. The cortex was homogenized in ice-cold 3% perchloric acid to make a 20% homogenate. After centrifugation at 3000 rpm for 15 min, the supernatant was neutralized and was used to determine ATP enzymatically with hexokinase and glucose-6-phosphate dehydrogenase [22].

Enzyme assay

γ-Glutamyltransferase (EC 2.3.2.2), aminopeptidase (EC 3.4.11.2) and Na^+/K^+-ATPase (EC 3.6.1.3) were assayed as described previously [9,23].

Transport studies

The uptake of substrates was measured by a rapid filtration technique. Briefly, brush-border membrane vesicles were preincubated for 10 min at 25°C before the initiation of uptake. The reaction was initiated rapidly by the addition of a buffer (80 or 20 μl) containing the labeled substrate to 20 μl of membrane suspension at 25°C. At the stated times, incubation was stopped by diluting the reaction mixture with 1 ml of ice-cold stop solution. The composition of the stop solution was 150 mM KCl, 20 mM Hepes-Tris (pH 7.5) and 0.1 mM $HgCl_2$ for tetraethylammonium uptake, and 150 mM NaCl, 20 mM Hepes-Tris (pH 7.5) and 0.1 mM phloridzin for D-glucose uptake. The contents of the tube were immediately poured onto Millipore filters (HAWP, 0.45 μm, 2.5 cm diameter), and the filters were washed once with 5 ml of ice-cold stop solution. The radioactivity of labeled substrate on the filter was determined by liquid scintillation counting.

Materials

Tetra[1-^{14}C]ethylammonium bromide (3.7 mCi/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA) and D-[1-^{3}H]glucose (8.3 Ci/mmol) was from Amersham International (Buckinghamshire, UK). Hepes and Mes were obtained from Nacalai Tesque (Kyoto, Japan). Hexokinase and glucose-6-phosphate dehydrogenase were obtained from Toyobo (Osaka, Japan). Adenosine 5′-triphosphate disodium salt and β-nicotinamide adenine dinucleotide phosphate sodium salt (NADP) were from Kojin (Tokyo, Japan). Phloridzin and cimetidine were obtained from

TABLE I

Effect of ischemia on the specific activities and recoveries of γ-glutamyltransferase, aminopeptidase and Na^+/K^+-ATPase in the homogenate and brush-border membrane

Enrichment indicates the ratio of the specific activities in the brush-border membrane (BBM) and the homogenate. Recovery indicates the percentage of the enzyme activity found initially in the homogenate. Each value is the mean ± S.E. of six experiments except that values of γ-glutamyltransferase represent the mean ± S.E. of five experiments. Specific activity (S.A.) (nmol/min per mg protein).

	γ-Glutamyltransferase		Aminopeptidase		Na^+/K^+-ATPase		Protein	
	control	ischemia	control	ischemia	control	ischemia	control	ischemia
Homogenate S.A.	973 ±72	731 * ±78	77 ±6	55 * ±5	68 ±6	67 ±3		
BBM S.A.	11989 ±624	9120 * ±692	1202 ±56	844 * ±52	63 ±8	76 ±8		
Enrichment	12.4 ±0.4	12.6 ±0.5	15.9 ±1.0	15.7 ±1.0	0.90 ±0.07	1.14 ±0.14		
Recovery	29.0 ±1.6	37.7 * ±1.5	35.7 ±2.8	47.2 * ±2.3	2.0 ±0.3	3.2 ±0.5	2.1 ±0.2	2.8 ±0.3

* $P < 0.05$, significant difference from control using analysis of variance followed by Fischer's *t*-test.

Sigma (St. Louis, MO). All other chemicals used for the experiments were of the highest grade available.

Results

Effect of the duration of ischemia on ATP content in renal cortex

The ATP content in renal cortex during 50 min of ischemia was measured. At 5 min, ischemia decreased the ATP content by 50% (control, 1.07 ± 0.03 μmol/g cortex, mean ± S.E. of three rats). At 20 min, the ATP content was 25% of that in the control and then was relatively constant up to 50 min.

