Biochimica et Biophysica Acta, 943 (1988) 288–296
Elsevier

BBA 74094

Inactivation of the intestinal uptake system for β-lactam antibiotics by diethylpyrocarbonate

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(Received 9 November 1987)
(Revised manuscript received 29 March 1988)

Key words: β-Lactam antibiotic; Transport system; Diethylpyrocarbonate; Brush-border membrane vesicle; (Rabbit small intestine)

The uptake system for β-lactam antibiotics in the rabbit small intestine was investigated using brush-border membrane vesicles. After treatment of membrane vesicles with the reagent diethylpyrocarbonate (DEP), the uptake of orally active β-lactam antibiotics with an α-amino group in the substituent at position 6 or 7 of the penam or cephem nucleus was significantly inhibited, whereas DEP-treatment had no inhibitory effect on the uptake of β-lactam antibiotics without an α-amino group. The kinetic analysis revealed an apparent competitive inhibition indicating a decreased affinity of the transport system for α-amino-β-lactam antibiotics. Substrates of the intestinal dipeptide transport system – dipeptides and α-amino-β-lactam antibiotics – could protect the transport system from irreversible inhibition by DEP, whereas β-lactam antibiotics without an α-amino group as well as amino acids or bile acids had no effect. Incubation of DEP-treated vesicles with hydroxylamine led to a partial restoration of the transport activity indicating that DEP may have led to a modification of a histidine residue of the transport protein. From the data presented we conclude that a specific interaction of the α-amino group in the substituent at position 6 or 7 of the penam or cephem nucleus presumably with a histidine residue of the transport protein is involved in the translocation process of orally active α-amino-β-lactam antibiotics across the intestinal brush-border membrane.

Introduction

β-Lactam antibiotics are taken up by the small intestine to various extents depending upon their structure [1]. β-Lactam antibiotics with an α-amino group in the substituent at position 6 or 7 of the cephem or penam nucleus are well absorbed from the intestinal lumen and are taken up by the intestinal transport system for dipeptides [2–6]. On the other side, most modern β-lactam antibiotics with high antibacterial activity are barely absorbed from the gastrointestinal tract [1]. Evidence has been presented that orally active β-lactam antibiotics share the nutrient transport systems for dipeptides in the brush-border membranes of the small intestine and the kidney [2–7]. The intestinal transport system for β-lactam antibiotics and dipeptides in the small intestine of rats and rabbits was recently characterized by photoaffinity labeling and a membrane protein with an apparent molecular weight of 127000 was identified as a specific binding protein for β-lactam antibiotics and dipeptides [8–11]. It was demonstrated that

Abbreviations: DEP, diethylpyrocarbonate; HPLC, high pressure liquid chromatography; Tris, trishydroxyaminomethane; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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all β-lactam antibiotics, whether they are enterally absorbed or not, bind from the luminal side to the dipeptide transport system. Hitherto unknown structural features determine whether a β-lactam antibiotic molecule bound to the transport system is also translocated across the intestinal brush border membrane. The orally active α-amino-β-lactam antibiotics are transported across the intestinal brush-border membrane by a transport system, which is stimulated by an inward-directed H^+ gradient ($pH_{out} < pH_{in}$) [4,5]. Histidine residues at the active site of the carrier protein are essential for various H^+-dependent transport processes [12–16]. Recently, it was shown that the H^+-dependent transport system for dipeptides in the rabbit renal brush-border membrane could be inhibited by the group specific reagent diethylpyrocarbonate (DEP). It was demonstrated that a histidine residue of the transport protein is essential for the H^+-dependent uptake process [12]. In the present study we investigated the effect of DEP on the intestinal transport system for orally active β-lactam antibiotics.

Materials and Methods

Materials

Cephalexin, cephradine, cefadroxil, cephalothin, cephaloridine, amoxicillin, ampicillin and benzylpenicillin were purchased from Sigma (Munich, F.R.G.). Cefotaxime (Claforan®) was from Hoechst Aktiengesellschaft (Frankfurt, F.R.G.), Ceftizoxime (Ceftix®) from Fujisawa Pharmaceutical Co. (Osaka, Japan), Cefoperazone (Cefobis®) from Pfizer GmbH (Karlsruhe, F.R.G.) and Cefotiam (Spizef®) from Takeda Chemical Industries (Osaka, Japan). The structural formulas of the different cephalosporins are given in Fig. 1. Amino acids, taurocholate, peptides and diethylpyrocarbonate were obtained from Serva (Serva Heidelberg, F.R.G.). [^{3}H]Benzylpenicillin (specific radioactivity 15–18 Ci/mmol) was from Amersham Buchler GmbH (Braunschweig, F.R.G.). Cellulose nitrate filters (type HAWP 0.45 μm, 25 mm diameter) for the transport studies were from Millipore (Eschborn, F.R.G.). Solvents for high pressure liquid chromatography were from Merck (Darmstadt, F.R.G.). All other substances were from commercial sources and of analytical grade.

