Biochimica et Biophysica Acta, 769 (1984) 449–454
Elsevier

BBA 71938

CARRIER-MEDIATED TRANSPORT OF CEPHALEXIN VIA THE DIPEPTIDE TRANSPORT SYSTEM IN RAT RENAL BRUSH-BORDER MEMBRANE VESICLES

KEN-ICHI INUI, TOMONOBU OKANO, MIKIHISA TAKANO, HIDEYUKI SAITO and RYOHEI HORI *

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

(Received August 30th, 1983)

Key words: Brush-border membrane; Countertransport; Cephalexin; Aminocephalosporin antibiotic; Dipeptide transport; (Rat kidney)

Carrier-mediated transport of aminocephalosporin antibiotics by renal brush-border membrane vesicles has been studied in relation to the transport systems for dipeptides and amino acids. Dipeptides such as L-carnosine (β-alanyl-L-histidine) and L-phenylalanylglycine competitively inhibited the uptake of cephalexin, but amino acids did not. Cephalexin uptake was stimulated by the countertransport effect of L-carnosine in the normal and papain-treated vesicles, and by the effect of L-phenylalanylglycine only in the papain-treated vesicles. In the papain-treated vesicles, the hydrolysis of dipeptides was markedly decreased, and the specific activity for cephalexin transport was increased approx. 2-fold because of the partial removal of membrane proteins. These results suggest that carrier-mediated transport of cephalexin can be transported by the system for dipeptides in renal brush-border membranes.

Introduction

The cephalosporin antibiotics, the most widely used antimicrobial agents, are excreted principally by the kidney. In a preceding paper [1], we have reported the existence of a carrier-mediated transport system for aminocephalosporin antibiotics such as cephalexin and cephradine in brush-border membranes isolated from rat renal cortex. This transport system is saturable, is inhibited by structural analogs and sulfhydryl reagents, undergoes a countertransport effect, and is different from the transport system for *p*-aminohippurate [2]. Aminocephalosporins are amphoteric molecules, having both amino and carboxyl groups, and are completely ionized at the physiological pH. It is well known that renal brush-border membranes contain active and/or facilitated transport systems for dipeptides [3–6] as well as amino acids [7]. Since there are some physicochemical similarities between aminocephalosporins and nutrients such as amino acids and dipeptides, nutrient transport mechanisms may be available for the transport of aminocephalosporins. The present study was undertaken to determine whether the carrier-mediated transport of cephalexin could be facilitated via carrier transport mechanisms of amino acids and/or dipeptides in brush-border membranes. The results suggest that cephalexin can be transported by dipeptide carrier across the renal brush-border membranes.

Materials and Methods

Materials

Cephalexin was kindly supplied from Shionogi & Co. (Osaka, Japan). Glycylglycine, glycyl-L-leucine, glycyl-L-phenylalanine, glycyl-L-proline and L-carnosine (β-alanyl-L-histidine) were

* To whom correspondence should be addressed.

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

purchased from Peptide Institute Protein Research Foundation (Osaka, Japan). L-Phenylalanylglycine and papain (from papaya latex Type III) were obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation of brush-border membrane vesicles

Brush-border membrane vesicles were isolated from the renal cortex of male Wistar albino rats (190–230 g) as described previously [1,2], and were suspended in a buffer comprising 100 mM mannitol/20 mM Hepes-Tris (pH 7.5) (buffer A). In the preparation of papain-treated brush-border membranes, papain (53.1 unit/ml) was activated in buffer A/1 mM EDTA/5 mM cysteine for 30 min at room temperature under bubbling with nitrogen gas [8]. Papain digestion of the vesicles (0.05 unit/mg protein) was carried out at 37°C for 30 min in the same buffer. The mixture was diluted 5-fold with ice-cold buffer A, and centrifuged at $30000 \times g$ for 30 min. The pellet was washed in the same volume of buffer A and centrifuged again. The final pellet was suspended in buffer A and used for transport studies.

Transport studies

The uptake of cephalexin was measured by a rapid filtration technique detailed previously [1], with a modification in the ice-cold solution to stop the reaction and to wash the filter as follows; 200 mM NaCl/20 mM Hepes-Tris (pH 7.5)/0.1 mM $HgCl_2$ instead of buffer A. Based on the previous results, a stop solution containing sulfhydryl reagent was used in order to minimize the loss of cephalexin from vesicles before filtration. By this modification, cephalexin entrapped on the filter was increased approx. 60%.