Enzyme activities in isolated brush-border membrane

Table I shows the specific activities and recoveries of marker enzymes for brush-border membrane (γ-glutamyltransferase and aminopeptidase) and basolateral membrane (Na^+/K^+-ATPase) in the homogenate and final brush-border membrane isolated from the control and ischemic kidneys. The specific activities of γ-glutamyltransferase and aminopeptidase in the brush-border membrane were more than 10-fold higher than those in the homogenate (enrichment), and the purity of the membranes isolated from control and the ischemic kidneys was similar. However, the specific activities of these enzymes were reduced in the homogenate and brush-border membrane from ischemic kidneys. There was no significant difference between the control and ischemic kidneys on the specific activities of Na^+/K^+-ATPase in the homogenate and final brush-border membrane. The yield of brush-border membrane protein from ischemic kidneys was also similar to that from control kidneys.

TABLE II

Effect of reperfusion on the specific activities and recoveries of γ-glutamyltransferase, aminopeptidase and Na^+/K^+-ATPase in the homogenate and brush-border membrane

Enrichment indicates the ratio of the specific activities in the brush-border membrane (BBM) and the homogenate. Recovery indicates the percentage of the enzyme activity found initially in the homogenate. Each value is the mean ± S.E. of four experiments. Specific activity (S.A.) (nmol/min per mg protein).

	γ-Glutamyltransferase		Aminopeptidase		Na^+/K^+-ATPase		Protein	
	control	reper-fused	control	reper-fused	control	reper-fused	control	reper-fused
Homogenate S.A.	997 ±73	814 ±51	91 ±3	72 * ±2	75 ±1	88 * ±4		
BBM S.A.	11675 ±662	8291 * ±389	1086 ±17	701 * ±10	41 ±4	173 * ±7		
Enrichment	11.7 ±0.2	10.2 * ±0.4	12.0 ±0.2	9.7 * ±0.3	0.55 ±0.05	2.00 * ±0.12		
Recovery	30.7 ±2.3	14.0 * ±1.2	31.4 ±2.3	13.4 * ±1.0	1.5 ±0.2	2.7 * ±0.2	2.6 ±0.2	1.4 * ±0.1

* $P < 0.05$, significant difference from control using analysis of variance followed by Fischer's *t*-test.

Table II shows the effect of reperfusion on the specific activities and recoveries of γ-glutamyltransferase, aminopeptidase and Na^+/K^+-ATPase in the homogenate and brush-border membrane. The specific activities of γ-glutamyltransferase and aminopeptidase in brush-border membrane were increased about 10-fold relative to those in the homogenate. However, the enrichment of these enzymes in the membrane isolated from reperfused kidneys was slightly lower than that from the control kidneys. The specific activities of these enzymes were reduced in the homogenate and brush-border membrane from reperfused kidneys compared with control kidneys. On the other hand, the enrichment of Na^+/K^+-ATPase in the membrane isolated from reperfused kidneys was about four times higher than that in the control kidneys. In addition, the protein recovery of brush-border membrane isolated from reperfused kidneys was about a half of that from the control kidneys.

Transport of tetraethylammonium and D-*glucose*

Fig. 1 shows the tetraethylammonium uptake by brush-border membrane vesicles isolated from control and ischemic kidneys. As reported previously [9], tetraethylammonium uptake by renal brush-border membrane vesicles was actively driven against its concentration gradient by an outwardly directed H^+ gradient (overshoot phenomenon). The tetraethylammonium uptake by ischemic vesicles in the presence of an H^+ gradient was slightly, but significantly, decreased compared with that by control vesicles at 30 s, 1 and 2 min. Equilibrium values of tetraethylammonium uptake by the two membrane vesicles were similar, which suggests that the intravesicular volume was not changed by ischemia.

