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Contribution of Passive Transport Mechanisms to the Intestinal Absorption of β-Lactam Antibiotics

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Abstract—The transport characteristics of aminopenicillins (ampicillin and amoxicillin), aminocephalosporins (cephalexin, cephradine and cefadroxil) and cefazolin have been compared with those of an actively transported substance (D-glucose) and a passively transported substance (L-glucose). Although the initial uptake of the aminocephalosporins was stimulated in the presence of an inward H^+ gradient, there was no overshoot in the uptake of any of the drugs tested, even in the presence of an H^+ gradient. Also, the time course and the degree of uptake of these drugs were similar to those of L-glucose, especially in the absence of an H^+ gradient. These results suggest that the β-lactam antibiotics tested, like L-glucose, pass through the rat intestinal brush border membrane mainly by passive diffusion. However, the differences in absorption between these drugs, like the differences in their disappearance from a proximal loop of rat intestine, cannot be explained by a simple permeation process alone.

There have been many studies of intestinal absorption of amino β-lactam antibiotics (Penzotti & Poole 1974; Iseki et al 1984, 1985, 1988; Kimura et al 1985; Okano et al 1986a, b; Tsuji et al 1987). However, the extent to which passive or carrier-mediated transport systems participate in the absorption process remains controversial. The present study attempts to clarify the contribution of passive transport mechanisms in that we have compared the permeabilities of the β-lactam antibiotics ampicillin, amoxicillin, cephalexin, cephradine, cefadroxil and cefazolin with an actively transported substance (D-glucose) and a passively transported substance (L-glucose), using the rat intestinal brush border membrane. We have also examined the relationship between the uptake of these β-lactam antibiotics by membrane vesicles and their absorption from a proximal loop of rat intestine.

Materials and Methods

Materials

Ampicillin anhydrous (Takeda Chemical Industries, Osaka, Japan), amoxicillin trihydrate (Kyowa Hakko Kogyo Co., Tokyo, Japan), cephalexin monohydrate (Shionogi & Co., Tokyo, Japan), cephradine (Sankyo Co., Tokyo, Japan), cefadroxil (Banyu Co., Tokyo, Japan) and cefazolin (Fujisawa Pharmaceutical Co., Osaka, Japan) were kindly donated. D-[^{14}C(u)]glucose (sp. act. 0·5 GBq $mmol^{-1}$) and L-[1-^{14}C]glucose (sp. act. 1·7 GBq $mmol^{-1}$) were purchased from NEN Research Products (Boston, MA, USA). All other chemicals were of the highest grade available and used without further purification.

Preparation of brush border membrane vesicles

The entire small intestine of male Wistar rats (180–230 g) was excised under anaesthesia with diethyl ether, and brush border membrane vesicles were isolated according to the calcium chloride precipitation method of Kessler et al (1978) and as described previously (Iseki et al 1989). The buffers used were: A, 20 mM N-2-hydroxyethylpiperazine-N′-2-ethansulphonic acid (Hepes)/Tris and 100 mM D-mannitol, pH 7·5; B, 20 mM 2-(N-morpholino) ethansulphonic acid (Mes)/Tris, 100 mM KCl, and 100 mM D-mannitol, pH 6·0; and C, 20 mM Hepes/Tris, 100 mM KCl, and 100 mM D-mannitol, pH 7·5. Brush border membrane vesicles were used for transport studies within 4 h of preparation. The purity of the membrane was routinely assessed by measuring the specific activity of alkaline phosphatase (E.C.3.1.3.1.), which is increased over 12-fold in the final membrane suspension compared with the concentration in the homogenate of intestinal scrapings. The membrane was equilibrated for 1 h at 0°C before use.

Transport studies

The uptake of substrates was measured by a rapid filtration technique. In the standard assay, the reaction was initiated by the addition of 100 μL of the test buffer (A, B or C) containing the substrate to 20 μL of membrane suspension (approximately 6 mg of protein mL^{-1}) at 25°C. At a stated time the reaction was stopped by diluting the reaction mixture with 5 mL of an ice-cold buffer containing 150 mM NaCl, 20 mM Hepes/Tris (pH 7·5). The mixture was immediately filtered through a Millipore filter (HAWP, 0·45 μm, 2·5 cm diameter), which was washed once with 8 mL of the same ice-cold buffer. In a separate experiment, non-specific adsorption onto a Millipore filter was determined using the test buffer instead of brush border membrane suspension. This value was subtracted from the uptake data.

