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KINETICS AND MECHANISM OF *IN VITRO* UPTAKE OF AMINO-β-LACTAM ANTIBIOTICS BY RAT SMALL INTESTINE AND RELATION TO THE INTACT-PEPTIDE TRANSPORT SYSTEM

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Abstract—By utilizing the everted jejunum of rats, the initial uptake rates of several antibiotics were measured over a wide range of concentrations. The uptakes followed mixed-type kinetics involving saturable and non-saturable processes in parallel. The pertinent kinetic parameters for the uptake of each antibiotic were determined. The effect of cephalexin on the uptake of cyclacillin obeyed competitive inhibition kinetics, and the inhibition constant K_i was found to be equal to the Michaelis constant K_t for the uptake of cephalexin itself. In a similar way, the uptake of cephalexin was inhibited by cyclacillin. Uptakes of both cyclacillin and cephalexin were reduced significantly by several metabolic inhibitors. From the effect of temperature on the uptakes of cyclacillin and cephalexin, activation energies of 24.8 and 23.1 kcal/mole were obtained respectively. These results indicate the involvement of an active transport mechanism for cyclacillin and cephalexin. It was found that several dipeptides markedly inhibited the uptakes of cyclacillin and cefadroxil. Furthermore, the uptake of glycylglycine, a typical dipeptide, was inhibited by cyclacillin, cefadroxil, cephalexin, and cephradine. The kinetics of mutual inhibition of the uptakes of cyclacillin and glycylglycine were consistent with competitive-type inhibition. This is the first report which establishes, from a kinetic point of view, the involvement of a common transport system in the *in vitro* uptakes of the dipeptides and the antibiotics.

Numerous studies on the GI absorption of amino-β-lactam antibiotics have been conducted by an *in vitro* technique employing rat everted sacs or an *in situ* perfusion technique in rat intestine [1–15]. These studies have established various characteristics of the intestinal transport of these antibiotics. Kinetic studies in our laboratory [11–15] utilizing the *in situ* rat intestinal perfusion technique indicated that amino-β-lactam antibiotics, such as amoxicillin, cyclacillin, cefadroxil, cefaclor,† cefatrizine,† cefroxadine,† cephalexin and cephradine are absorbed by a carrier-mediated process. The mutual inhibitions in the *in situ* absorptions of these antibiotics were interpreted successfully in terms of competitive inhibition kinetics in the transport process.† It was also found that cyclacillin and cephalexin markedly inhibited the absorption of a dipeptide, carnosine.† These results suggest that the transport system for amino-β-lactam antibiotics is closely related to that for dipeptides. Quay [16] and Addison *et al.* [17] also noted an inhibitory effect of cephalexin on the transport of phenylalanylglycine and glycylsarcosylsarcosine.

The present investigation was undertaken to determine accurately the kinetics of intestinal transport of amino-β-lactam antibiotics by measuring the *in vitro* uptake of the substrate into the everted intestinal tissue of the rat jejunum. The major goals of this work were (1) to show whether our claim [11–15] that amino-β-lactam antibiotics are transported mainly via a carrier-mediated process is correct, (2) to elucidate whether these antibiotics share a common carrier system, and (3) to clarify the characteristics of the carrier system and its relation with the dipeptide transport system.

MATERIALS AND METHODS

Chemicals

Amoxicillin and cefazolin (Fujisawa Pharmaceutical Co., Osaka, Japan), cyclacillin (Takeda Chemical Ind., Osaka, Japan), cefaclor and cephalexin (Shionogi Co., Osaka, Japan), cefatrizine (Banyu Pharmaceutical Co., Tokyo, Japan), cefroxadine (Ciba Geigy, Takarazuka, Japan), cefadroxil (Bristol Myers Co., Tokyo, Japan), and cephradine (Sankyo Co., Tokyo, Japan) were gifts from the cited companies. [^{14}C]Inulin (2.1 mCi/g), [^{3}H]inulin (174 μCi/g), and [^{14}C]glycylglycine (54 mCi/g) were purchased from Amersham International Ltd., Buckinghamshire. All other chemicals were of the highest grade available commercially.

Animals

Male albino Wistar rats, 220 ± 20 g, were fasted for 20 hr prior to the experiment, but water was given freely.

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† E. Nakashima, A. Tsuji, S. Kagatani and T. Yamana, *J. Pharmacobio-Dynamics* **7**, 452 (1984).

