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Transport of phenethicillin into rat intestinal brush border membrane vesicles: role of the monocarboxylic acid transport system

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Abstract

Transport characteristics of phenethicillin (PEPC) was examined using brush border membrane vesicles (BBMV) prepared from rat small intestine. Uptake of PEPC in the presence of an inwardly directed H^+ gradient (pH_{in} 7.5 and pH_{out} 5.5) exhibited an overshoot phenomenon, indicating that PEPC is transported uphill into BBMV. Uptake of PEPC was inhibited by various monocarboxylic acids, not by oligopeptides or the β-lactams which are the substrates of the oligopeptide transporter. Moreover, when the uptake of L-lactic acid, a model substrate of the monocarboxylic acid transporter(s), was measured in the presence of PEPC, the uptake of L-lactic acid was inhibited by PEPC in a competitive manner. It was concluded that PEPC is transported into rat small intestinal BBMV mainly via the monocarboxylic acid transporter(s), not via the oligopeptide transporter.

Keywords: Phenethicillin; Monocarboxylic acid transporter; Brush border membrane; Rat; Intestine

1. Introduction

It is well accepted that some β-lactam antibiotics as well as di/tripeptides are transported by the H^+-coupled oligopeptide transporter which exists at the brush border membrane (BBM) of the small intestine (Oh et al., 1993; Tsuji and Tamai, 1996). Previously, it was thought that only β-lactams with an α-amino group in the side chain, e.g. cephalexin (CEX), cephradine (CED) and cyclacillin (ACPC), are transported by the oligopeptide transporter, which makes these β-lactams orally active. However, it was revealed

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afterwards that ceftibuten (CETB) and cefixime (CFIX) which do not possess an α-amino group in the side chain are also transported by the oligopeptide transporter (Tsuji et al., 1987b; Muranushi et al., 1989; Yoshikawa et al., 1989). Since these β-lactams are also orally active, it is now believed that the α-amino group in the side chain is not a prerequisite for the transport of β-lactams via the oligopeptide transporter (Oh et al., 1993). Moreover, it is reported that ACE (angiotensin converting enzyme) inhibitors and renin inhibitors, which possess peptide-like structures, are also transported by the oligopeptide transporter (Tsuji and Tamai, 1996). Therefore, the oligopeptide transporter appears to be involved in the intestinal absorption of a wide variety of peptide-mimetic drugs.

On the other hand, it has been reported recently that the monocarboxylic acid transporter(s), which is also present at BBM of the small intestine, is involved in the absorption of various anionic drugs (Tsuji and Tamai, 1996). This transporter is partly responsible for the absorption of cefdinir which is a mono-anionic orally active β-lactam (Tsuji et al., 1993). Phenethicillin (PEPC) is another orally active β-lactam which is water soluble, mono-anionic at physiological pH, and lacks an α-amino group in the side chain. It is highly likely that PEPC may be absorbed from the intestine via either the oligopeptide or the monocarboxylic acid transport system. However, the transport characteristics of PEPC have not been reported, yet. Although propicillin (PPPC), which is very similar to PEPC in chemical structure, has been used as an inhibitor to the oligopeptide and monocarboxylic acid transporters, the results are not conclusive. PPPC exhibited affinity to both oligopeptide and monocarboxylic acid transporters (Tsuji et al., 1987b; Simanjuntak et al., 1991; Tsuji et al., 1993). However, it has not been clarified that PPPC is transported by one of these transporters or by both of them. Therefore, in the present study, transport of PEPC was studied using brush border membrane vesicles (BBMV) prepared from rat small intestine. Stereoselectivity in the transport of PEPC was also studied because PEPC is used clinically as the mixture of two epimers (Hoogmartens et al., 1982; Itoh and Yamada, 1995).