Analytical methods

Cephalexin was analyzed by a high-pressure liquid chromatograph LC-3A (Shimadzu Co., Kyoto, Japan) with Chemcosorb ODS 15 cm × 4.6 mm (Chemco Scientific Co., Ltd., Osaka, Japan) as previously described [1]. Protein was determined, after precipitation with ice-cold 10% (w/v) trichloroacetic acid, by the method of Lowry et al. [9] with bovine serum albumin as a standard. Amino acids and dipeptides were determined by a high-pressure liquid chromatograph LC-3A amino acid analysis system (Shimadzu Co., Kyoto, Japan) equipped with ISC-07/S1504 cation-exchange column, step gradient unit SGR-1A, fluorescence spectromonitor RF-530 (post-column mode with *o*-phthalaldehyde). The conditions were as follows: mobile phase, 0.07 M sodium citrate (pH 4.25) and 0.2 M sodium citrate (pH 7.0); flow rate, 0.5 ml/min; temperature, 55°C; wavelengths, excitation 348 nm, emission 450 nm.

Results

Effect of amino acids and dipeptides on cephalexin uptake

The effect of amino acids and dipeptides (25 mM) on the initial uptake of cephalexin (2.5 mM)

TABLE I

EFFECT OF AMINO ACIDS (A) AND DIPEPTIDES (B) ON CEPHALEXIN UPTAKE BY BRUSH-BORDER MEMBRANE VESICLES

The vesicles (20 μl, (A) 140 μg protein, (B) 188 μg protein) were incubated at 25°C for 1 min with the substrate mixture (20 μl) containing 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 200 mM NaCl, 5 mM cephalexin and either 50 mM amino acid or dipeptide. Final concentration: 100 mM NaCl, 2.5 mM cephalexin, 25 mM amino acid or dipeptide. Each value represents the mean ± S.E. of 3–6 determinations except for duplicates for amino acids.

Addition		Cephalexin uptake	
		nmol/mg protein per min	%
(A)	Amino acids		
	None	2.35 ± 0.04	100
	Glycine	2.54 (2.44, 2.64)	108
	L-Proline	2.41 (2.18, 2.64)	103
	L-Glutamic acid	2.26 (2.18, 2.33)	96
	L-Phenylalanine	2.26 (2.03, 2.49)	96
(B)	Dipeptides		
	None	2.20 ± 0.07	100
	Glycylglycine	2.08 ± 0.08	95
	L-Phenylalanylglycine	1.82 ± 0.02 [a]	83
	Glycyl-L-leucine	1.72 ± 0.03 [b]	78
	Glycyl-L-phenylalanine	1.70 ± 0.18 [c]	77
	Glycyl-L-proline	1.64 ± 0.11 [b]	75
	L-Carnosine	1.33 ± 0.04 [d]	60

[a] $P < 0.01$;
[b] $P < 0.005$;
[c] $P < 0.025$;
[d] $P < 0.001$, when compared with control value.

by brush-border membrane vesicles is summarized in Table I. A number of dipeptides, except glycylglycine, showed a significant inhibitory effect on cephalexin uptake, although the uptake was not inhibited by free amino acids. The inhibitory effect of dipeptides was similar in the presence of NaCl or KCl gradient (data not shown). The results suggest that cephalexin and dipeptides may be transported by a common system in brush-border membranes.

Kinetic analysis of cephalexin uptake and inhibition by dipeptides

In order to characterize the inhibition by dipeptides, kinetic analysis of cephalexin uptake and its inhibition by L-carnosine or L-phenylalanylglycine were examined. In Fig. 1 are shown Lineweaver-Burk plots of the initial rates of cephalexin uptake at substrate concentrations between 1 and 20 mM in the presence or absence of dipeptides (20 mM). Uptake rate was corrected for the nonsaturable component as described previously [1]. The values of K_m and V_{max} for the control experiment were 2.6 mM and 3.6 nmol/mg protein per min, respectively. Because of a modification in the stop solution for transport assay, the value of V_{max} appeared to increase in comparison with the previous results [1]. The inhibition of cephalexin uptake by L-carnosine and L-phenylalanylglycine was competitive, with apparent K_i values of 18.4 and 22.9 mM, respectively.