Preparations

Everted segments of the jejunum isolated from rats were used in all *in vitro* experiments. The methods of surgical operations and fixing of the everted intestine on a polyethylene tube were those described by Hoshi *et al.* [18]. The animals were anesthetized with ether, and the proximal portion of the jejunum, about 30 cm long, was excised. During the surgical operation, anesthesia was maintained by ether inhalation. The isolated portion was everted in the usual way, divided into small segments of 4 cm length, and fixed over a polyethylene tube of 4 mm outer diameter and about 4.5 cm length. Each polyethylene tube bore two lines at a distance of 3 cm, and the everted intestine was tied with cotton threads at the portions of these marks. A 20-cm length thread was ligated at the edge of intestine in order to take up the intestine immediately from the medium at the desired time.

The everted intestinal segments thus prepared were preincubated in a standard buffer solution (having the composition described below) under O_2 at 37° for 5 min. After preincubation, the intestine was immediately taken up and placed in 25 ml of antibiotic medium in a 100-ml volumetric flask. During the experiment, the flask was shaken reciprocally at the rate of 100 times/min. During the incubation, O_2 was supplied at a constant rate (60 ml/min).

In the present study, the following solution was used as the standard buffer solution: sodium chloride, 129.18 mM; potassium chloride, 5.09 mM; calcium chloride, 1.364 mM; potassium phosphate, monobasic, 1.273 mM; magnesium sulfate, 1.273 mM in 10 mM phosphate buffer at pH 7.0. Upon completion of the incubations, the tissues were washed in ice-cold isotonic solution for exactly 5 sec, blotted on filter paper, and then used for assay. In the study of the effect of metabolic inhibitors, the preincubation was omitted, and the incubation was performed for 10 min.

Analytical procedures

For the determination of antibiotics, the intestinal tissue was cut off at the marks from the polyethylene tube and was homogenized in a homogenizer (Ultra-Turrax, Ika-Werk, Janke & Kunkel) with saline to give a 20% (w/v wet weight) homogenate. After centrifugation at 15,600 *g* for 10 min, the amount of cyclacillin or cephalexin in the supernatant fraction was determined by microbiological assay as described previously [15].

For the assay of the samples containing two antibiotics in the mutual inhibition study, the uptake amount in the supernatant fraction was determined after deproteinization with saturated ammonium sulfate, by both high-performance liquid chromatographic (HPLC) and/or fluorometric assays as described below. Ammonium sulfate (0.7 g) was added to 1 ml of the supernatant fraction, and the mixture was allowed to stand at 4° for 10 min. After being shaken at 4° for 15 min, the mixture was allowed to stand at 4° for 5 min. Then deproteinized supernatant fluid was obtained by centrifugation at 4° and 15,600 *g* for 10 min. The supernatant fraction was used for HPLC assay.

The liquid chromatograph (model Trirotor-II, Japan Spectroscopic Co., Tokyo, Japan) was equipped with a u.v. detector (model UVIDEC 100-III, Japan Spectroscopic Co.) set at 210 and 254 nm for cyclacillin and aminocephalosporins respectively. A chromatograph equipped with a reversed phase column (4.0 mm i.d. × 30 cm, μ-Bondapack C_{18}, Water Associates, Milford, MA) packed in this laboratory was used. The mobile phase was 1–6% acetonitrile–10 mM ammonium acetate. The injection volume was 50 μl. Peak height was used for quantification. Standard curves were generated after similar deproteinization with saturated ammonium sulfate by the use of blank tissue samples containing known amounts of the antibiotics.

The reported fluorometric method [19] was used with a slight modification for tissue samples containing cefadroxil, cefroxadine, cephalexin, and cephradine in the presence of cyclacillin. A sample corresponding to 0.2 g of tissue was deproteinized with 3 ml of 3% trichloroacetic acid. After centrifugation at 1700 *g* for 10 min, an aliquot of the supernatant (2 ml) was used for determining extracellular space, and another (0.5 ml) was used for the determination of antibiotics. The fluorescent products were obtained by treatment of the sample as described by Aikawa *et al.* [19]. The fluorescent products were extracted with a disposable extraction column (Bond Elute C_{18}, Analytichem International, Harbor City, CA) instead of with an organic solvent. The fluorescent product obtained from the column was washed with 2 ml of 0.2 N HCl and then partitioned twice back into 2 ml of a methanol–borate buffer (1:1, v/v) mixture by centrifugation at 190 *g*. The borate buffer was prepared as described elsewhere [19]. Furthermore, the column was washed with 2 ml of the borate buffer, and the total solution recovered by centrifugation was assayed. Fluorescence intensity was measured by a model FP-4 fluorescence spectrophotometer (Japan Spectroscopic Co.) with excitation at 360, 355, 355, and 350 nm, and emission at 450, 440, 435, and 440 nm, for cefadroxil, cefroxadine, cephalexin, and cephradine respectively. The measured fluorescence intensity of cephalexin determined by the present column extraction method was 102.0 ± 1.5% of the value obtained by the original assay method [19], and the S.E.M. was very much smaller than in the latter case.