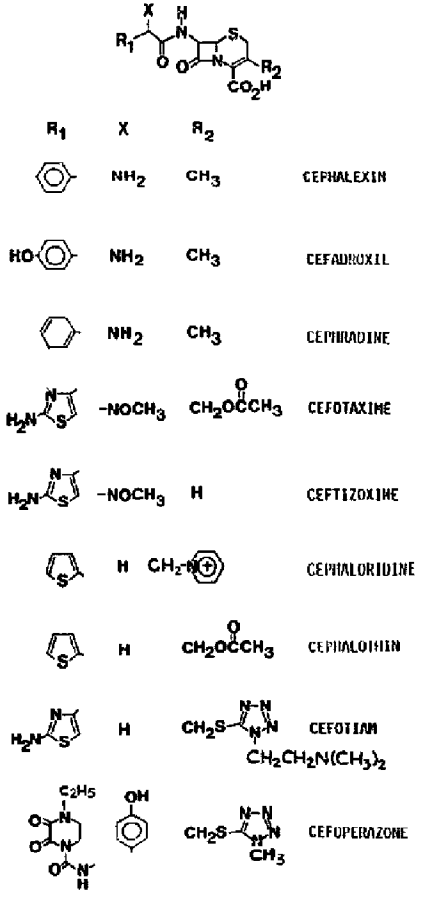

Fig. 1. Structural formulas of cephalosporins.

Animals

White rabbits (3–3.5 kg) (Tierzucht Kastengrund, Hoechst Aktiengesellschaft, Frankfurt, F.R.G.) were maintained on standard diets and tap water ad libitum.

Preparation of brush-border membrane vesicles from rabbit small intestine

Brush-border membrane vesicles from rabbit small intestine were prepared by the Mg^{2+}-

precipitation method as described previously [9,11,19]. The final pellet of the vesicle preparation was suspended in the desired volume of 10 mM Tris-Hepes buffer (pH 7.4)/300 mM mannitol with the aid of a No. 27 gauge needle. The quality of the vesicles was tested by measurement of Na^+-dependent D-glucose uptake. The uptake of 100 μM D-[^{14}C]glucose (1 μCi/sample) in 20 mM Tris-Hepes buffer (pH 7.4)/140 mM NaCl was measured in the presence of an inward Na^+ gradient. The uptake at 15 s showed an 24–35-fold overshoot compared to equilibrium. The vesicles were stored in 4 mg aliquots in liquid nitrogen up to 4 weeks without loss in Na^+-dependent D-glucose uptake or loss in marker enzyme activities. Protein concentration was determined according to Bradford [20] using the Bio-Rad kit (Bio-Rad, Munich, F.R.G.). The enrichment of the specific activities of the brush-border marker enzymes leucine aminopeptidase (EC 3.4.11.2) and γ-glutamyltransferase (EC 2.3.2.2) were 26 ± 5-fold and 23 ± 4-fold, respectively.