2. Materials and methods

2.1. Materials

Carindacillin (CIPC), ceftibuten (CETB), cyclacillin (ACPC) and cefixime (CFIX) were kindly donated by Pfizer Pharmaceutical (Tokyo, Japan), Shionogi Pharmaceutical (Osaka, Japan), Takada Pharmaceutical (Tokyo, Japan) and Fujisawa Pharmaceutical (Osaka, Japan), respectively. Phenethicillin potassium salt (PEPC, S/R $\approx$ 1.2), sodium salicylate, D-lactic acid, cephalexin (CEX), cephradine (CED), oligopeptides (GLY–GLY, GLY–PRO, GLU–ALA, LYS–VAL, ALA–ALA–ALA) and carbonyl cyanide *p*-(trifluoromethoxy)phenyl hydrazone (FCCP) were purchased from Sigma (St. Louis, MO). The epimeric mixture of R-PEPC and S-PEPC will be termed RS-PEPC in the present study. ^{14}C-labelled L-lactic acid was purchased from DuPont NEN Products (MA, USA). L-Lactic acid, acetic acid, propicillin potassium salt (PPPC, S/R $\approx$ 1.2), HEPES (2-[4(2-hydroxylethyl)-1-piperazinyl]ethanesulfonic acid), MES (2-morpholinoethanesulfonic acid, monohydrate) and Tris (tris(hydorxymethyl) aminomethane) were purchased from Wako Pure Chemicals (Osaka, Japan). All other chemicals were of the highest grade available.

2.2. Preparation of rat intestinal brush border membrane vesicles

BBMV were prepared by the calcium precipitation method as described below. All preparation procedures were performed on ice or under refrigeration (0–4°C). Centrifugations were performed using a Hitachi himac CP70G centrifuge with a RP50T rotor (Hitachi, Tokyo, Japan) or a KR-200B with a RA-3 rotor (Kubota, Tokyo, Japan).

Four to six male Sprague–Dawley rats weighing 270–400 g (Nippon SLC, Hamamatsu-shi, Japan) were fasted for about 15 h prior to the experiment but had free access to water. The

jejunum was removed and flushed with ice-cold saline, and the mucosa was scraped. The scraping (approximately 5 g of wet weight) was placed in 45 ml of 'Buffer A' consisting of 10 mM mannitol and 2 mM Hepes/Tris (pH 7.1), and the mixture was homogenized with a Waring Blender (Excel Auto Homogenizer, Model ED-2, Nihon Seiki Seisakusho, Tokyo, Japan) at 18000 rpm for 2 min. Aliquots were removed from this initial homogenate for protein and enzyme determinations. Calcium chloride was added to a final concentration of 10 mM, and the homogenate was well stirred and allowed to stand for 15 min. Fifty milliliters of Buffer A containing 10 mM calcium chloride was added to the homogenate and centrifuged at $500 \times g$ for 12 min. The supernate was centrifuged at $15000 \times g$ for 12 min and the resulting pellet was suspended in 25 ml of Buffer A with a glass/teflon Potter homogenizer at 1000 rpm for ten strokes. Calcium chloride was added to this homogenate to give a final concentration of 10 mM, and the homogenate was well stirred and allowed to stand for 15 min. Twenty-five milliliters of Buffer A containing 10 mM calcium chloride was added to the homogenate, and the homogenate was centrifuged at $750 \times g$ for 12 min. The supernate was centrifuged at $30000 \times g$ for 12 min, and the resulting pellet was suspended in 100 ml of 'Buffer B' consisting of 100 mM mannitol and 20 mM Hepes/Tris (pH 7.5) with a glass/teflon Potter homogenizer at 1000 rpm for ten strokes. The homogenate was centrifuged at $48000 \times g$ for 20 min and the resulting pellet was suspended in 1 ml of Buffer B. The homogenate was passed through a 20-gauge needle ten times, and 45 ml of Buffer B was added. This homogenate was centrifuged at $2000 \times g$ for 5 min and the resulting supernate was centrifuged at $48000 \times g$ for 20 min. The resulting pellet was suspended in 0.5 ml of the solution consisting of 100 mM mannitol, 100 mM KCl and 10 mM Hepes/Tris (pH 7.5), and passed through a 27-gauge needle ten times. Aliquots of this suspension were removed for protein and enzyme analyses. Prepared vesicles were stored at -80°C and used for uptake experiments within 7 days.

The purity of the BBMV was evaluated by comparing the activities of alkaline phosphatase (a marker enzyme of the brush border membrane) and Na^+/K^+-ATPase (a marker enzyme of the basolateral membrane) to those of the initial homogenate. Protein concentrations of BBMV samples were determined using a Bio-Rad Protein assay kit (Bio-Rad Laboratories, Richmond, CA).