Radiochemical assay

Radioactivity of [^{14}C]inulin, [^{3}H]inulin, and [^{14}C]glycylglycine was determined by direct liquid scintillation counting in vials containing 10 ml of dioxane-based scintillation fluid. Quenching was corrected by the external standard method. Tissue samples were oxidized with a sample oxidizer (model ASC-113, Aloka Co., Tokyo, Japan) to $^{14}CO_2$, and radioactivity was determined by liquid scintillation counting (model LSC-651 liquid scintillation counter, Aloka Co.).

Calculation of results

Uptake was expressed as moles(min · g wet tissue weight)$^{-1}$ after correction of the concentration of antibiotics or peptide on the basis of inulin space

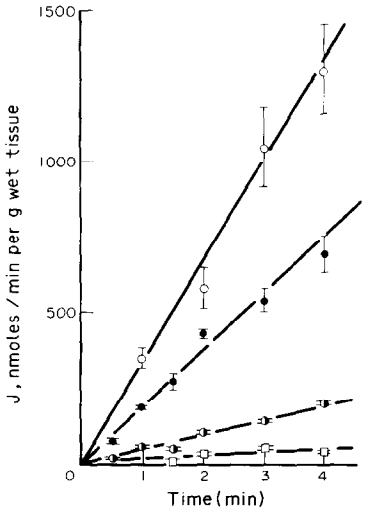

Fig. 1. Time course of the uptake of cyclacillin (●), cefazoline (□), cephalexin (◑), and glycylglycine (○) by everted jejunum of rats. The antibiotic concentration was 1 mM. Each point is the mean ± S.E.M. of at least three determinations. The solid lines were drawn by the least-squares method.

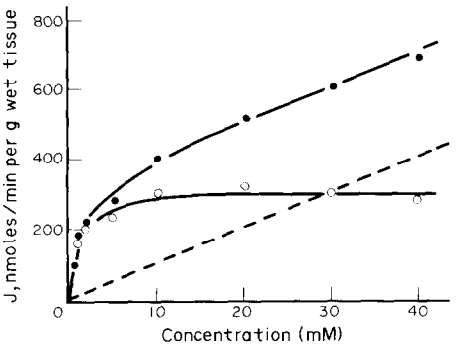

Fig. 2. Influence of cyclacillin concentration in the incubation medium on the uptake by isolated everted jejunum of rat. The curves were generated from equation 1, using the NONLIN fitted parameters listed in Table 1. Solid lines indicate the total uptake (●) and mediated uptake (○). The dotted line indicates simple diffusion. Each point is the mean of at least three experiments.

[20]. All estimations of uptake were made from the mean values of uptake obtained with at least three preparations. The S.E.M. of uptake was usually about 15%.

RESULTS

Kinetic properties of drug uptake

Time courses. Time courses of uptake into the rat small intestinal tissue were examined for cyclacillin, cefazolin, cephalexin, and glycylglycine. Figure 1 shows that the uptake amounts of these compounds increased linearly with time up to at least 4 min. Therefore, in the subsequent experiments, a 2-min incubation was adopted to determine the initial uptake of cyclacillin or glycylglycine, and 4 min for cephalexin. As can be seen in Fig. 1, glycylglycine was taken up most rapidly, cyclacillin less rapidly, and cephalexin very much less rapidly than the other two compounds. As expected, the uptake of cefazolin, which has no amino group and is poorly absorbed by the oral route, was almost negligible.

Concentration dependency of initial uptake. Uptake rates of the antibiotics (cyclacillin, cephalexin) and a peptide (glycylglycine) were measured over the concentration range up to 40 mM. Figure 2 shows the typical plot of relationship between the initial uptake rate and concentration for substrate in the medium for cyclacillin (the values are means of four to five experiments). Similar results were obtained for the other two compounds. As noted previously from studies of the *in situ* absorption kinetics [11–15], the initial uptake rates of the antibiotics and glycylglycine across the *in vitro* everted jejunum can be expressed by the following equation:

$$J = \frac{J_{max}[S]}{K_t + [S]} + K_d[S] \quad (1)$$

where J is the initial uptake rate, $[S]$ is the initial concentration of substrate in the medium, J_{max} is the maximum uptake rate through a carrier-mediated process, K_t is the Michaelis constant, and K_d is the coefficient for non-mediated and passive uptake. Himukai *et al.* [21] reported that *in vitro* uptake of glycylglycine into the intestine of the guinea pig includes two kinetic components of transport, in accordance with equation 1. The solid lines in Fig. 2 were generated by using equation 1 with the NONLIN [22] fitted parameters, and it is clear that equation 1 gives an excellent description of the experimental data on uptake into the everted rat gut. The obtained values of J_{max}, K_t and K_d are listed in Table 1.