Absorption from the intestinal loop

The drugs were dissolved in a modified Ringer solution (Schultz et al 1966), pH 7·4 (100 μg mL^{-1}). Two intestinal loops (10 cm) separated by 1 cm, starting 10 cm below the pylorus of male Wistar rats (180–230 g) were prepared according to Levine & Pelikan (1961). Since drug absorption from both loops was the same, longitudinal specialization of

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the proximal intestine tested can be ignored. After washing the loop gently with 10 mL of the Ringer solution, 1 mL of drug solution was injected into loops using a syringe. After 30 min the loops were removed. The contents were then emptied into a 25 mL volumetric flask and the mucosal surface of the loop was rinsed with the Ringer solution to a volume of 25 mL. For the determination of the tissue concentration, the homogenate was prepared in a Teflon homogenizer with 10 mL of 0·9% NaCl.

Analytical methods

D-[^{14}C(u)]glucose or L-[1-^{14}C]glucose on the filter was determined by liquid scintillation counting. Ampicillin, amoxicillin, cephalexin and cephradine were determined by HPLC fluorometrically (Miyazaki et al 1983). Cefadroxil and cefazolin were determined by HPLC using UV detection (Iseki et al 1987a). Protein was measured by the method of Lowry et al (1951) with bovine serum albumin as the standard.

Results

Uptake of D-glucose and L-glucose by brush border membrane vesicles

Fig. 1 shows the uptake of D-glucose (1 mM) by brush border membrane vesicles in the presence of a Na^+ or K^+ gradient. The initial rate of uptake was clearly increased in the presence of an inward Na^+ gradient and there was also an overshoot effect. The effects of Na^+ and K^+ gradients on the uptake of the lower concentration of D-glucose (0·1 mM) were similar, and are not shown. The uptake of L-glucose was virtually the same as that of D-glucose in the absence of a Na^+ gradient. The concentration of preincubation mannitol was not adjusted to avoid osmolarity differences (inside/outside vesicles) in experiments, as we considered the osmotic effect on uptake would be negligible in these experiments; mannitol is membrane permeable, and we have compared the uptake of L-glucose in the presence of a NaCl or KCl 100 mM gradient at equal osmolarity. The uptake values after 1 min incubation in the presence of the NaCl gradient, the KCl gradient and absence of gradient were $0·44 \pm 0·22$, $0·45 \pm 0·11$, $0·47 \pm 0·14$ (nmol (mg protein)$^{-1}$, mean $\pm$ s.d., n = 3), respectively. These results suggest that

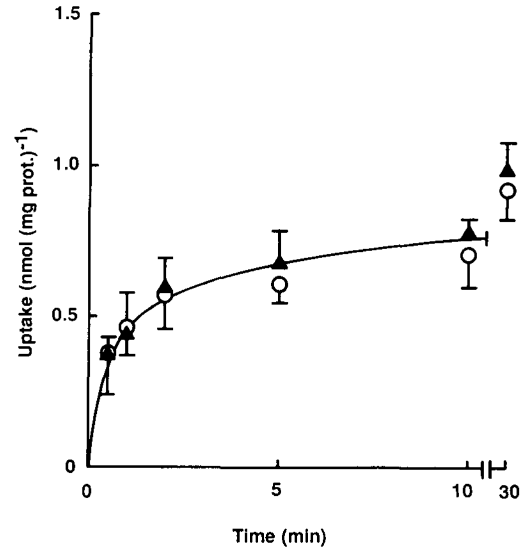

FIG. 2. Uptake of ampicillin (1 mM) by rat intestinal brush border membrane vesicles. Membrane vesicles were preincubated at 25°C in buffer C for 2 min. The vesicles (20 μL) were incubated with buffer B (100 μL) (○) or buffer C (100 μL) (▲) containing 1·2 mM ampicillin at 25°C. Each point represents the mean ± s.e.m. of 4–9 measurements with different preparations of vesicles.