2.3. Uptake experiment

Uptake of PEPC and L-lactic acid into BBMV was measured at 25°C by the rapid filtration method. The uptake was initiated by adding 180 μl of the incubation medium to 20 μl of the BBMV suspension. The composition of the incubation medium is listed in the legend of the figures. At appropriate time, the reaction was stopped by the addition of 1 ml of ice-cold stop solution consisting of 100 mM mannitol, 100 mM KCl and 10 mM Mes/Tris (pH 5.5). The mixture was immediately filtered (Millipore filter, HAWP, 0.45 μm of pore size, 25 mm in diameter) followed by washing with 5 ml of ice-cold stop solution. For measuring the L-lactic acid uptake, the filter was transferred into a scintillation vial and 10 ml of scintillation cocktail (Aquasol®-2, Packard Instrument, Meriden, CT) was added. Radioactivity was measured with a liquid scintillation counter (Beckmann LS5000TD). For measuring the PEPC uptake, the filter was transferred into a glass tube and the PEPC retained in the vesicles was extracted with 400 μl of distilled water using a vortex mixer. A 200-μl aliquot was injected onto HPLC.

Adsorption of the drug to the vesicles was measured by adding L-lactic acid or PEPC after the stop solution was added to the vesicle suspension. Adsorbed L-lactic acid or PEPC was measured in the same manner as described above. Adsorbed amount was subtracted and the corrected value was considered as the uptake amount in the present study.

2.4. HPLC conditions for PEPC determinations

A high-performance liquid chromatograph was used to determine the concentrations of PEPC epimers. Cosmosil® ($5C_{18}$-AR, 2.1 mm (i.d.) $\times$ 150 mm; Nakalai Tesque, Kyoto, Japan) was used as

an analytical column. The mobile phase was 0.1 M CH_3COONH_4–CH_3OH (62:38 (vol/vol)) with a flow rate of 0.25 ml/min. PEPC epimers were detected at 220 nm.

2.5. Kinetic analysis

In order to calculate kinetic parameters for the uptake of each PEPC epimer into the BBMV, the following equation (Eq. (1)) was used and the parameter values were obtained by a non-linear least squares method (MULTI; Yamaoka et al., 1981).

$$v = \frac{V_{max} \cdot [S]}{K_m + [S]} + K_d \cdot [S] \quad (1)$$

where v is the initial uptake rate, V_{max} is the maximum uptake rate, K_m is the Michaelis constant, K_d is the coefficient of passive diffusion and [S] is the initial concentration of the drug.

3. Results

For the present membrane preparations, the enrichment factors of alkaline phosphatase and Na^+/K^+-ATPase were 15.5 ± 1.2 (mean $\pm$ S.D., $n = 5$) and 1.14 ± 0.92 (mean $\pm$ S.D., $n = 5$), respectively. According to the present HPLC method, PEPC epimers were baseline separated and no peaks interfering with either of the epimers were observed on the chromatograms of blank samples. The detection limit was approximately 0.02 nmol.

Time course of the uptake of PEPC in the presence of a H^+ gradient is shown in Fig. 1. The uptake of PEPC showed an overshoot phenomenon, suggesting that PEPC was actively transported into BBMV under the present experimental conditions. The uptake of PEPC was not stereoselective since the S-epimer to R-epimer ratios of the PEPC inside the vesicles ($S/R = 1.20 \pm 0.04$, mean $\pm$ S.D., $n = 7$) were equal to that in the incubation medium ($S/R = 1.2$).

The uptake of PEPC at equilibrium (uptake in 30 min) was measured at various concentrations of mannitol in the incubation medium. The results are shown in Fig. 2. Since the uptake of PEPC was not stereoselective (Fig. 1), the results are shown as the total of PEPC epimers. The uptake amount at equilibrium decreased with an increase of the osmolarity of the incubation medium, indicating that PEPC was transported into the osmotically active intravesicular space. The intercept on the y-axis was relatively small compared to the uptake amount, suggesting that the adsorption of PEPC to the vesicles was smaller than the uptake into the vesicles.

Effect of extravesicular pH on the initial uptake of PEPC (uptake in 10 s) was measured with the intravesicular pH being fixed at 7.5. The results are shown in Fig. 3. Changes in the extravesicular pH showed a marked effect on PEPC uptake, with the uptake of PEPC being greater at lower pH. Moreover, when the uptake of PEPC was measured in the presence of 50 μM FCCP, a protonophore which dissipates the H^+ gradient, the initial uptake (uptake in 10 s) was reduced to approximately 70% of the control (Fig. 4).