To obtain the carrier-mediated kinetic component

Table 1. Kinetic parameters for the *in vitro* rat intestinal uptake of cyclacillin, cephalexin, and glycylglycine

Substrate	Michaelis–Menten kinetic parameters		First-order rate constant
	K_t (mM)	J_{max} (nmoles/min/g wet tissue)	K_d (nmoles/min/g wet tissue/mM)
Cyclacillin	1.15 ± 0.23	316 ± 34	10.2 ± 1.5
Cephalexin	6.85 ± 1.54	286 ± 60	13.9 ± 1.5
Glycylglycine	6.02 ± 1.07	2770 ± 370	20.3 ± 8.2

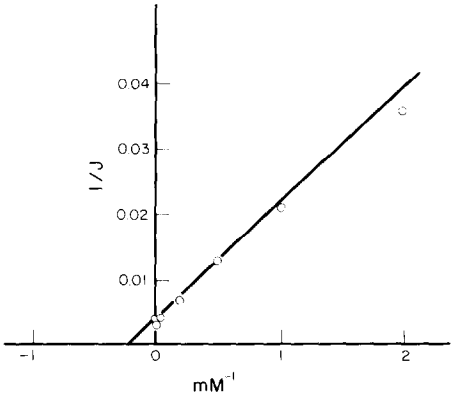

Fig. 3. Lineweaver–Burk plots of uptake of cephalexin. The values are corrected for non-mediated uptake. The line was calculated using the parameters listed in Table 1. Each point is the mean of at least three determinations.

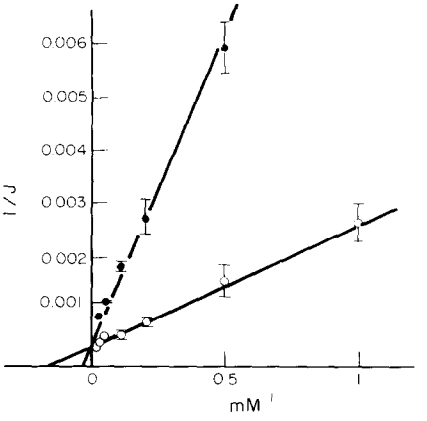

Fig. 5. Lineweaver–Burk plots of uptake of glycylglycine in the absence (○——○) and in the presence (●——●) of 5 mM cyclacillin. The values are corrected for non-mediated uptake. The line for the control experiment was calculated using the parameters listed in Table 1. The line for uptake in the presence of cyclacillin is a weighted least-squares regression. Each point is the mean ± S.E.M. of at least three determinations.

of the three compounds, all data were corrected for the respective K_d values. After the correction, the uptake rates of the two antibiotics and glycylglycine appeared to conform to a simple Michaelis–Menten kinetic process, as indicated by the linear Lineweaver–Burk plots (Figs. 3–5).

Inhibition between permeants

Mutual inhibition kinetics between cephalexin and cyclacillin. Uptake of cyclacillin was determined in the presence of cephalexin (10–40 mM) over the cyclacillin concentration range of 0.5–40 mM. The Lineweaver–Burk plots of uptake of cyclacillin in the absence and presence of cephalexin after correction for the simple diffusion component are illustrated in Fig. 4. Cephalexin produced an increase of apparent K_t for cyclacillin without altering J_{max} (Fig. 4). The Dixon plot is shown in Fig. 6, giving a linear relationship.

Mutual inhibition kinetics between cyclacillin and glycylglycine. The results of a mutual inhibition study of cyclacillin and glycylglycine are depicted in Fig. 5 and Figs. 7–9 as Lineweaver–Burk plots and Dixon plots. In the presence of cyclacillin, glycylglycine uptake was inhibited competitively (Fig. 5), since the effect of cyclacillin was to increase apparent K_t for glycylglycine without altering J_{max}.

Furthermore, in the presence of glycylglycine, cyclacillin uptake was inhibited competitively (Fig. 7). The K_i values of glycylglycine for cyclacillin uptake were 1.45 and 2.5 mM for 10 and 5 mM glycylglycine respectively. That of cyclacillin for gly-

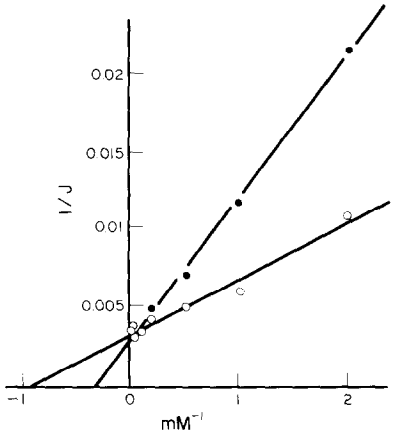

Fig. 4. Lineweaver–Burk plots of uptake of cyclacillin in the absence (○——○) and in the presence (●——●) of cephalexin (10 mM). Values are corrected for non-mediated uptake. The line for the control experiment was calculated using the parameters listed in Table 1. The line for uptake in the presence of cephalexin is a weighted least-squares regression.