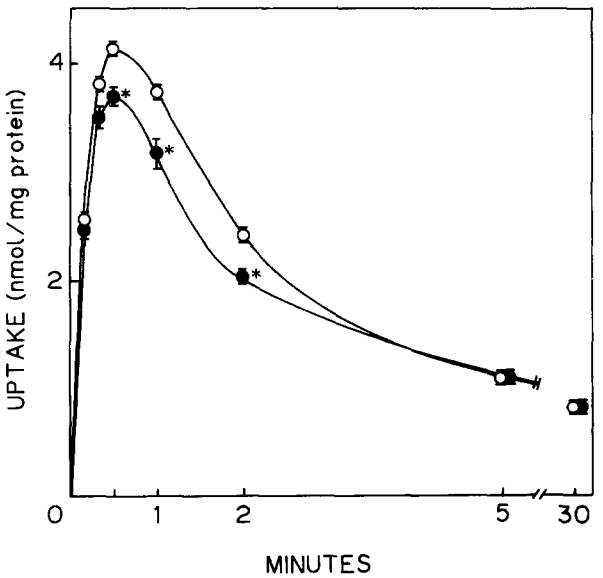

Fig. 1. Effect of ischemia on tetraethylammonium uptake by Brush-border membrane vesicles in the presence of H^+ gradient. Brush-border membrane vesicles (20 μl, 115–182 μg of protein), suspended in 100 mM mannitol, 100 mM KCl and 10 mM Mes (pH 6.0), were incubated at 25°C with the substrate mixture (80 μl) comprising 100 mM mannitol, 100 mM KCl, 10 mM Hepes (pH 7.5) and 0.3125 mM [^{14}C]tetraethylammonium. Membrane vesicles were isolated from control (○) or ischemic (●) kidneys. Each point represents the mean ± S.E. of three separate experiments performed in two to three determinations. * $P < 0.05$, significant difference from control using analysis of variance followed by Fischer's *t*-test.

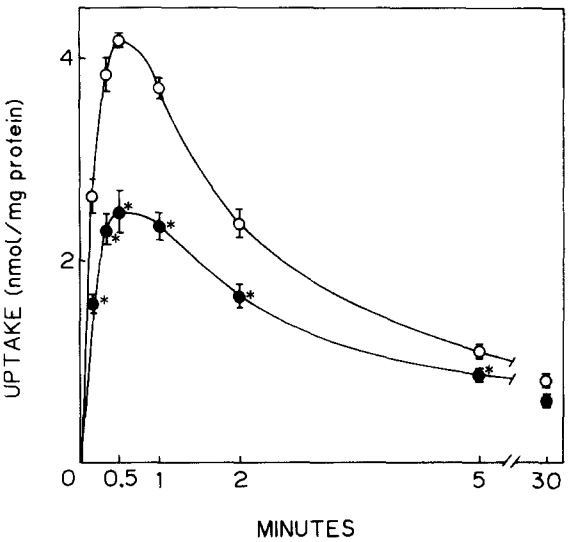

Fig. 2. Effect of reperfusion on tetraethylammonium uptake by brush-border membrane vesicles in the presence of H^+ gradient. Brush-border membrane vesicles (20 μl, 70–172 μg of protein), suspended in 100 mM mannitol, 100 mM KCl and 10 mM Mes (pH 6.0), were incubated at 25°C with the substrate mixture (80 μl) comprising 100 mM mannitol, 100 mM KCl, 10 mM Hepes (pH 7.5) and 0.3125 mM [^{14}C]tetraethylammonium. Membrane vesicles were isolated from control (○) or reperfused (●) kidneys. Each point represents the mean ± S.E. of three separate experiments performed in two or three determinations. * $P < 0.05$, significant difference from control using analysis of variance followed by Fischer's *t*-test.

Fig. 2 shows the tetraethylammonium uptake by brush-border membrane vesicles isolated from the reperfused kidneys. The uptake of tetraethylammonium was measured under the same condition as that in Fig. 1. When compared with the uptake by the vesicles from ischemic kidneys, reperfusion markedly decreased the initial rate and overshoot magnitude of tetraethylammonium uptake.