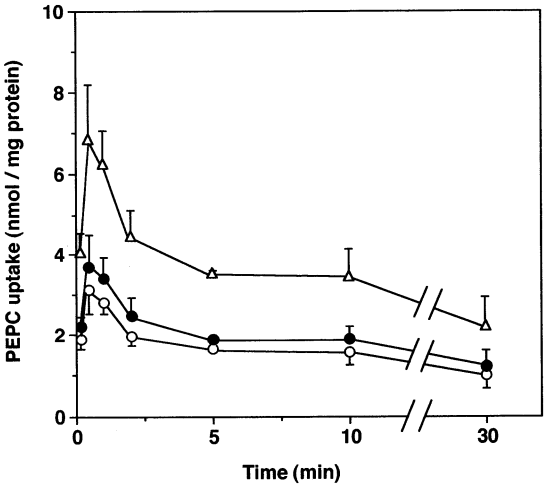

Fig. 1. Time course of the uptake of PEPC (○, R-PEPC; ●, S-PEPC; and △, R-PEPC plus S-PEPC) into BBMV. Vesicles (20 μl, 152 μg of protein) suspended in 100 mM mannitol, 100 mM KCl and 10 mM Hepes/Tris (pH 7.5) were incubated in the incubation medium (180 μl) containing 5 mM RS-PEPC, 100 mM mannitol, 100 mM KCl, 10 mM Mes/Tris (pH 5.5). Each point represents the mean $\pm$ S.D. of three determinations.

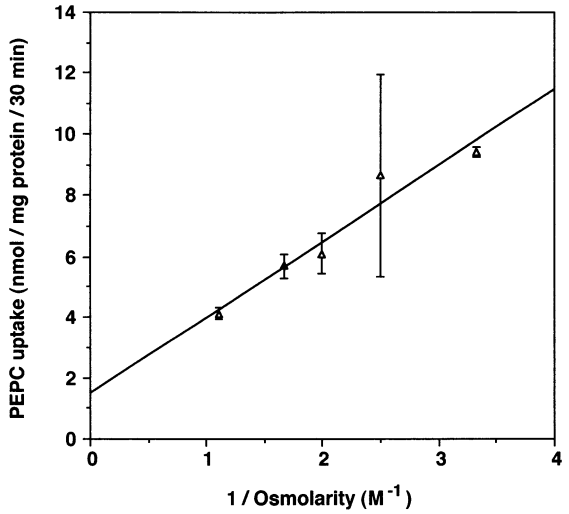

Fig. 2. Effect of the medium osmolarity on PEPC uptake into BBMV. Vesicles (20 μl, 130 μg of protein) were incubated for 30 min in the incubation medium (180 μl) containing 100–500 mM mannitol, 100 mM KCl, 10 mM Mes/Tris (pH 5.5) and 10 mM RS-PEPC. Each point represents the mean ± S.D. of three determinations. The results are shown as the total of PEPC epimers.

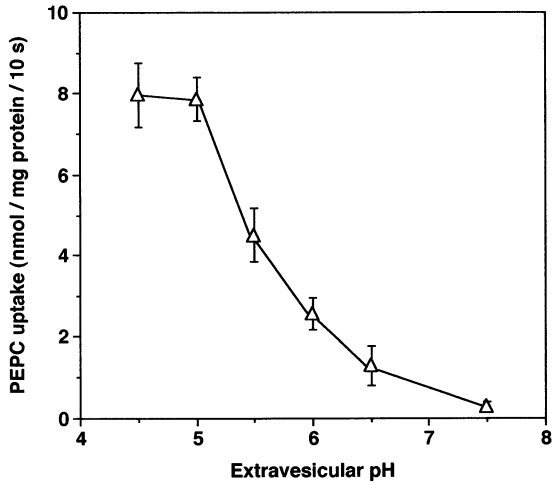

Fig. 3. Effect of the extravesicular pH on the initial uptake of PEPC into BBMV. Uptake of PEPC in 10 s was measured in the same manner as described in the legend of Fig. 1, except that the pH of the incubation medium was varied between 4.5 and 7.5. Each point represents the mean ± S.D. of three determinations. The results are shown as the total of PEPC epimers.