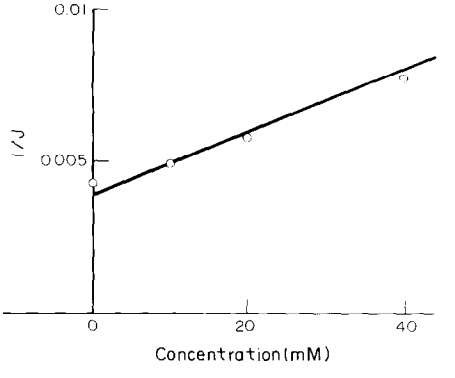

Fig. 6. Dixon plots of uptake of cyclacillin by the everted jejunum of rat as a function of cephalexin concentration. The values are corrected for non-mediated uptake. Each point is the mean of three to four determinations. The line was calculated using the parameters listed in Table 1 on the assumption that the uptake of cyclacillin was inhibited competitively in the presence of cephalexin.

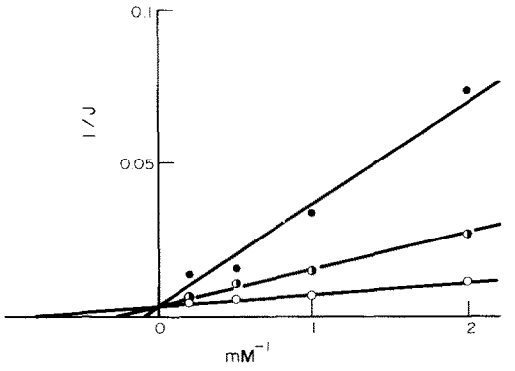

Fig. 7. Lineweaver–Burk plots of uptake and cyclacillin in the absence (○) or in the presence of 5 mM (◑) and 10 mM (●) glycylglycine. Values are corrected for non-mediated uptake. The line for the control experiment was calculated using the parameters listed in Table 1. The lines for uptake in the presence of glycylglycine are weighted least-squares regression lines. Each point is the mean of at least three determinations.

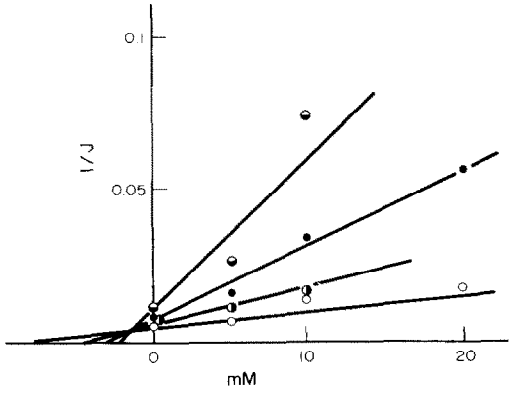

Fig. 8. Dixon plots of uptake of cyclacillin by the everted jejunum of rat as a function of glycylglycine concentration. The values are corrected for non-mediated uptake. The lines are weighted least-squares regression lines. The concentrations of cyclacillin are 0.5 mM (◒), 1.0 mM (●), 2.0 mM (◑), and 5.0 mM (○). Each point is the mean of three determinations.

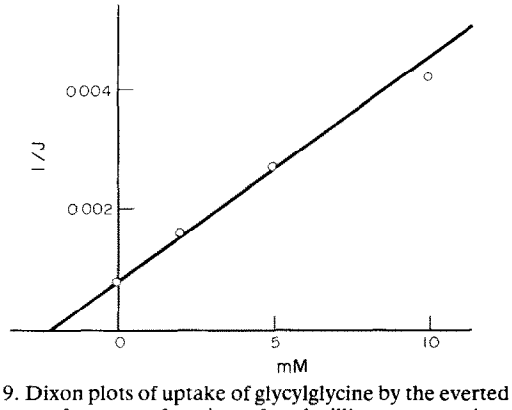

Fig. 9. Dixon plots of uptake of glycylglycine by the everted jejunum of rat as a function of cyclacillin concentration. The values are corrected for non-mediated uptake. Each point is the mean ± S.E.M. of three to four determinations. The line was calculated using the parameters listed in Table 1 on the assumption that the uptake of glycylglycine was inhibited competitively in the presence of cyclacillin.