The uptake of D-glucose by brush-border membrane vesicles isolated from ischemic and reperfused kidneys was measured in the presence of an inward Na^+ gradient. As shown in Fig. 3A, the initial rate and overshoot magnitude of D-glucose uptake decreased in the vesicles isolated from ischemic kidneys compared with the control kidneys. D-Glucose uptake in the presence of an inward K^+ gradient was not different between the two groups. Fig. 3B shows the D-glucose uptake by brush-border membrane vesicles isolated from reper-

fused kidneys. Reperfusion further reduced the initial rate and overshoot magnitude of Na^+-dependent D-glucose uptake more than ischemia did.

Fig. 4A shows curves for the concentration dependence of tetraethylammonium uptake by brush-border membrane vesicles isolated from control and reperfused kidneys. The non-saturable component was evaluated from the tetraethylammonium uptake in the presence of $HgCl_2$ [24]. The Eadie-Hofstee plots of the data after correction for non-saturable component showed a linear relationship in both membrane vesicles (Fig. 4B). Table III summarizes the kinetic parameters; V_{max} is the maximum uptake rate by carrier-mediated process, K_m is the Michaelis constant, and K_d is the coefficient of simple diffusion. The V_{max} value was significantly decreased in brush-border membrane vesicles isolated from reperfused kidneys compared with that in control vesicles. On the other hand, there was no change in K_m and K_d values.

To investigate whether the decreased tetraethylammonium uptake by brush-border membrane vesicles from reperfused kidneys was due to a defect of the transport carrier, or a change in the dissipation rate of an H^+ gradient (a driving force), we measured the specific tetraethylammonium uptake in the presence or absence of an H^+ gradient. The specifically mediated uptake was evaluated by subtracting the uptake in the presence of cimetidine, a potent inhibitor on tetraethylammonium transport in the brush-border membrane [16], from that in the absence of cimetidine. As

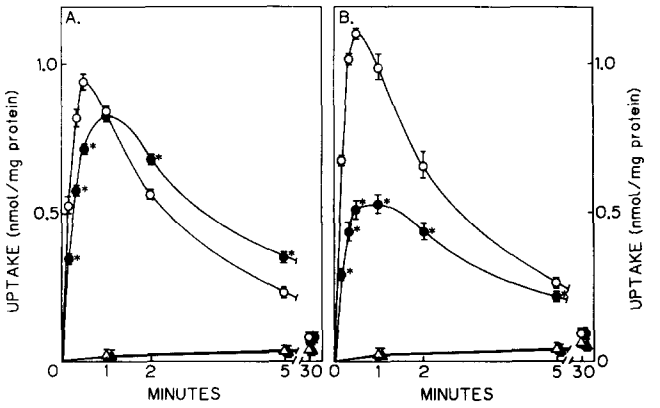

Fig. 3. (A) Effect of ischemia on D-glucose uptake by brush-border membrane vesicles. Brush-border membrane vesicles (20 μl, 124–126 μg of protein), suspended in 300 mM mannitol and 10 mM Hepes (pH 7.5), were incubated at 25°C with the substrate mixture (20 μl) comprising 100 mM mannitol, 10 mM Hepes (pH 7.5), 200 mM NaCl (○, ●) or KCl (△, ▲), and 0.1 mM D-[^{3}H]glucose. Membrane vesicles were isolated from control (○, △) or ischemic (●, ▲) kidneys. Each point represents the mean ± S.E. of three determinations from a typical experiment. * $P < 0.05$, significant difference from control using analysis of variance followed by Fischer's t-test. (B) Effect of reperfusion on D-glucose uptake by brush-border membrane vesicles. Brush-border membrane vesicles (20 μl, 53–128 μg of protein), suspended in 300 mM mannitol and 10 mM Hepes (pH 7.5), were incubated at 25°C with the substrate mixture (20 μl) comprising 100 mM mannitol, 10 mM Hepes (pH 7.5), 200 mM NaCl (○, ●) or KCl (△, ▲), and 0.1 mM D-[^{3}H]glucose. Membrane vesicles were isolated from control (○, △) or reperfused (●, ▲) kidneys. Each point represents the mean ± S.E. of three separate experiments performed in three determinations except that the points with 100 mM KCl represent the mean ± S.E. of three determinations from a typical experiment. * $P < 0.05$, significant difference from control using analysis of variance followed by Fischer's t-test.