Transport experiments

Transport studies were performed at 30°C using the membrane filtration technique [21,22] as described previously [9,11,19]. Membrane vesicles were thawed at 37°C and then kept at 0°C. Typically, 20 μl (100 μg) of vesicles were added as a drop on the wall of disposable polyethylene tubes (11 × 70 mm) containing 180 μl of medium. The composition of the media is described in the legends to figures. Uptake was initiated by mixing for 2 s on a Vortex-mixer. At the desired time uptake was stopped by the addition of 1 ml of ice-cold stop solution (10 mM Tris-Hepes (pH 7.4)/140 mM KCl) and the resulting solution was immediately pipetted on to the middle of a prewetted cellulose nitrate filter. After washing with 5 ml of ice-cold stop solution the amount of transported ligand was determined either by liquid scintillation counting of radioactively labeled ligands or by HPLC. For transport experiments with unlabeled substrates a disc of 1 cm was cut out from the filters and the trapped compounds were extracted with 300 μl of water. 200 μl of each probe were subjected to analysis by HPLC using a Waters chromatography system (M 740 data module, M 490 detector, M 680 automated gradient controller, M 501/510 HPLC pumps, M 712 automated sample processor, Waters Instruments, Eschborn, F.R.G.). β-Lactam antibiotics were analyzed with a flow rate of 1 ml/min on a Bischoff 250 × 4 mm column (Bischoff, Leonberg, F.R.G.) filled with LiChrosorb RP 18 (7 μm, Merck, Darmstadt, F.R.G.). The mobile phase was acetonitrile/30 mM sodium phosphate buffer (pH 7.0) with the following ratios: 16:84 for cephalexin 11:89 for cephradine, 5:95 for amoxicillin, 10:90 for ampicillin, 16:84 for cephaloridine and 16:84 for cefoperazone. Cephalosporins were measured at 262 nm and penicillin at 210 nm.

For efflux studies, the membrane vesicles were adjusted to a protein concentration of 10 mg/ml with 20 mM potassium phosphate buffer (pH 6.4)/280 mM mannitol and incubated for 60 min at 30°C with the desired concentration of the respective β-lactam antibiotic. After equilibration time, 10-μl aliquots (100 μg) were pipetted into reaction tubes and mixed with 5 μl (10 mM) of freshly prepared DEP solutions (0.1 ml DEP + 0.1 ml ethanol + 11.3 ml 10 mM potassium phosphate buffer (pH 6.4)/280 mM mannitol) or with 5 μl of 20 mM potassium phosphate buffer (pH 6.4)/280 mM mannitol/0.87% ethanol (for controls). After incubation at 30°C for 10 min, efflux was initiated by mixing of 10 μl of loaded vesicles with 190 μl of 10 mM sodium phosphate buffer (pH 7.4)/300 mM mannitol. At defined time intervals, efflux was stopped by addition of 1 ml of ice-cold stop solution. Determination of β-lactam antibiotics trapped on the filters was performed as described above. In all transport experiments the indicated values are the mean ± S.D. of 3–6 individual determinations using a single membrane preparation. All experiments have been carried out under identical conditions with at least three different membrane preparations.

Treatment of intestinal brush-border membrane vesicles with DEP

Treatment of brush-border membrane vesicles from rabbit small intestine was performed as described [12]. Membrane vesicles (10 mg/ml) were vigorously mixed with freshly prepared solutions of DEP (0.1 ml DEP + 0.1 ml ethanol + 11.3 ml 20 mM potassium phosphate (pH 6.4)/280 mM

mannitol) in the concentration range of 1–20 mM DEP. The ethanol concentration was kept below 2% and in control experiments without DEP-treatment a corresponding amount of ethanol was added to the vesicles. After 10 min of incubation at 20°C, the vesicles were diluted with 35 ml of ice-cold 10 mM Tris-Hepes buffer (pH 7.4)/300 mM mannitol. After centrifugation at 48000 × g for 30 min the vesicles were resuspended in 1.5 ml of 10 mM Tris-Hepes buffer (pH 7.4)/300 mM mannitol and centrifuged again. The resulting pellets were suspended in 10 mM Tris-Hepes buffer (pH 7.4)/300 mM mannitol and adjusted to a protein concentration of 10 mg/ml.

In substrate protection experiments the membrane vesicles were incubated with the protecting substrates in 20 mM potassium phosphate buffer (pH 6.4)/280 mM mannitol for 60 min. Then vesicles were treated with 5 mM of DEP for 10 min, whereas for controls the vesicles were only treated with the corresponding amount of ethanol instead of ethanol. After 10 min the reaction was stopped by dilution with 35 ml of ice-cold 10 mM Tris-Hepes buffer (pH 7.4)/300 mM mannitol and the vesicles were centrifuged and prepared for transport measurements as described above. The protective effect by a substrate was expressed as percentage of uptake into DEP-treated vesicles compared to the corresponding control vesicles.