Hydrolysis of dipeptides by normal and papain-treated vesicles

In order to estimate the extent of dipeptide hydrolysis during the uptake experiments, the hydrolysis of glycylglycine, L-phenylalanylglycine and L-carnosine by the normal and papain-treated brush-border membranes was studied. As shown in Fig. 2, L-carnosine was extremely resistant to the hydrolysis compared with glycylglycine and L-phenylalanylglycine [6]. With normal vesicles, the hydrolysis of glycylglycine and L-phenylalanylglycine were 4 and 11% for 1 min, and 92 and 87% for 30 min, respectively. However, papain treatment allowed almost complete absence of dipeptide hydrolysis, consistent with the results of Berteloot et al. [10,11]. Therefore, the lack of inhibitory effect of glycylglycine in Table I may be due to low affinity for the dipeptide transport

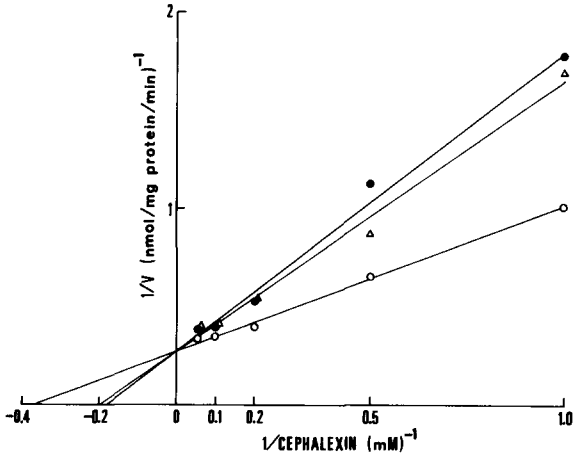

Fig. 1. Lineweaver-Burk plot of cephalexin uptake by brush-border membrane vesicles. Cephalexin uptake for 1 min at concentrations between 1 and 20 mM was determined in the presence of 20 mM dipeptide as described for Table I: control (○), L-phenylalanylglycine (△) and L-carnosine (●). Uptake rate was corrected for the nonsaturable component as described previously [1]. Each point represents the mean ± S.E. of triplicate determinations.

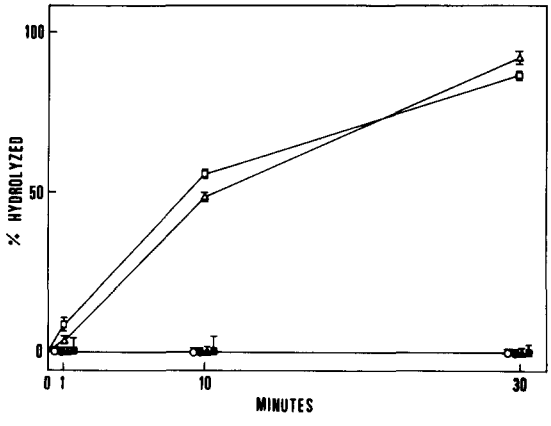

Fig. 2. Hydrolysis of dipeptides by normal and papain-treated brush-border membrane vesicles. The vesicles (50 μl, normal 349 μg protein, papain-treated 184 μg protein) were incubated at 25°C with the substrate mixture (50 μl) containing 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 200 mM NaCl and 50 mM dipeptide. At the stated time points, the incubation was stopped by adding 100 μl of ice-cold 10% trichloroacetic acid. Normal vesicles (open symbol), papain-treated vesicles (closed symbol); glycylglycine (△, ▲); L-phenylalanylglycine (□, ■); L-carnosine (○, ●). Each point represents the mean ± S.E. of triplicate determinations.