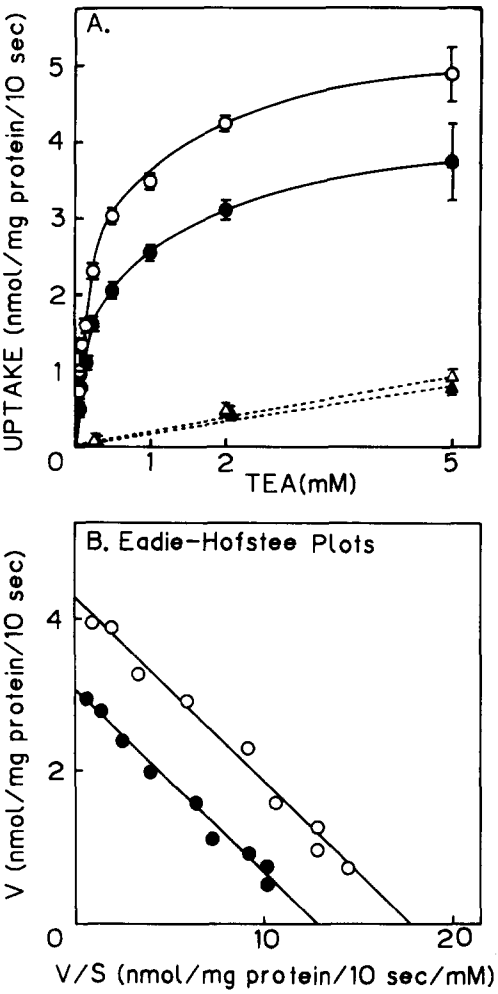

Fig. 4. Concentration dependence of tetraethylammonium uptake by brush-border membrane vesicles. Brush-border membrane vesicles (20 μl, 66–91 μg of protein), suspended in 100 mM mannitol, 100 mM KCl and 10 mM Mes (pH 6.0), were incubated at 25°C with the substrate mixture (80 μl) comprising 100 mM mannitol, 100 mM KCl, 10 mM Hepes (pH 7.5), and [^{14}C]tetraethylammonium. (A) The uptake of tetraethylammonium (TEA) for 10 s at concentrations between 0.05 and 5 mM was determined by brush-border membrane vesicles isolated from control (○, △) and reperfused (●, ▲) kidneys. Solid lines indicate the total uptake (○, ●), and broken lines indicate the non-saturable uptake (△, ▲), which was evaluated from tetraethylammonium uptake in the presence of 0.1 mM $HgCl_2$. Each point represents the mean ± S.E. of three determinations from a typical experiment. (B) Eadie-Hofstee plots of tetraethylammonium uptake after correction for the non-saturable component. Brush-border membrane vesicles were isolated from control (○) and reperfused (●) kidneys.

TABLE III

Kinetic parameters of tetraethylammonium uptake by brush-border membrane vesicles from control and reperfused kidneys

The K_m and V_{max} values were calculated from the Eadie-Hofstee plots and K_d values from tetraethylammonium uptake in the presence of $HgCl_2$. Each value represents the mean ± S.E. of three separate experiments performed in three determinations.

Vesicles	K_m (mM)	V_{max} (nmol/mg protein per min)	K_d (nmol/mg protein per min per mM)
Control	0.21 ± 0.02	23.0 ± 1.3	1.25 ± 0.09
Reperfused	0.24 ± 0.01	16.1 ± 1.3 *	1.09 ± 0.21

* $P < 0.05$, significant difference from control using analysis of variance followed by Fischer's t-test.

shown in Fig. 5, tetraethylammonium uptake by brush-border membrane vesicles from reperfused kidneys decreased both in the presence and absence of the H^+ gradient.