Table 2. Effects of cyclacillin (10 mM) on the uptake of aminocephalosporins (1 mM) by the everted jejunum of rat*

Substrate	Inhibitor	J (nmoles/min/g wet tissue)	P
Cefradroxil	None	95.9 ± 14.6 (3)	
	Cyclacillin	20.7 ± 3.5 (3)	<0.01
Cefroxadine	None	105.0 ± 11.0 (3)	
	Cyclacillin	17.5 ± 2.9 (4)	<0.01
Cephalexin	None	41.4 ± 9.1 (4)	
	Cyclacillin	4.5 ± 2.6 (4)	<0.01
Cephradine	None	62.2 ± 12.1 (4)	
	Cyclacillin	10.8 ± 1.2 (4)	<0.01

* Each result is the mean ± S.E.M. with the number of experiments in parentheses.

cylglycine uptake was 1.06 mM. This value is in good agreement with the K_t value of cyclacillin as listed in Table 1.

Effect of cyclacillin on aminocephalosporin transport. Uptakes of 1 mM cefadroxil, cefroxadine, cephalexin, and cephradine were determined in the presence of 10 mM cyclacillin. The results are shown in Table 2. The uptakes of cephalosporins were strongly inhibited by cyclacillin ($P < 0.01$). The extent of inhibition of cephalexin uptake by cyclacillin agrees well with the expected inhibition predicted by the method of Finch and Hird [23].

Effect of dipeptides on the uptakes of aminocephalosporins. To investigate the relationship of the transport systems for antibiotics and dipeptides, the effect of glycylglycine on the uptake of aminocephalosporins was studied.

Table 3 shows the uptake of 1 mM cefadroxil and cephalexin in the presence of glycylglycine. Uptake of the two aminocephalosporins was inhibited significantly by glycylglycine ($P < 0.02$). As shown in Tables 3 and 4, all the dipeptides tested in this study significantly inhibited the uptake of cyclacillin and cefadroxil.

Effect of the antibiotics on the uptake of glycylglycine. It was shown that the uptake of [^{14}C]glycylglycine was decreased significantly in the presence of 30 mM cyclacillin, cefadroxil, cephalexin, or cephradine ($P < 0.01$) (Table 5). In the case of cefroxadine, no significant inhibitory effect was

Table 3. Effects of glycylglycine (30 mM) on the uptake of aminocephalosporins (1 mM) by the everted jejunum of rat*

Substrate	Inhibitor	J (nmoles/min/g wet tissue)	P
Cefadroxil	None	95.9 ± 14.6 (3)	
	Glycylglycine	18.9 ± 8.7 (3)	<0.02
Cephalexin	None	41.4 ± 9.1 (4)	
	Glycylglycine	4.5 ± 1.2 (4)	<0.01

* Each result is the mean ± S.E.M. with the number of experiments in parentheses.

Table 4. Effects of dipeptides (30 mM) on uptake of cyclacillin (1 mM) and cefadroxil (1 mM) by the everted intestine of rats*

Inhibitor	J (mmoles/min/g wet tissue) Cyclacillin	Cefadroxil
None	156.5 ± 15.1	95.9 ± 14.7
Gly-Leu	6.4 ± 5.5†	7.2 ± 12.4‡
Gly-Sar	19.0 ± 2.3†	26.5 ± 7.3‡
Leu-Gly	2.7 ± 9.5†	13.4 ± 6.8‡
Phe-Gly	9.6 ±10.6†	22.8 ± 9.0†
Carnosine	31.3 ± 9.7†	21.6 ±17.3†

* Each result is the mean ± S.E.M. of three determinations. Gly-leu, glycylleucine, Gly-Sar, glycylsarcosine; Leu-Gly, laucylglycine; and Phe-Gly, phenylalanylglycine.
† $P < 0.01$.
‡ $P < 0.05$.

Table 6. Effects of metabolic inhibitors on the uptake of cyclacillin (1 mM) and cephalexin (1 mM) by the everted intestine of rats*

Inhibitor	J (mmoles/min/g wet tissue) Cyclacillin	Cephalexin
None	1.51 ± 0.21	0.523 ± 0.057
Sodium azide (30 mM)	0.847 ± 0.079†	0.225 ± 0.042†
2,4-Dinitrophenol (0.5 mM)	0.498 ± 0.076†	0.268 ± 0.098‡
Sodium cyanide (5 mM)	0.688 ± 0.021†	0.285 ± 0.107‡

* Each result is the mean ± S.E.M. with the number of experiments in parentheses.
† $P < 0.01$.
‡ $P < 0.05$.

observed, presumably due to the limited solubility of the drug.