In order to examine further the transport characteristics of PEPC, the initial uptake of PEPC was measured at various concentrations and the kinetic parameters were calculated. The results are shown in Fig. 5. A non-linear relationship was observed between the concentration and the initial uptake rate. Kinetic parameters were calculated according to Eq. (1) with a non-linear least squares method, and the parameter values obtained are as follows; $K_m = 17.9$ mM and $V_{max} =$ 25.4 nmol/mg protein per 10 s for R-PEPC and $K_m = 20.5$ mM and $V_{max} = 29.8$ nmol/mg protein per 10 s for S-PEPC. The parameter values were similar between the epimers, indicating that the transport of PEPC is not stereoselective. The K_d values were obtained by measuring the uptake at 4°C and the values were 0.080 and 0.082 nmol/mg protein per 10 s per mM for R-PEPC and S-PEPC, respectively.

Effects of various monocarboxylic acids, β-lactams and oligopeptides on the uptake of PEPC were studied in the presence of a H^+ gradient. The results are summarized in Table 1. Neither the oligopeptides (20 mM GLY–GLY, GLY–PRO, GLU–ALA, LYS–VAL or ALA–ALA–ALA) nor the compounds which are the substrates of the oligopeptide transporter, i.e.

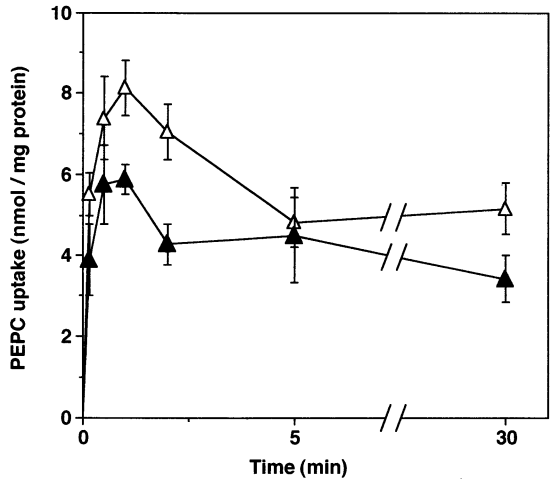

Fig. 4. Effect of FCCP on PEPC uptake into BBMV. Uptake of PEPC was measured in the same manner as described in the legend of Fig. 1, except that the incubation medium contained 0.5% ethanol alone (△) or 0.5% ethanol and 50 μM FCCP (▲). Each point represents the mean ± S.D. of three determinations. The results are shown as the total of PEPC epimers.

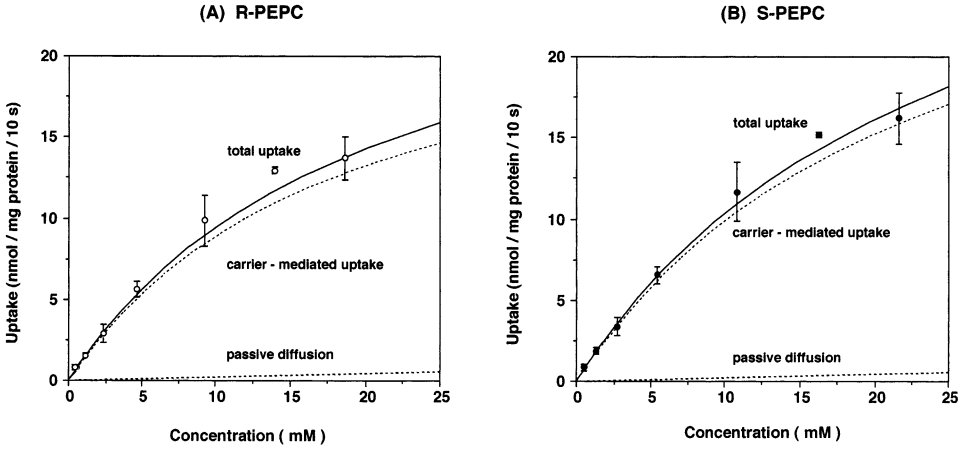

Fig. 5. Concentration dependence of the initial uptake of PEPC into BBMV. Uptake of PEPC in 10 s was measured in the same manner as described in the legend of Fig. 1, except that the concentration of RS-PEPC was varied between 1 and 40 mM. Each point represents the mean ± S.D. of three determinations. The curves show the results of non-linear regression fitting.

ACPC, CETB, CEX, CED and CFIX, inhibited the uptake of PEPC. These results suggested that the uptake of PEPC may not be mediated by the oligopeptide transporter.