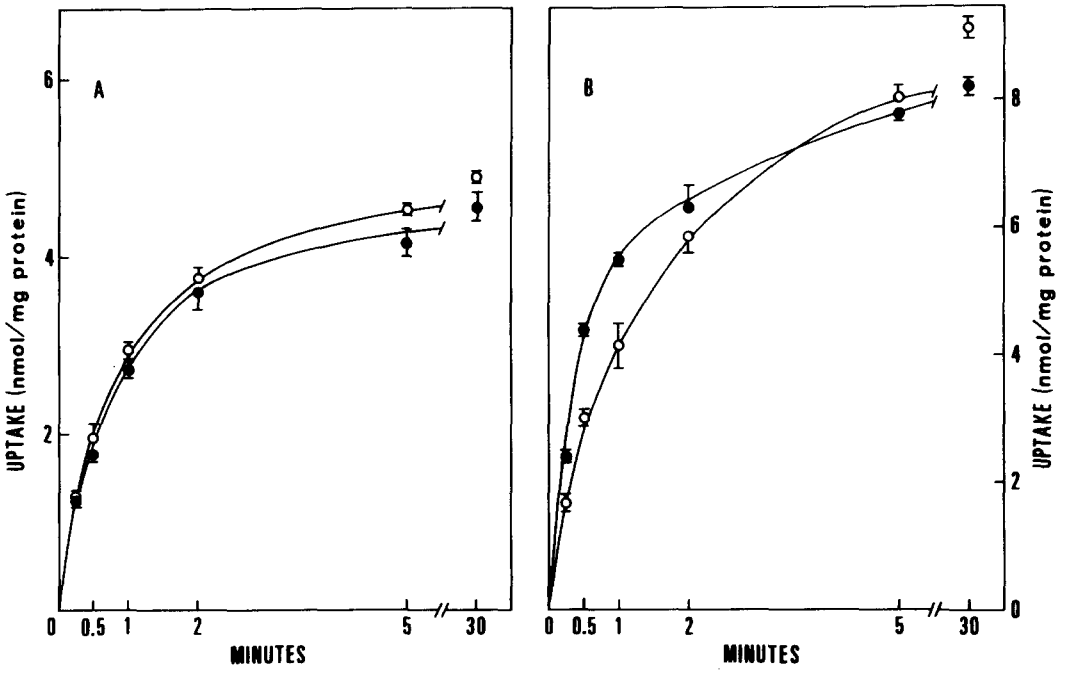

Fig. 3. Countertransport effect of L-phenylalanylglycine on cephalexin uptake by normal (A) and papain-treated (B) brush-border membrane vesicles. The vesicles were preincubated in 100 mM mannitol and 20 mM Hepes-Tris (pH 7.5), with (●) or without (○) 25 mM L-phenylalanylglycine for 30 min, and then aliquots (20 μl) were incubated with the substrate mixture (200 μl) containing 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 100 mM NaCl and 2.5 mM cephalexin. Each point represents the mean ± S.E. of triplicate determinations.

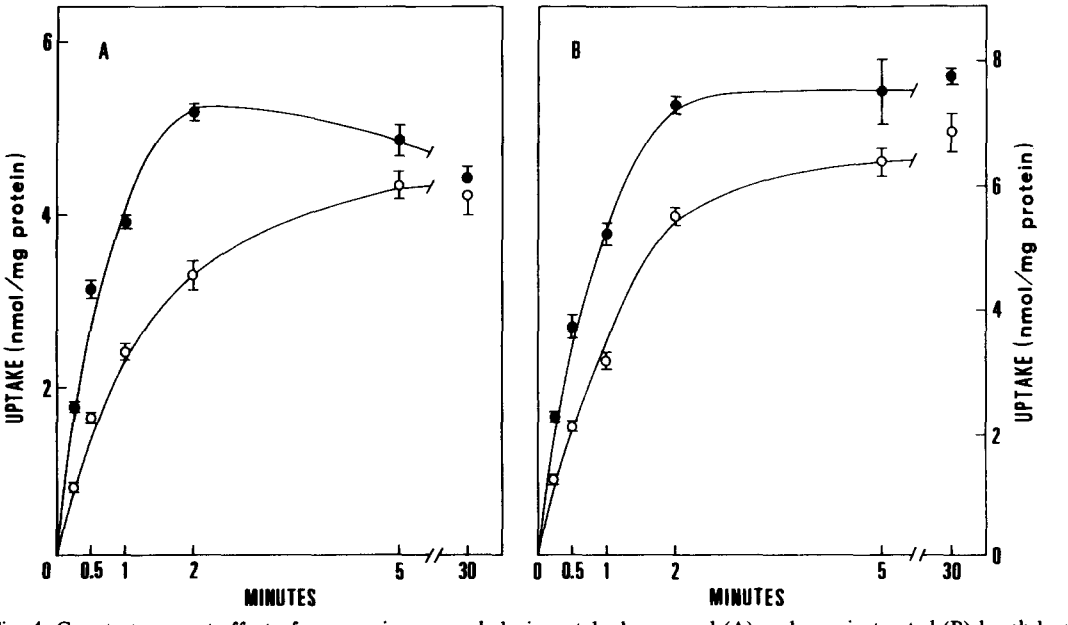

Fig. 4. Countertransport effect of L-carnosine on cephalexin uptake by normal (A) and papain-treated (B) brush-border membrane vesicles. The vesicles were preincubated in 100 mM mannitol and 20 mM Hepes-Tris (pH 7.5), with (●) or without (○) 25 mM L-carnosine for 30 min, and then aliquots (20 μl) were incubated with the substrate mixture (200 μl) containing 100 mM mannitol, 20 mM Hepes-Tris (pH 7.5), 100 mM NaCl and 2.5 mM cephalexin. Each point represents the mean ± S.E. of triplicate determinations.

system, rather than hydrolysis of this dipeptide [4,6].