Effects of metabolic inhibitors and temperature effect. The effects of metabolic inhibitors and of temperature on the uptakes of cyclacillin and cephalexin were examined with an initial concentration of 1 mM antibiotic in the medium. The effects of metabolic inhibitors are shown in Table 6. There was marked inhibition in the uptakes of both antibiotics by 0.5 mM 2,4-dinitrophenol, 5 mM sodium cyanide, and 30 mM sodium azide when compared with the control uptakes ($P < 0.05$).

The uptake rates of cyclacillin and cephalexin at a 1 mM concentration were measured at four different temperatures from 21 to 37° at pH 7.0. The Arrhenius plots are shown in Fig. 10. From these slopes, the apparent activation energies of the initial uptakes were determined to be 24.8 and 23.1 kcal/mole for cyclacillin and cephalexin respectively.

DISCUSSION

In our previous papers [11–15], it was reported that the disappearance rates of aminopenicillins, such as amoxicillin and cyclacillin [11, 12, 14, 15], and aminocephalosporins, such as cefaclor,§ cefadroxil,§ cefatrizine,§ cefroxadine,§ cephalexin§ [13], and cephradine§ [13], from the rat *in situ* intestinal perfusate could be described by mixed-type kinetics consisting of Michaelis–Menten and first-order kinetic processes. The present results show that similar kinetics were applicable to the *in vitro* initial uptake into rat small intestinal tissues (Fig. 2).

Cyclacillin and cephalexin have the same K_d value *in vitro*, as expected from the fact that both antibiotics have similar molecular weights and structures. It is likely that the uptake corresponding to the K_d term is a passive diffusion driven by a concentration gradient. To clarify the nature of the saturable mechanism, it is necessary to subtract this first-order uptake rate from the total disappearance rate.

Lineweaver–Burk type plots and a Dixon plot for the saturable uptake for both antibiotics after such correction of the data revealed that cyclacillin and cephalexin share a common transport system. The extent of inhibition of cyclacillin uptake by

Table 5. Effects of antibiotics (30 mM) on the uptake of glycylglycine (1 mM) by the everted intestine of rats*

Inhibitor	J (nmoles/min/g wet tissue)	P
None	400.0 ± 20.1 (5)	
Cyclacillin	44.0 ± 1.2 (3)	<0.01
Cefadroxil	181.8 ± 6.6 (3)	<0.01
Cefroxadine†	358.4 ± 24.8 (3)	NS‡
Cephalexin	239.1 ± 12.5 (3)	<0.01
Cephradine	227.2 ± 13.6 (3)	<0.01

* Each value is the mean ± S.E.M. with the number of experiments in parentheses.
† Cefroxadine (5 mM) was used because of its limited solubility.
‡ Not significant.

§ E. Nakashima, A. Tsuji, S. Kagatani and T. Yamana, manuscript submitted for publication.

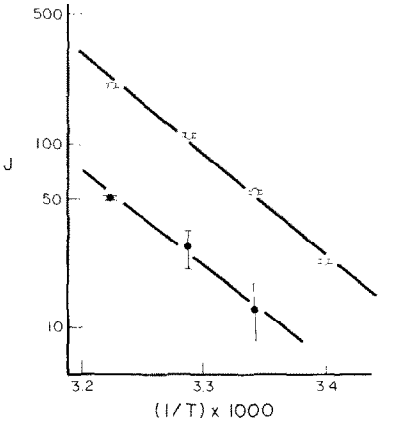

Fig. 10. Temperature dependences of uptake of cyclacillin (○) and cephalexin (●) by the everted jejunum of rat. Each vertical bar represents the S.E.M. of at least three determinations. The solid lines were drawn by a weighted nonlinear least-squares method.

cephalexin agreed well with the value predicted by the method of Finch and Hird [23]. The K_i for inhibition of uptake of cyclacillin by cephalexin was 6.89 mM (Fig. 4). This value is equal to the K_t (6.85 mM) of cephalexin itself (Fig. 3). Since the Dixon plot was linear and the K_i value was equal to K_m of cephalexin, it can be concluded that cephalexin inhibited the uptake of cyclacillin competitively over the cephalexin concentration range of 0–40 mM (Fig. 6).