Results

Effect of DEP on the uptake of β-lactam antibiotics by rabbit small intestinal brush-border membrane vesicles

The uptake of cephalexin into brush-border membrane vesicles from rabbit small intestine in the presence of an inward H^+ gradient (pH_{out} = 6.0, pH_{in} = 7.4) was linear for up to 60 s. Repeated experiments showed that 20, 30 or 60 s incubation time yielded similar results. The initial uptake rates of cephalexin transport into brush-border membrane vesicles from rabbit small intestine in the presence of an inward H^+ gradient was measured in the concentration range up to 30 mM. Fig. 2 shows that the uptake of cephalexin is a saturable transport process obeying Michaelis-Menten kinetics. The maximum uptake rate was 1.8 nmol · mg^{-1} · 30 s^{-1}. The apparent Michaelis-Menten constant K_M was 3.5 mM. Since uptake is similar at 15 and 25 mM cephalexin, a 'diffusional' component of uptake is not present. A correction for 'diffusional' uptake was, therefore, not necessary as opposed to experiments on rat intestine [3,5].

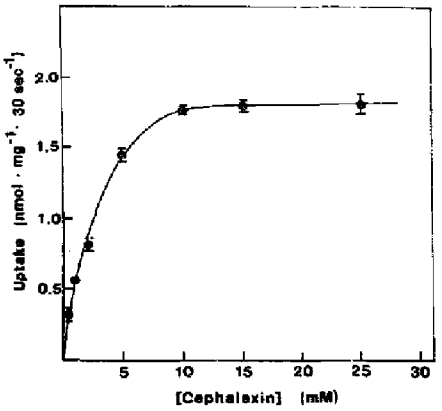

Fig. 2. Concentration dependence of the uptake of cephalexin into brush-border membrane vesicles from rabbit small intestine. The uptake of the indicated concentrations of cephalexin into brush-border membrane vesicles (100 μg) in 10 mM citrate-Tris buffer (pH 6.0)/140 mM KCl was measured for 30 s in the presence of an inward H^+ gradient. The vesicles were preloaded with 10 mM Tris-Hepes buffer (pH 7.4)/300 mM mannitol.

In order to investigate the effect of DEP on cephalexin transport, brush border membrane vesicles from rabbit small intestine were treated with 10 mM DEP at pH 6.4 for 10 min. After washing of the membranes the uptake of the α-aminocephalosporin cephalexin was measured. Fig. 3A shows that the uptake of cephalexin into DEP-treated vesicles in the presence of an inward H^+ gradient was significantly inhibited compared to untreated vesicles. In order to establish whether the inhibition resulted from a dissipating effect of DEP on the H^+ gradient, the uptake of cephalexin into control and DEP-treated vesicles was measured in the absence of an H^+ gradient. Fig. 3B shows that under these conditions transport was also inhibited. The different values at 30 min in control and DEP-treated vesicles do not reflect differences in the intravesicular volumes. Control

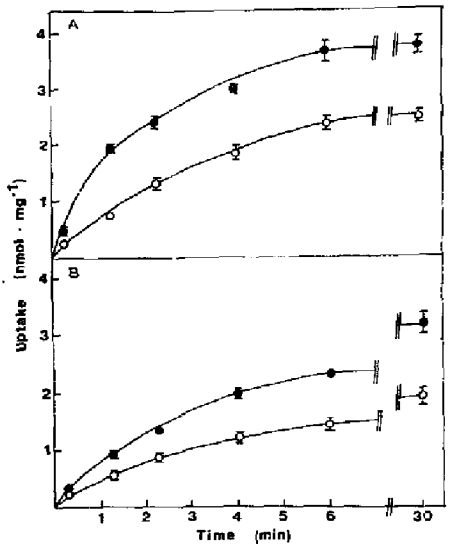

Fig. 3. Effect of DEP on the uptake of cephalexin into intestinal brush-border membrane vesicles. Brush-border membrane vesicles from rabbit small intestine were treated with 10 mM DEP in 20 mM potassium phosphate buffer (pH 6.4)/280 mM mannitol/0.9% ethanol for 10 min. Control vesicles were treated similarly without DEP. After washing, the membrane vesicles (20 µl, 100 µg) loaded with 10 mM Tris-Hepes buffer (pH 7.4)/300 mM mannitol were incubated at 30 °C either with 180 µl of 10 mM citrate-Tris buffer (pH 6.0)/140 mM KCl containing 2 mM cephalexin (A) or 180 µl of 10 mM Tris/HCl buffer (pH 7.4)/140 mM NaCl containing 2 mM cephalexin (B). Uptake was measured as described in the experimental section. ●, Uptake by control vesicles; ○, uptake by DEP-treated vesicles.