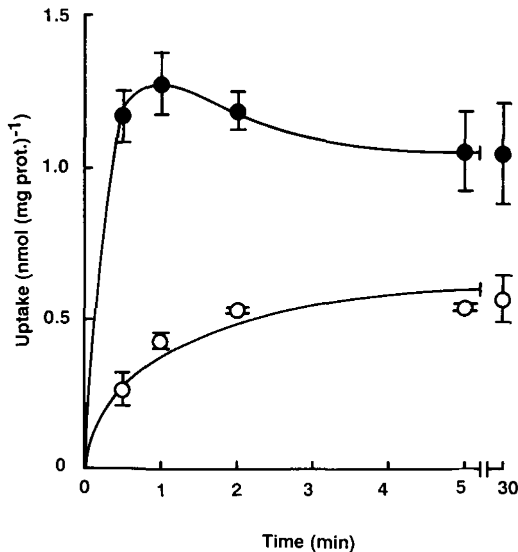

FIG. 1. Uptake of D-glucose (1 mM) by rat intestinal brush border membrane vesicles. Membrane vesicles were preincubated at 25°C in buffer A for 2 min. The vesicles (20 μL) were incubated with buffer A (100 μL) containing 120 mM NaCl (●) or 120 mM KCl (○) and 1·2 mM D-glucose at 25°C. Each point represents the mean ± s.e.m. of three measurements.

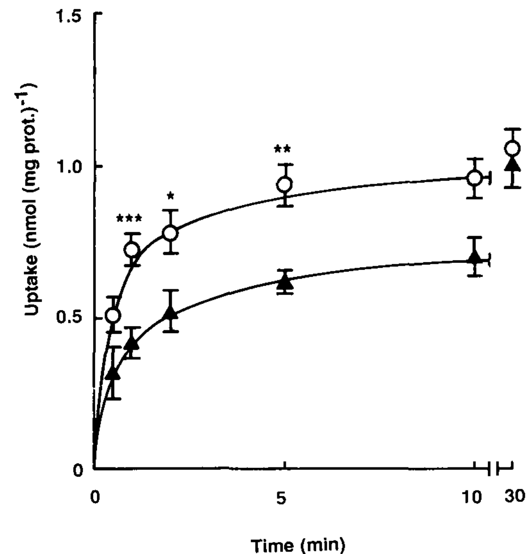

FIG. 3. Uptake of cephalexin (1 mM) by rat intestinal brush border membrane vesicles. Membrane vesicles were preincubated at 25°C in buffer C for 2 min. The vesicles (20 μL) were incubated with buffer B (100 μL) (○) or buffer C (100 μL) (▲) containing 1·2 mM cephalexin at 25°C. Each point represents the mean ± s.e.m. of 7–12 measurements with different preparations of vesicles. $*P < 0·025$, $**P < 0·005$, $***P < 0·001$, significantly different from control with no H^+ gradient.

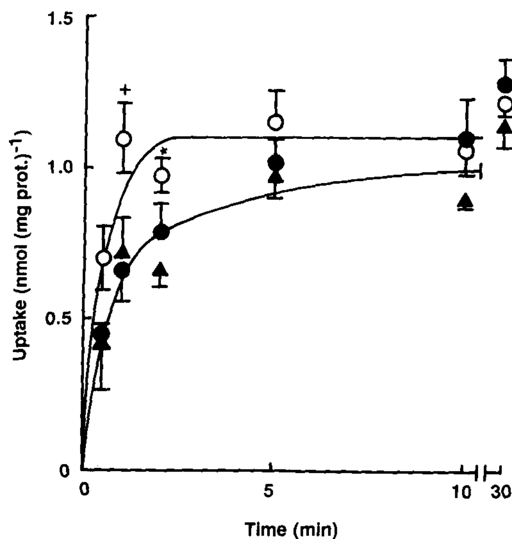

FIG. 4. Uptake of cephradine (1 mM) by rat intestinal brush border membrane vesicles. Membrane vesicles were preincubated at 25°C in buffer B (●) or buffer C (○, ▲) for 2 min. The vesicles (20 μL) were incubated with buffer B (100 μL) (○, ●) or buffer C (100 μL) (▲) containing 1·2 mM cephradine at 25°C. Each point represents the mean ± s.e.m. of 4-6 measurements with different preparations of vesicles. *$P<0·05$, significantly different from control with no H^+ gradient (pH 7·5). †$P<0·025$, significantly different from control with no H^+ gradient (pH 6·0).