All the findings obtained in this kinetic study on the uptakes of cyclacillin and cephalexin are consistent with the view that both antibiotics are transported across the rat small intestine by a carrier-mediated and active system. There is already much evidence showing that cyclacillin is absorbed actively through the rat small intestine; this is probably because cyclacillin is rapidly transported across the intestine and thus the carrier-mediated uptake can be determined rather easily. However, other amino-β-lactam antibiotics exhibit relatively slow absorption rates, and their absorption mechanisms are, therefore, less easy to identify. For example, discrepant results have been reported from different laboratories regarding the absorption mechanism of cephalexin. Quay [1, 16] concluded that cephalexin is actively transported across the *in vitro* rat jejunum from the findings that cephalexin and *l*-phenylalanylglycine competed at the intestinal tissue site and that the unidirectional flux of cephalexin transport was inhibited by 2,4-dinitrophenol, a metabolic inhibitor. In contrast, Kimura *et al.* [6] reported that cephalexin was transported into the intestinal tissue passively. Yasuhara *et al.* [4] and Kimura *et al.* in their most recent study [10] found no saturation in the absorption of cephalexin and no effect of *l*-phenylalanylglycine on the absorption in *in situ* perfusion experiments. Since the ratios of serosal-to-mucosal concentration (S/M ratio) were lower than unity, Yasuhara *et al.* and Umeniwa *et al.* claimed that ampicillin [7], amoxicillin [7], and cephalexin [4, 7] were transported passively, even through the tissue accumulation of cephalexin was inhibited significantly by 2, 4-dinitrophenol. One reason for these discrepancies regarding the absorption mechanism of amino-β-lactam antibiotics could be that the most investigations have been carried out at one concentration only, or at a very limited number of concentrations. As far as we know, there has been no kinetic study to explore the transport mechanism by measuring the initial uptake rates. If a transport experiment is carried out at a concentration where the contribution of simple diffusion to the total transport is much greater than that of the mediated transport, it is difficult to identify the mediated and active transport. In the present kinetic study, we therefore selected suitable experimental conditions on the basis of the kinetic parameters of the mediated and non-mediated transport processes. The apparent activation energies of intestinal uptakes of both cyclacillin and cephalexin at a 1 mM concentration were so high that passive diffusion could be ruled out in relation to this uptake. Furthermore, the remarkable effects of metabolic inhibitors on the uptake of cyclacillin and cephalexin indicate strongly that the two antibiotics were transported actively.

The present finding of the involvement of active transport in cephalexin uptake is consistent with that of Quay [1, 16] and inconsistent with those of Yasuhara *et al.* [4] and Umeniwa *et al.* [7]. The discrepancy from other investigations [4, 7] may be attributed to the fact that cephalexin has low affinity to the carrier (K_t = 6 mM) and thus the saturable phenomena may be very difficult to detect, depending on the experimental system. Furthermore, the *in vitro* everted sac method sometimes gives unreliable S/M ratios because of the difficulty of transport across the gut muscle, as stated previously by Perrier and Gibaldi [24].

The uptake system for cyclacillin was closely related to the dipeptide transport system. As shown in Table 4, the uptake of cyclacillin was remarkably uninhibited by several dipeptides. In the case of cefadroxil, which has a larger disappearance rate from the *in situ* intestine [15] than cephalexin, a similar inhibitory effect was obtained.

The mutual inhibition between the uptake of cyclacillin and that of the dipeptide glycylglycine was competitive. Although the K_t value of glycylglycine was slightly different from the values reported previously for the guinea pig intestinal uptake [18], there is a good agreement between the K_i and K_t values of cyclacillin for the inhibition of the uptake of glycylglycine. Uptakes of other aminocephalosporins such as cefadroxil, cefroxadine, cephalexin, and cephradine were inhibited significantly by glycylglycine (Table 3). Furthermore, animocephalosporins inhibited the uptake of glycylglycine (Table 5). Since it was reported that almost all dipeptides are transported across the brush border by a single transport system [25, 26], it is reasonable to consider that amino-β-lactam antibiotics, which are absorbed well after oral administration, are absorbed via the dipeptide transport system. The present results thus led us to conclude that the amino-β-lactam antibiotics, cyclacillin, cefadroxil, cefroxadine, cephalexin and cephradine, share a single mediated transport mechanism both *in situ* and *in vitro*, and that this transport system is identical, as suggested previously by Quay [1, 16] and Addison *et al.* [17], with the dipeptide transport system.

Whether or not such a carrier-mediated transport system is present in the human intestine has not been clarified as yet. It seems reasonable, however, to speculate that the complete absorption of amoxicillin [27], cefadroxil [28], cefroxadine [28], cephalexin [29], and cephradine [29], and 80% absorption of cyclacillin [30] after oral administration in humans may be due to the contribution of a saturable carrier system rather than simply to membrane transport of the very poorly lipid-soluble and zwitterionic amino derivatives of β-lactam antibiotics.

Kimura and co-workers [31] reported that the intestinal transport of cyclacillin was dependent on the external sodium concentration of the medium. On the other hand, glycylglycine uptake was reported to be completely independent of the presence of sodium in the guinea pig intestine [21]. More detailed studies on the kinetics of the sodium dependency of antibiotic uptakes are required for characterization of the transport system.

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