experiments have shown that the DEP-treated vesicles are not yet at equilibrium after 30 min. Uptake measurements using 0.5–5 h incubation time have shown that equilibrium in DEP-treated vesicles is reached after 1–2 h of incubation. The kinetic analysis of this inhibition, both in the presence and in the absence of an H^+ gradient, revealed an apparent competitive inhibition (Fig. 4). The DEP-treatment of brush-border membrane vesicles had no effect on the maximal uptake rate, but increased the Michaelis-Menten constant K_M. This indicates that treatment of rabbit small intestinal brush-border membrane vesicles with DEP

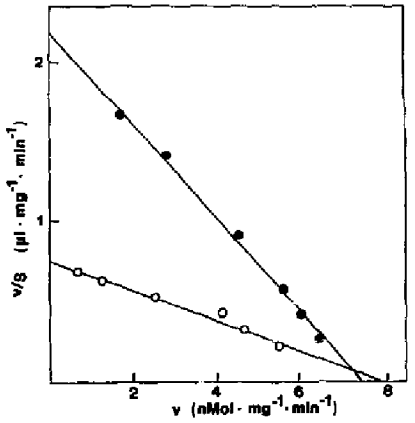

Fig. 4. Effect of DEP on the kinetics of cephalexin uptake by intestinal brush-border membrane vesicles. Brush-border membrane vesicles from rabbit small intestine were treated at 20 °C for 10 min with 5 mM DEP. After washing the uptake of 1, 2, 5, 10, 15 and 25 mM cephalexin was measured in control (●) and DEP-treated (○) vesicles for 1 min in presence of an inward H^+ gradient.

decreased the affinity of the transport system for α-amino-β-lactam antibiotics. Since most orally active β-lactam antibiotics carry an α-amino group

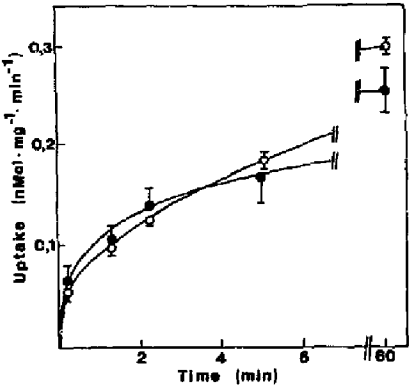

Fig. 5. Effect of DEP on the uptake of [^{3}H]benzylpenicillin by intestinal brush-border membrane vesicles. Brush-border membrane vesicles from rabbit small intestine were treated for 10 min with 10 mM DEP. After washing the uptake of 100 µM (1 µCi/point) was measured into control (●) and DEP-treated (○) vesicles in presence of an inward H^+ gradient.

TABLE I

EFFECT OF DEP ON THE UPTAKE OF VARIOUS β-LACTAM ANTIBIOTICS BY INTESTINAL BRUSH-BORDER MEMBRANE VESICLES

Brush-border membrane vesicles form rabbit small intestine were treated for 10 min with 10 mM DEP. After washing the uptake of 2 mM solutions of the indicated β-lactam antibiotics (5 mM for amoxicillin) into control and DEP-treated vesicles was measured for 1 min in presence of an inward H^+ gradient.

β-Lactam antibiotic	Uptake (nmol·mg^{-1}·min^{-1})	
	Control	DEP-treated
Cephalexin	2.97±0.48	1.62±0.34 (54.5%)
Cephradine	3.30±0.23	1.01±0.21 (30.6%)
Amoxicillin	0.75±0.01	0.28±0.02 (37.3%)
Cefoperazone	0.54±0.05	0.64±0.02 (118%)
Cephaloridine	0.75±0.05	0.95±0.05 (126%)

TABLE II

RESTORATION OF DEP-INDUCED INHIBITION OF H^+-DEPENDENT CEPHALEXIN UPTAKE INTO INTESTINAL BRUSH-BORDER MEMBRANE VESICLES BY HYDROXYLAMINE

Brush-border membrane vesicles from rabbit small intestine were treated with the indicated reagents. After washing and centrifugation the uptake of 2 mM cephalexin was measured for 1 min in the presence of an inward H^+ gradient.