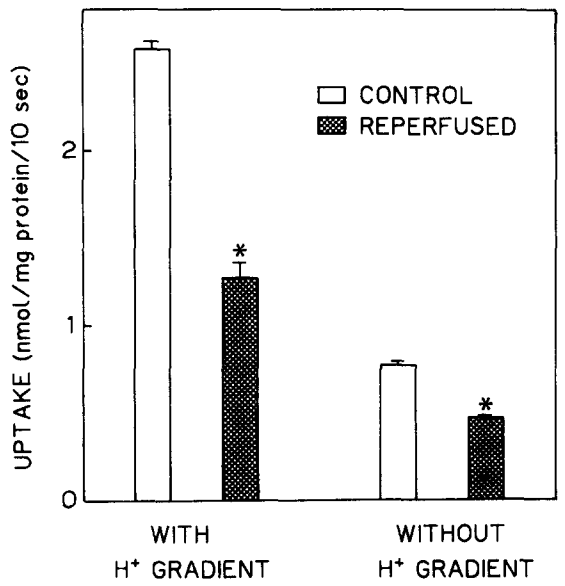

Fig. 5. Effect of reperfusion on tetraethylammonium uptake by brush-border membrane vesicles in the presence or absence of an H^+ gradient. Brush-border membrane vesicles (20 μl, 74–162 μg of protein), suspended in 100 mM mannitol, 100 mM KCl and either 10 mM Mes (pH 6.5) or 10 mM Hepes (pH 7.5), were incubated at 25°C with the substrate mixture (80 μl) comprising 100 mM mannitol, 100 mM KCl, 10 mM Hepes (pH 7.5) and 0.625 mM [^{14}C]tetraethylammonium, with or without 0.625 mM cimetidine. Final concentrations: 0.5 mM [^{14}C]tetraethylammonium and 0.5 mM cimetidine. Membrane vesicles were isolated from control (open column) or reperfused (dotted column) kidneys. Specifically mediated uptake of tetraethylammonium was calculated by subtracting the uptake in the presence of cimetidine from that in the absence of cimetidine under each uptake condition. Each column represents the mean ± S.E. of six determinations from two separate experiments. * $P < 0.05$, significant difference from control using analysis of variance followed by Fischer's t-test.

Discussion

A significant component of ischemic cellular injury develops with restoration of blood flow [25]. During reperfusion, various changes occur, such as the disturbance of cellular calcium homeostasis [26] and enhanced generation of oxygen free radicals [27], which may have adverse effects on cellular membranes. The brush-border membrane of the proximal tubular cell is the earliest and most prominent site of morphologic damage in renal ischemia [2]. We examined the effect of ischemia (50 min) and reperfusion (15 min) on tubular transport of tetraethylammonium, an organic cation, and D-glucose by using isolated brush-border membrane vesicles.

Failure of cells to maintain ATP levels is the hallmark of ischemia [28]. In this study, the ATP concentration measured in the renal cortex decreased to 25% of the preischemic values after 20 min of ischemia and remained low up to 50 min. The activities of brush-border membrane enzymes and oxygen consumption rates in the tubules are reportedly lowered to the lowest levels after 15 min of reflow of blood [29,30]. In addition, loss of brush-border and increased number of cytoplasmic vacuoles are evident after 15 min of reperfusion [3,4]. These findings suggest that the maximum damage occurs at 15 min after reperfusion and therefore 15 min of reperfusion was chosen in the present study.