Table 1
Effects of various compounds on PEPC uptake

Inhibitors	Concentration (mM)	PEPC uptake (% of control)
Control		100
L-Lactic acid	40	69.7 ± 11.3*
D-Lactic acid	40	65.4 ± 1.9*
Acetic acid	40	78.3 ± 4.3*
Salicylic acid	20	69.0 ± 9.6*
CIPC	1	13.3 ± 6.1**
PPPC	20	52.1 ± 10.8*
CETB	5	103.8 ± 21.3
CETB	10	148.1 ± 19.8
ACPC	20	120.4 ± 10.4
CED	20	93.5 ± 14.3
CEX	20	105.4 ± 34.5
CFIX	20	133.4 ± 9.6
GLY–GLY	20	95.6 ± 20.6
GLY–PRO	20	103.1 ± 19.3
LYS–VAL	20	85.5 ± 10.7
GLU–ALA	20	107.7 ± 10.9
ALA–ALA–ALA	20	109.5 ± 13.7

*, ** Significantly different from the control (*$P<0.01$, **$P<0.001$).

In contrast, L-lactic, D-lactic, acetic and salicylic acids, which are the substrates of the monocarboxylic acid transporter(s), significantly inhibited the uptake of PEPC, indicating that PEPC may share the common transport system, i.e. the monocarboxylic acid transporter(s), with these monocarboxylic acids. Moreover, the uptake of PEPC was significantly inhibited in the

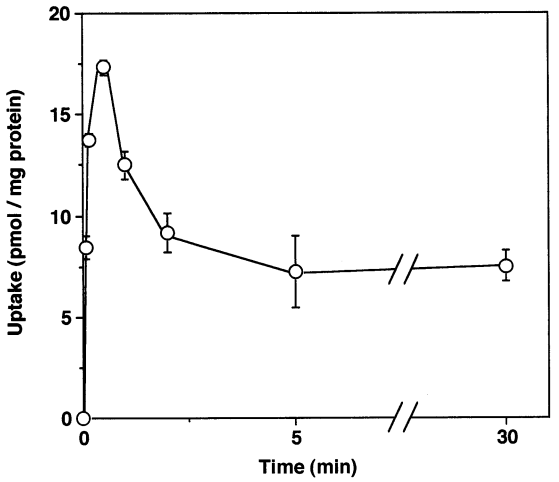

Fig. 6. Time course of L-lactic acid uptake into BBMV. Uptake of L-lactic acid (11 μM) was measured as a function of time in the presence of a H^+ gradient (pH_{in} 7.5, pH_{out} 5.5). Each point represents the mean ± S.D. of three determinations.

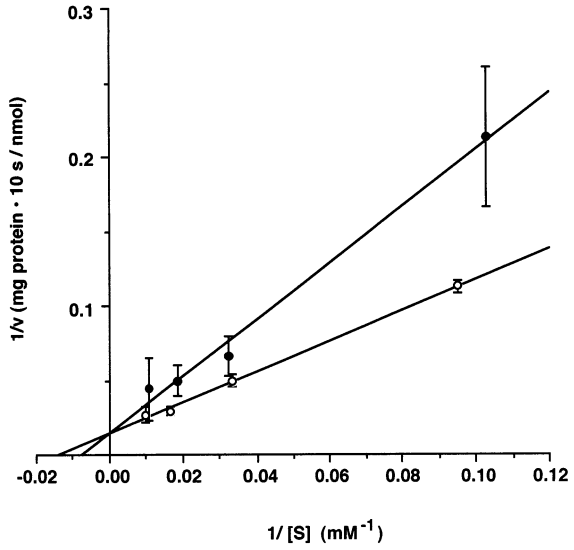

Fig. 7. Lineweaver–Burk plots of the initial uptake of L-lactic acid in the presence of a H^+ gradient. The uptake of L-lactic acid (10–100 mM) in 10 s was measured in the absence (○) or presence (●) of 17.6 mM RS-PEPC. Each point represents the mean ± S.D. of four experiments. Solid lines were obtained by linear regression analyses.

presence of PPPC or CIPC, suggesting that these penicillins may also share the monocarboxylic acid transporter(s).