Reagent	Uptake (nmol·mg^{-1}·min^{-1})	% inhibition
None	2.31±0.13	0
+5 mM DEP, 10 min	0.97±0.09	58
+100 mM NH_2OH, 30 min	1.62±0.06	29
+5 mM DEP, 10 min, then +100 mM NH_2OH, 30 min	1.38±0.06	40

in the substituent at position 6 or 7 of the penam or cephem nucleus, we investigated the effect of DEP on the uptake of β-lactam antibiotics without such an α-amino group. Fig. 5 shows that the uptake of [^{3}H]benzylpenicillin (100 μM), which shares the intestinal uptake system for α-amino-cephalosporins [8,11,26], was not significantly inhibited by treatment of the vesicles with DEP. The uptake of other β-lactam antibiotics without an α-amino group such as cefotiam, cefoperazone, cephaloridine or ceftazidime (concentration 2 mM), was also never inhibited by DEP in contrast to the uptake of α-aminocephalosporins and α-aminopenicillins (Table I). Fig. 6 shows the progressive inhibition of cephalexin uptake by increasing concentrations of DEP with a half-maximal inhibition at about 3 mM DEP. The group specific reagent DEP selectively modifies histidine residues in the pH range 5.5–7.3 [17,18]. This modification of histidine residues can be reversed by hydroxylamine [17]. The effect of hydroxylamine on control and DEP-treated membrane vesicles is described in Table II. Treatment of brush-border membrane vesicles with 10 mM DEP at pH 6.4 for 10 min caused an 58% inhibition of cephalexin uptake. When DEP-treated vesicles were subsequently exposed to 0.2 M hydroxylamine, the inhibition of cephalexin transport activity was reduced to 40%, whereas the incubation of vesicles with hydroxylamine alone produced an inhibition of 29%. This partial reversibility of the DEP-induced inhibition suggests in accordance with the work from other groups that the DEP has modified a histidine residue of the transport protein rather than a sulfhydryl or primary amino group [13,14,17].

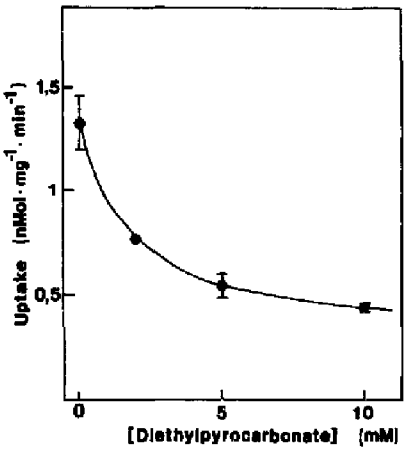

Fig. 6. Effect of DEP-concentration on cephalexin uptake by intestinal brush-border membrane vesicles. Brush-border membrane vesicles from rabbit small intestine were treated at 20 °C for 10 min with 0, 2, 5 and 10 mM DEP. After washing, the uptake of 2 mM cephalexin was measured for 1 min in the presence of an inward H^+ gradient.

Protection of DEP induced inhibition of cephalexin uptake by various substrates

In order to investigate whether the inhibition of the intestinal transport system by DEP-treatment of brush-border membrane vesicles could be prevented by the presence of substrates for this transport system during incubation with DEP, the effect of various compounds on the DEP-induced inhibition was investigated. Vesicles were incubated with the respective substrates for 1 h to attain equilibrium and were then treated with DEP. After washing of the vesicles the H^+-dependent cephalexin uptake into DEP-treated and untreated vesicles was measured. Table III shows that amino acids such as glycine and proline or bile salts such as taurocholate–compounds which do not share the intestinal transport system for β-lactam antibiotics and dipeptides [23–25]–had no protective effect on the inhibition by DEP. Dipeptides however, the natural substrates for this transport system, were cleary and significantly protective. Among the β-lactam antibiotics, the orally active α-aminocephalosporins and α-aminopenicillins could protect the transport system from inhibition by DEP. Cephalosporins for parenteral use such as cefotiam, ceftizoxime,

TABLE III

EFFECT OF VARIOUS SUBSTRATES ON THE DEP-INDUCED INHIBITION OF CEPHALEXIN UPTAKE BY INTESTINAL BRUSH-BORDER MEMBRANE VESICLES

Brush-border membrane vesicles from rabbit small intestine were incubated with the respective concentrations of substrates in 20 mM potassium phosphate buffer (pH 6.4)/280 mM mannitol for 10 min at 30°C. After 10 min DEP was added to a final concentration of 5 mM, whereas control vesicles were treated only with buffer. After 10 min of incubation the vesicles were washed and centrifuged. The uptake of 2 mM cephalexin was measured for 1 min in presence of an inward H^+ gradient. The uptake is given as the ratio (in %) of uptake into DEP-treated vesicles and uptake into the corresponding control vesicles, which have been incubated only with substrates. The 1 min-uptake value of 2 mM cephalexin under these conditions was 1.36 ± 0.12 nmol·mg^{-1}·min^{-1}. The data are the mean ± S.D. of at least three determinations from one membrane preparation. * β-lactam antibiotics with an α-amino group.