Countertransport effect of dipeptides on cephalexin uptake by normal and papain-treated vesicles

Furthermore, in order to confirm the existence of a common carrier transport system between cephalexin and dipeptide in brush-border membranes, we studied the countertransport effect of dipeptide on cephalexin uptake in the normal and papain-treated vesicles. In the case of L-phenylalanylglycine preload, there was observed the enhancement of cephalexin uptake in the papain-treated vesicles, with no enhancement in the normal vesicles (Fig. 3). The lack of countertransport effect in the normal vesicles is probably due to the hydrolysis of L-phenylalanylglycine during the preincubation of 30 min, in contrast to little hydrolysis of L-phenylalanylglycine in the papain-treated vesicles. Therefore, we also examined the countertransport effect of L-carnosine, which was extremely resistant to the hydrolysis by brush-border membranes. As shown in Fig. 4, the normal and papain-treated vesicles preloaded with L-carnosine clearly showed transient enhancement of cephalexin uptake. Although higher values of cephalexin uptake were obtained in the papain-treated vesicles compared with the normal vesicles (Figs. 3 and 4), the increase of the specific activity closely matched the extent of protein removal during papain digestion (40–50% removal). These results suggest that the cephalexin and dipeptides can share a common carrier-mediated transport system, and that papain digestion seems not to remove any protein essential in this transport system.

Discussion

Recent studies show that dipeptide transport in brush-border membranes is Na^+-independent, carrier-mediated and may be a passive process [3–6,10–13]. As described previously [1], carrier-mediated cephalexin uptake is also an Na^+-independent process, and therefore is compatible with the dipeptide transport system.

From the curves for the concentration dependence of cephalexin uptake, the contributions of carrier-mediated system and nonsaturable system on cephalexin uptake at 2.5 mM for 1 min were approx. 65 and 35%, respectively. Based on our previous results of cephalexin [1] and *p*-aminohippurate [2] uptake by brush-border membrane vesicles, the nonsaturable uptake of cephalexin could be estimated as the uptake by an organic anion transport system and simple diffusion.

In the papain-treated vesicles, carrier-mediated transport system for cephalexin seemed not to be altered, although the hydrolysis of dipeptides was markedly decreased. Our results are compatible with those of Berteloot et al. [10,11] and Ganapathy et al. [5,12], who have reported that the papain-treated brush-border membrane vesicles are useful for studying the transport of intact dipeptides without interference from hydrolytic events.

In the renal handling of cephalexin by the clearance technique in vivo, we have reported that the mechanisms responsible for the elimination are glomerular filtration, tubular secretion and tubular reabsorption, and that the degree of cephalexin reabsorption was much larger than the estimate based on its lipid solubility [14]. Therefore, carrier-mediated transport of cephalexin via the dipeptide transport system may contribute to tubular reabsorption of cephalexin in vivo. Thus, it is very interesting to note that a foreign compound such as cephalexin can be transported via the nutrient reabsorption system in renal brush-border membranes.

In conclusion, we have demonstrated that carrier-mediated transport of aminocephalosporins such as cephalexin can share a common carrier transport system with dipeptides in renal brush-border membranes.

Acknowledgement

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

References

1 Inui, K., Okano, T., Takano, M., Kitazawa, S. and Hori, R. (1983) Biochem. Pharmacol. 32, 621–626
2 Hori, R., Takano, M., Okano, T., Kitazawa, S. and Inui, K. (1982) Biochim. Biophys. Acta 692, 97–100
3 Ganapathy, V., Mendicino, J., Pashley, D.H. and Leibach,

F.H. (1980) Biochem. Biophys. Res. Commun. 97, 1133–1139
4 Ganapathy, V., Mendicino, J.F. and Leibach, F.H. (1981) J. Biol. Chem. 256, 118–124
5 Ganapathy, V., Mendicino, J. and Leibach, F.H. (1981) Biochim. Biophys. Acta 642, 381–391
6 Ganapathy, V. and Leibach, F.H. (1982) Biochim. Biophys. Acta 691, 362–366
7 Murer, H. and Kinne, R. (1980) J. Membrane Biol. 55, 81–95
8 Klip, A., Grinstein, S. and Semenza, G. (1979) FEBS Lett. 99, 91–96
9 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
10 Berteloot, A., Khan, A.H. and Ramaswamy, K. (1981) Biochim. Biophys. Acta 649, 179–188
11 Berteloot, A., Khan, A.H. and Ramaswamy, K. (1982) Biochim. Biophys. Acta 686, 47–54
12 Ganapathy, V., Mendicino, J.F. and Leibach, F.H. (1981) Life Sci. 29, 2451–2457
13 Ganapathy, V. and Leibach, F.H. (1982) Life Sci. 30, 2137–2146
14 Kamiya, A., Okumura, K. and Hori, R. (1983) J. Pharm. Sci. 72, 440–443