Time course of L-lactic acid uptake in the presence of a H^+ gradient is shown in Fig. 6. An overshoot phenomenon was observed, indicating that L-lactic acid is transported uphill into BBMV. Initial uptake of L-lactic acid (uptake in 10 s) was measured at various concentrations (10–100 mM) of L-lactic acid in the absence or presence of 17.6 mM PEPC. The results are shown in Fig. 7. Lineweaver–Burk plots clearly showed that PEPC competitively inhibit the uptake of L-lactic acid. The Michaelis constant (K_m) of L-lactic acid was 69.7 mM and the inhibition constant (K_i) of PEPC was 20.8 mM.

4. Discussion

It has been reported that CETB (Muranushi et al., 1989; Yoshikawa et al., 1989) and CFIX (Tsuji et al., 1987b) as well as amino-β-lactams (Oh et al., 1993; Tsuji and Tamai, 1996) are transported across intestinal BBM via the oligopeptide transporter. According to our study, the transport of CETB into rat intestinal BBMV showed an overshoot phenomenon in the presence of an inwardly directed H^+ gradient (pH_{in} 7.5 and pH_{out} 5.5), and the uptake amount and transport characteristics of CETB were similar to those reported previously (data not shown; Yoshikawa et al., 1989). Transport characteristics of L-lactic acid in the present study were also similar to those reported previously (Storelli et al., 1980; Tiruppathi et al., 1988). Moreover, the enrichment factors of the marker enzymes were comparable to those reported by other groups (Yoshikawa et al., 1989; Simanjuntak et al., 1990; Tamai et al., 1995). Therefore, it was considered that the membrane vesicles were well prepared in the present study and that both oligopeptide and monocarboxylic acid transport systems are present in our BBMV preparations.

The uptake of PEPC into BBMV showed an overshoot in the presence of an inwardly directed H^+ gradient (pH_{in} 7.5 and pH_{out} 5.5) (Fig. 1), and PEPC was transported into the osmotically active space inside the vesicles (Fig. 2). On the other hand, the initial uptake rate increased with an decrease of the extravesicular pH (Fig. 3), whereas the presence of FCCP reduced the uptake of PEPC (Fig. 4). All these observations supported that PEPC is transported uphill into BBMV and that the transport is driven by a H^+ gradient.

Stereoselective transport via the oligopeptide transporter into intestinal BBMV is reported for certain β-lactam antibiotics. It is reported that ceftibuten, the cis-isomer, has higher affinity to the oligopeptide transporter than its trans-isomer (Yoshikawa et al., 1989) and that L-CEX exhibits greater affinity than D-CEX (Tamai et al., 1988). Moreover, the uptake of cephalexin, loracarbef and oligopeptides into Caco-2 cells are also stereoselective (Thwaites et al., 1994; Wenzel et al., 1995). Transport via the monocarboxylic acid transporter(s) is also stereoselective since the

transport rate differs between L- and D-lactic acids (Ogihara et al., 1996). In contrast, stereoselectivity in the transport of PEPC into BBMV appears to be small (Fig. 1).

The apparent Michaelis constant values (K_m = 17.9 and 20.5 mM for R-PEPC and S-PEPC, respectively) seemed to be greater than those reported for other β-lactams that are transported by the oligopeptide transporter; the reported K_m values are 0.17, 0.83 and 0.79 mM for CETB, CFIX and loracarbef, respectively (Tsuji et al., 1987b; Yoshikawa et al., 1989; Hu et al., 1994). On the other hand, the K_m values of PEPC epimers appear to be similar to those of the substrates of the monocarboxylic acid transporter(s); the reported K_m values are 4.43 mM for nicotinic acid in rat intestinal BBMV (Simanjuntak et al., 1990) and 12.7 and 15.2 mM for L-lactic acid and pravastatin, respectively, in rabbit intestinal BBMV (Tiruppathi et al., 1988; Tamai et al., 1995). It should be noted that the K_m values obtained in the present study are apparent values since the transport was measured in the presence of the other epimer. It will be necessary to obtain the true parameter values in the absence of the other epimer.

Studies on the inhibitory effects of various compounds on the uptake of PEPC revealed that none of the oligopeptides nor the compounds that are the substrates of the oligopeptide transporter inhibit the uptake of PEPC (Table 1). Since the inhibitor concentrations were much greater than the reported K_m values of these inhibitors, it was concluded that PEPC may not be transported by the oligopeptide transporter.