Substrate	Uptake (% of control)
None	22.4 ± 4.1
50 mM glycine	27.8 ± 4.7
50 mM L-proline	30.1 ± 3.8
1 mM taurocholate	28.8 ± 3.9
50 mM glycyl-L-proline	60.4 ± 9.0
50 mM glycylglycine	63.5 ± 7.0
50 mM triglycine	40.2 ± 6.8
50 mM tetraglycine	38.9 ± 6.4
50 mM glycylsarcosine	102.8 ± 12
25 mM L-carnosine	93.0 ± 3.2
12 mM amoxicillin *	79.0 ± 7.5
12 mM ampicillin *	48.9 ± 2.7
25 mM cephalexin *	85.0 ± 7.1
25 mM cefadroxil *	65.0 ± 3.1
25 mM cephradine *	51.0 ± 4.0
25 mM cefotaxime	22.3 ± 6.5
20 mM ceftizoxime	28.1 ± 6.1
20 mM cefotiam	34.2 ± 2..
20 mM cephalothin	31.3 ± 2.1
20 mM cephaloridine	30.3 ± 3.6

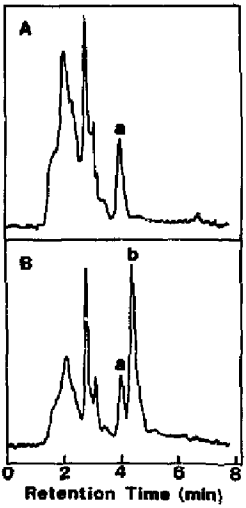

Fig. 7. HPLC-chromatograms of cephalexin taken up by intestinal brush-border membrane vesicles without and with DEP-treatment in presence of cephradine. Brush-border membrane vesicles from rabbit small intestine were preloaded for 1 h at 30°C with 25 mM cephradine in 20 mM potassium phosphate buffer (pH 6.4)/280 mM mannitol. Subsequently one half of this suspension was incubated for 10 min with 10 mM DEP, whereas the other half was only treated with buffer for control. After dilution the vesicles were centrifuged and uptake of 2 mM cephalexin for 1 min was determined in the presence of an inward H^+ gradient. The cephalexin taken up by the vesicles was eluted from the filters with water and determined by HPLC. (A) HPLC-chromatogram of control vesicles. (B) HPLC-chromatogram of DEP-treated vesicles. Peak a, position of cephalexin; Peak b, position of cephradine.

cefotaxime, cephalothin or cephaloridine, however, could not significantly prevent the DEP-induced inhibition. During analysis of the substrate protection experiments by HPLC we detected that α-amino-β-lactam antibiotics were trapped within the vesicles after treatment with DEP. Fig. 7 shows the HPLC analysis of cephalexin taken up by membrane vesicles which had been incubated with cephradine before treatment with DEP. The cephradine (Fig. 7, peak b) taken up into the control vesicles was completely washed out by dilution with buffer (Fig. 7A). However, if the cephradine-loaded vesicles were treated with DEP, cephradine could only be partially washed out and remained trapped within the vesicles (Fig. 7B, peak b). The other peaks beside cephalexin and cephradine in the HPLC chromatograms are caused by components of the nitrocellulose filters and by components of the membrane vesicles, which are eluted during extraction of the β-lactam antibiotics trapped on the filters with water. In order to investigate whether DEP-treatment of brush-border membrane vesicles influences the efflux of β-lactam antibiotics, vesicles were equilibrated with β-lactam antibiotics carrying either an α-amino group (cephalexin) or not (benzylpenicillin). After treatment of these preloaded vesicles with DEP the efflux of the respective β-lactam antibiotics from these vesicles was measured. Fig. 8 shows that in DEP-treated vesicles the time-dependent efflux of β-lactam antibiotics with an α-amino group was significantly decreased as compared to control vesicles (Fig. 8A). The efflux of β-lactam antibiotics without an α-amino group remained unchanged (Fig. 8B). The different values at 30 min in control and DEP-treated vesicles do not reflect an increased binding of cephalexin to DEP-treated membrane vesicles. Measurement of cephalexin uptake into control and DEP-treated vesicles in dependence of the osmolarity of the medium revealed no increased binding.