The specific activities of γ-glutamyltransferase and aminopeptidase were reduced in homogenate and brush-border membranes isolated from ischemic and reperfused kidneys. These data are compatible with those reported by others [19,29]. The specific activities of these enzymes in brush-border membranes were increased about 10-fold or more relative to those in the homogenate, but the apparent purity of the membranes isolated from reperfused kidneys was slightly lower than that from control kidneys. In addition, the amount of brush-border membrane protein recovered from reperfused kidneys was reduced to a half of that from control kidneys. As Venkatachalam et al. [31] showed, the brush-border microvilli, fused with one another during ischemia, may have been interiorized into the proximal tubule cytoplasm soon after reflow of blood following ischemia. Another possibility is that reperfusion altered the composition of the brush-border membrane, which may affect the isolation procedures.

Recently, ischemia has been proposed to induce disruption of the cortical actin filament network, opening of cellular tight junctions, and loss of surface membrane protein and lipid polarity [32,33]. Redistribution of Na^+/K^+-ATPase (basolateral marker enzyme) into the apical membrane during ischemia occurs rapidly and in a duration-dependent fashion [34]. In the present study, the specific activity of Na^+/K^+-ATPase in

the ischemic brush-border membrane tended to be higher than that in the control membrane, though not significantly. In the reperfused brush-border membrane, the specific activity and enrichment of Na^+/K^+-ATPase were significantly increased. Spiegel et al. [35] reported that the enrichment of Na^+/K^+-ATPase in the brush-border membrane was increased during the reperfusion period.

There are few reports on the effect of renal ischemia on drug transport. In the present study, we examined the effect of ischemia and reperfusion on the transport of tetraethylammonium, a typical organic cation, in the brush-border membrane. Tetraethylammonium uptake by ischemic vesicles slightly decreased compared with that by control vesicles. In contrast to the ischemic vesicles, the tetraethylammonium uptake by brush-border membrane vesicles isolated from reperfused kidneys was decreased more markedly. To further obtain the information about the mechanism underlying the dysfunctioned tetraethylammonium transport by reperfusion, the concentration dependence of tetraethylammonium uptake was studied. The Eadie-Hofstee plots were linear in brush-border membrane vesicles isolated from control and reperfused kidneys, indicating the existence of one mode of H^+/organic cation antiport system in both cases. Kinetic analysis showed that the V_{max} value was decreased in the vesicles from reperfused kidneys without any change in K_m value. Further, we measured the tetraethylammonium uptake in the presence or absence of an outward H^+ gradient (a driving force). Tetraethylammonium uptake by the vesicles from reperfused kidneys decreased under both experimental conditions. Taken together, these results may indicate a defect of the membrane carrier for tetraethylammonium transport. However, because the extent of the decrease of tetraethylammonium uptake was somewhat larger when the uptake was measured in the presence of the H^+ gradient, the dissipation rate of the H^+ gradient may also be facilitated. We have previously shown that the decreased transport of tetraethylammonium in brush-border membrane vesicles isolated from rats with uranyl nitrate-induced acute renal failure was due to a defect of the transport carrier rather than a change in the dissipation rate of the H^+ gradient [36]. Therefore, ischemia-reperfusion may induce more widespread dysfunctions on the brush-border membrane than uranyl nitrate.

Na^+ gradient-dependent D-glucose uptake decreased in ischemic brush-border membrane vesicles, which is consistent with the observations by Molitoris and Kinne [37]. Our findings showed that D-glucose transport was more susceptible to ischemic injury than tetraethylammonium transport (Figs. 1 and 3A), which indicated that the susceptibility to ischemic injury is different among various transport systems. In addition, reperfusion further decreased tetraethylammonium uptake as well as D-glucose uptake compared with ischemia alone. Thus, drastic dysfunction in the brush-border membrane by ischemic renal failure should be induced during reperfusion rather than during ischemia itself.

In conclusion, renal ischemia decreased the transport of tetraethylammonium as well as D-glucose in brush-border membrane vesicles, but to different extents. Furthermore, reperfusion after ischemia resulted in severer alterations in the membrane enzymes and transport systems than ischemia alone. These findings should be useful for further investigation of tubular transport of drugs in ischemic renal failure.

Acknowledgment

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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