The uptake of PEPC into BBMV was reduced in the presence of acetic, salicylic, L-lactic or D-lactic acids (Table 1). Since these acids are the substrates of the monocarboxylic acid transporter(s) (Tiruppathi et al., 1988; Simanjuntak et al., 1990; Tamai et al., 1995; Tsuji and Tamai, 1996), the results indicated that PEPC may be transported into BBMV via the monocarboxylic acid transporter(s). It has been suggested quite recently that the monocarboxylic acid transporter(s) is responsible for the intestinal absorption of various anionic compounds. Since PEPC exists as a mono-anion at the physiological pH, it is highly likely that PEPC is transported by the monocarboxylic acid transporter(s) located at the intestinal brush border membrane.

Assuming a competitive inhibition, the extent of inhibition was calculated with the K_i value of L-lactic acid being equal to the K_m value (i.e. 69.7 mM). The uptake of PEPC in the presence of 40 mM L-lactic acid was predicted to be 65% of the control, which was similar to the observed uptake (69.7%) in the presence of L-lactic acid (Table 1). The result further supports that PEPC and L-lactic acid share the common transport system.

The inhibitory effects of L- and D-lactic acids on PEPC uptake were similar (Table 1), which appeared to disagree with the stereoselective transport of L- and D-lactic acids (Ogihara et al., 1996). However, the present results are consistent with the results on the inhibitory effects of L- and D-lactic acids on the uptake of mevalonic acid (Tamai et al., 1997), where similar inhibitory effects were observed for L- and D-lactic acids.

PPPC and CIPC, orally active β-lactams, inhibited the uptake of PEPC significantly (Table 1). According to our study, PPPC and CIPC also inhibited the L-lactic acid uptake and the inhibitory effects were in the order of PEPC < PPPC < CIPC (data not shown). Moreover, uptake of PPPC and CIPC showed overshoot in the presence of a H^+ gradient, suggesting that both β-lactams are actively transported into BBMV (data not shown). Therefore, these β-lactams also appear to be transported via the monocarboxylic acid transporter(s). Studies are underway to clarify further the transport characteristics of PPPC and CIPC.

The uptake of L-lactic acid in the presence of a H^+ gradient showed an overshoot (Fig. 6), and the uptake amount was similar to those reported previously (Storelli et al., 1980; Tiruppathi et al., 1988). The uptake of L-lactic acid was also measured in the absence or presence of PEPC. The results clearly showed that PEPC competitively inhibits the uptake of L-lactic acid (Fig. 7), with the inhibition constant (20.8 mM) being similar to the K_m values of PEPC (17.9 and 20.5 mM for R-PEPC and S-CBPC, respectively, as described above). These results strongly supported that PEPC and L-lactic acid share the common trans-

port system, i.e. the monocarboxylic acid transporter(s). Moreover, PEPC inhibited the uptake of acetic acid, another typical substrate of the monocarboxylic acid transporter(s), into BBMV (data not shown).

On the other hand, the K_m value of L-lactic acid in the present study was greater than the reported values. The reported K_m values are 12.7, 39.2 and 3.75 mM for rabbit brush border membrane vesicles (Tiruppathi et al., 1988), rat basolateral membrane vesicles (Orsenigo et al., 1997) and Caco-2 cells (Ogihara et al., 1996), respectively. Since the K_m value of L-lactic acid for rat intestinal brush border membrane has not been reported, we are unable to compare our value with the literature value. It is possible that the affinity of L-lactic acid to the monocarboxylic acid transporter(s) of rat intestinal BBM may be smaller than those of other animal species.

The involvement of the monocarboxylic acid transporter(s) is reported for the intestinal absorption of various anionic drugs (Tsuji and Tamai, 1996), including cefdinir which is an orally active β-lactam. It is suggested that both oligopeptide and monocarboxylic acid transporters are involved in the intestinal absorption of cefdinir (Tsuji et al., 1993). Since PEPC significantly inhibited the uptake of CETB in our study (data not shown), PEPC may also possess affinity to the oligopeptide transporter. This observation is consistent with the fact that PPPC, another β-lactam which is similar to PEPC in chemical structure, inhibits the transport of CFIX (Tsuji et al., 1987a). However, it is clearly shown in the present study that the monocarboxylic acid transporter(s) is mainly responsible for the transport of PEPC. Further studies will be needed to elucidate if the oligopeptide transporter contributes to the intestinal absorption of PEPC to any extent.

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