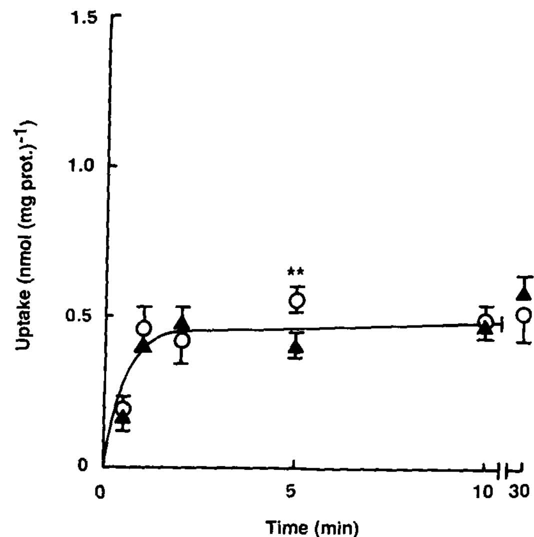

FIG. 5. Uptake of cefazolin (1 mM) by rat intestinal brush border membrane vesicles. Membrane vesicles were preincubated at 25°C in buffer C for 2 min. The vesicles (20 μL) were incubated with buffer B (100 μL) (○) or buffer C (100 μL) (▲) containing 1·2 mM cefazolin at 25°C. Each point represents the mean ± s.e.m. of 4-6 measurements with different preparations of vesicles. *$P<0·05$, significantly different from control with no H^+ gradient.

the membrane vesicles prepared in this study had an intact transport system, i.e. one which could clearly distinguish the passively and actively transported substances.

Uptake of β-lactam antibiotics by brush border membrane vesicles

The uptake of various β-lactam antibiotics (1 mM) by brush border membrane vesicles was measured in the presence and absence of an inward H^+ gradient and the results are illustrated in Figs 2-5 for ampicillin, cephalexin, cephradine, and cefazolin, respectively. The amoxicillin and cefadroxil data are not illustrated because they were similar to those of ampicillin and cephradine, respectively. The initial uptakes of the aminocephalosporins tested (cephalexin, cephradine and cefadroxil) were increased in the presence of an H^+ gradient. However, overshoot phenomena were not observed. On the other hand, the uptake of the aminopenicillins (ampicillin and amoxicillin) and of cefazolin, which lacks the aminobenzyl group, was independent of pH conditions at all times ($(pH)_i > (pH)_o$ compared with $(pH)_i = (pH)_o = 7·5$).

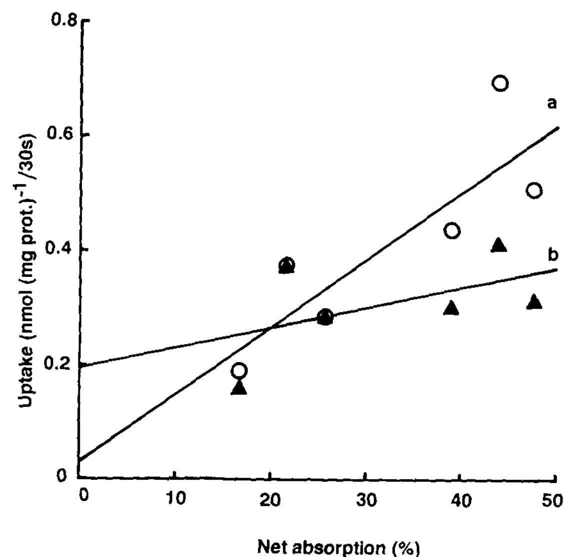

FIG. 6. Correlation of intestinal absorption and uptake by brush border membrane vesicles of β-lactam antibiotics in the presence (a; ○) or absence (b; ▲) of an H^+ gradient. Correlation coefficient is 0·851 for line a and 0·555 for line b.

Table 1. Absorption of β-lactam antibiotics from the intestinal loop.

	Disappearance (%)	Net absorption (%)	Tissue accumulation (%)
Ampicillin	23·0±6·0	21·6±3·2	1·4±0·5
Amoxicillin	43·8±2·7	25·7±3·3	18·2±1·2
Cephalexin	77·2±2·8	47·6±0·6	29·6±2·1
Cephradine	84·0±5·1	43·9±3·2	40·1±4·4
Cefadroxil	91·9±3·6	39·0±2·5	52·9±2·6
Cefazolin	16·8±0·7	16·8±0·7	N.D.

The dose was 1 mL of 100 μg mL^{-1} drug in modified Ringer solution (pH 7·4) per 10 cm loop. Each value represents the mean ± s.d. of 4-6 measurements. Net absorption is the difference between disappearance and tissue accumulation.