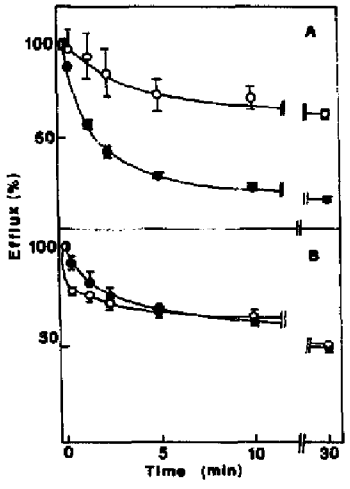

Fig. 8. Efflux of β-lactam antibiotics from control and DEP-treated intestinal brush-border membrane vesicles. Brush-border membrane vesicles from rabbit small intestine were preloaded for 1 h at 30 °C either with 2 mM cephalexin (A) or 100 μM (1 μCi/point) [^{3}H]benzylpenicillin (B) in 20 mM potassium phosphate buffer (pH 6.4)/280 mM mannitol. Subsequently vesicles were treated with 10 mM DEP for 10 min, whereas control vesicles only obtained buffer. After 10 min the vesicles were diluted 20-fold with buffer and efflux was measured from control (●) and DEP-treated (○) vesicles. The efflux rates are xpressed in % of the respective equilibrium values. (A) Efflux of cephalexin. (B) Efflux of benzylpenicillin.

Discussion

Most of the modern β-lactam antibiotics of the third generation are insufficiently transferred into blood after oral application. In contrast, the polar amino-β-lactam-antibiotics – α-aminocephalosporins and α-aminopenicillins – are very efficiently transported across the intestinal brush-border membrane using the H^+-dependent transport system for di- and tripeptides [2–6]. Kinetic and photoaffinity labeling studies showed that all β-lactam antibiotics, whether they are enterally absorbed or not, interact with the intestinal transport system for dipeptides and bind from the luminal side to the transport system [11]. The mechanisms responsible for transport across the intestinal brush-border membrane are not known.

In the present study we investigated whether the intestinal transport system shared by α-aminocephalosporins and dipeptides can be inhibited by DEP, a reagent specific for histidine residues in the pH range 5.5–7.3. After treatment of brush border membrane vesicles with DEP, the uptake of orally active β-lactam antibiotics with an α-amino group at position 6 or 7 of the penam

or cephem nucleus was significantly inhibited. The inhibition was competitive with an increase of the Michaelis-Menten constant indicating a decreased affinity of the transport system for α-amino-β-lactam antibiotics. This nature of inhibition of the intestinal transport system is different from the inhibition of the renal dipeptide transport system by DEP. DEP inhibits the intestinal transport system competitively, whereas the DEP-inactivation of the renal dipeptide transport system is noncompetitive [12]. This difference in inhibition by DEP supports other findings that the intestinal and the renal transport systems shared by β-lactam antibiotics and dipeptides are similar but not identical [27].

DEP-treatment of intestinal brush-border membranes had no inhibitory effect on the uptake of β-lactam antibiotics without an α-amino group. Since β-lactam antibiotics with and without α-amino functions bind to the same membrane protein [11], the different effect of DEP on the uptake of β-lactam antibiotics with or without an α-amino group may indicate that a DEP-modified amino acid residue of the transport protein specifically interacts with the α-amino-group. This interaction may be important for an effective transmembrane movement of carrier-bound β-lactam antibiotics. A maximal uptake rate of cephalexin occurs in the presence of an inward H^+ gradient with pH = 6 at the outside and pH = 7.4 at the inside. Since the pK_a values of the α-amino group in α-amino-β-lactam antibiotics are in the pH range of 6.89–7.35 [28,29] and that of the histidine residues in proteins are also near neutral pH, it is tempting to speculate that proton donor-acceptor relationships between the α-amino group in orally effective α-amino-β-lactam antibiotics and a histidine residue of the transport protein play an important role in the transport process of β-lactam antibiotics across the intestinal brush-border membrane.

Acknowledgement

The authors thank Dr. Gerhard Burckhardt for valuable discussions. Part of this work has been published in preliminary form [30].

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