The uptake of all drugs, like that of L-glucose tended to increase gradually with time, especially in the absence of an H^+ gradient.

Absorption of β-lactam antibiotics from the intestinal loop
Table 1 shows the drug absorption results following injection of the drug into the upper intestinal loop. Aminocephalosporins were well absorbed compared with aminopenicillins and cefazolin. These results are similar to absorption data in man following oral administration (Miyazaki et al 1977, 1979). Fig. 6 shows the relationship between the initial uptake by the membrane vesicles (30 s) and the net absorption from the intestinal loop. There tended to be a positive correlation in the presence of an inward H^+ gradient. There was a similar positive relationship between the initial uptake (e.g. see Figs 2 to 5) and the percentage disappearance from the intestinal loop (Table 1).

Discussion

It is well known that several amino β-lactam antibiotics are efficiently absorbed from the intestinal lumen after oral administration, even though they are completely ionized over the pH range in the gastrointestinal tract and generally have poor lipid solubility. The mechanisms of intestinal absorption of these drugs have therefore been extensively investigated. Some reports (Nakashima et al 1984; Okano et al 1986a, b; Tsuji et al 1987) suggested that a carrier-mediated system could transport aminocephalosporins since, like dipeptides, they have a zwitterionic structure. On the other hand, in our previous report (Iseki et al 1988) dealing with the effect of chlorpromazine on the membrane transport of these drugs and certain endogenous compounds, it was suggested that the permeation characteristics of the amino β-lactam antibiotics (ampicillin, cephradine and cephalexin) differ from those of the actively transported substances (glycylglycine and D-glucose). Moreover, in ampicillin and cephradine uptake, an overshoot phenomenon was not observed in the presence of an inward H^+ gradient, the driving force for dipeptide transport, even in the lower concentration (50 μM). On the other hand, there tended to be an overshoot in the uptake of glycylglycine (Iseki et al 1989). In the present study, therefore, we compared the uptake of several β-lactam antibiotics with that of a passively transported substance, L-glucose, using rat small intestinal brush border membrane vesicles. The membranes apparently possessed intact transport properties (see Fig. 1). There were no overshoot phenomena in the uptake of any of the β-lactam antibiotics tested even in the presence of an inward H^+ gradient, and the time course and the degree of uptake of these drugs were similar to those of L-glucose, especially in the absence of an H^+ gradient. It was also found that the initial uptake of all aminocephalosporins tested (cephalexin, cephradine and cefadroxil) was increased in the presence of an H^+ gradient. This increase was, however, much weaker than that observed by Okano et al (1986a, b) who observed uphill transport of cephradine in the rabbit intestine. Uptake of aminopenicillins (ampicillin and amoxicillin) and cefazolin, on the contrary, was not dependent on pH gradients ($(pH)_i > (pH)_o$ compared with $(pH)_i = (pH)_o = 7{\cdot}5$). The uptake differences between aminopenicillins and aminocephalosporins in the presence of an H^+ gradient are consistent with our previous results (Iseki et al 1989) using lower drug concentrations (50 μM, ampicillin and cephradine). We suggest, from both previous and present results, that all the β-lactam antibiotics tested can permeate through the rat intestinal brush border membrane largely by passive diffusion like L-glucose. It is also suggested that the increased initial uptake rate of aminocephalosporins seen with the H^+ gradient might contribute partially to their superior absorption (Table 1, Fig. 6). However, the reasons for the differences in an H^+ gradient effect on aminopenicillins and aminocephalosporins needs further investigation.

In the present study, there was only a weak positive correlation between the absorption from the upper intestinal loop and the initial uptake rate by the brush border membrane vesicles (Fig. 6). In our previous study, we found a soluble protein fraction which binds amino β-lactam antibiotics (ampicillin, amoxicillin, cephalexin, cephradine and cefadroxil) to rat intestinal epithelial cells, and we indicated that this binding process in the epithelial cells played an important role in the absorption of these efficiently absorbed zwitterionic penicillins and cephalosporins (Miyazaki et al 1982; Iseki et al 1987a, b). The present results suggest that the differences of absorption rate among these β-lactam antibiotics cannot be explained by simple permeation across the brush border membrane alone. Therefore, it is reasonable to suppose that at least two processes are involved. Binding and/or accumulation in the epithelial cells, could play a role in intestinal absorption and in the differences in absorption of these drugs.

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