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UPTAKE OF BENZYLPENICILLIN, CEFPIRAMIDE AND CEFAZOLIN BY FRESHLY PREPARED RAT HEPATOCYTES

EVIDENCE FOR A CARRIER-MEDIATED TRANSPORT SYSTEM

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Abstract—The kinetics and mechanism of the hepatic uptake of β-lactam antibiotics were studied by using freshly prepared rat hepatocytes. The initial uptake rates of benzylpenicillin and cefpiramide represented both saturable and nonsaturable transport processes, whereas that of cefazolin showed an apparently nonsaturable uptake process within the concentration range below 4 mM. The apparent nonsaturable uptake rate constants for benzylpenicillin, cefpiramide and cefazolin were 0.580, 0.047 and 0.289 nmoles/min/mg protein/mM respectively. The apparent values of K_t and V_{max} describing the saturable transport were 0.473 ± 0.158 mM and 2.02 ± 0.48 nmoles/min/mg protein for benzylpenicillin and 0.847 ± 0.254 mM and 0.70 ± 0.18 nmoles/min/mg protein for cefpiramide respectively. The Arrhenius plot of benzylpenicillin uptake of 200 μM presented a single straight line in the range of 22°–37° with an activation energy of 16.8 kcal/mole. An energy requirement was also demonstrated for benzylpenicillin uptake as metabolic inhibitors (antimycin A, NaCN, rotenone and 2,4-dinitrophenol) significantly reduced the initial uptake rate of benzylpenicillin ($P < 0.05$). Uptake of benzylpenicillin (200 μM) was not inhibited by ouabain (1 mM). Benzylpenicillin uptake was inhibited competitively by phenoxymethylpenicillin, cefpiramide and cefazolin with the inhibition constants, K_i, of 0.680, 0.583 and 11.7 mM respectively. Benzylpenicillin also inhibited competitively the uptake of cefpiramide with a K_i of 0.655 mM. From these results it was considered that a carrier-mediated uptake system participates in the hepatic uptake of at least four of the β-lactam antibiotics examined in this study.

It is well known that most of β-lactam antibiotics are easily eliminated from the body through the renal elimination pathway after administration to experimental animals and humans [1–3]. However, there are several derivatives of β-lactam antibiotics that are exclusively eliminated from the liver and excreted into the bile duct, e.g. nafcillin [4], cefoperazone [5] and cefpiramide [6]. High exposure of the hepatobiliary tract to these β-lactam antibiotics is advantageous if an infection is confirmed in this area of the body. Although the mechanism of renal β-lactam antibiotic elimination has been examined extensively [7–10], there has been little knowledge gained with respect to the hepatic elimination process [11, 12]. Since the concentrations of β-lactam antibiotics in bile are significantly higher than those in plasma, this class of antibiotics has been thought to be eliminated from the liver via an active transport process or a facilitated diffusion process [4, 12–14]. From the viewpoint of structure–activity relationship, animal experiments have indicated that the biliary excretion of penicillins and cephalosporins in rats increases from 5 to 90% of the dose with increasing lipophilicity [15] and/or molecular weight [16] of the drug. However, the mechanism of hepatic transport and biliary secretion has not yet been elucidated.

There are three important processes in hepatic elimination, i.e. uptake through a sinusoidal membrane, intracellular translocation and excretion through bile canalicular membranes. The purpose of the present study was to elucidate the mechanism of hepatic uptake of three β-lactam antibiotics, i.e. benzylpenicillin, cefpiramide and cefazolin (the structures of which are shown in Scheme 1), by isolated rat hepatocytes. It has been claimed that the use of freshly prepared hepatocytes is advantageous for investigating the mechanism of the hepatic plasma membrane transport process of bile acids [17, 18]. Some of the results appearing in the present paper have been reported previously in a preliminary manner [19].

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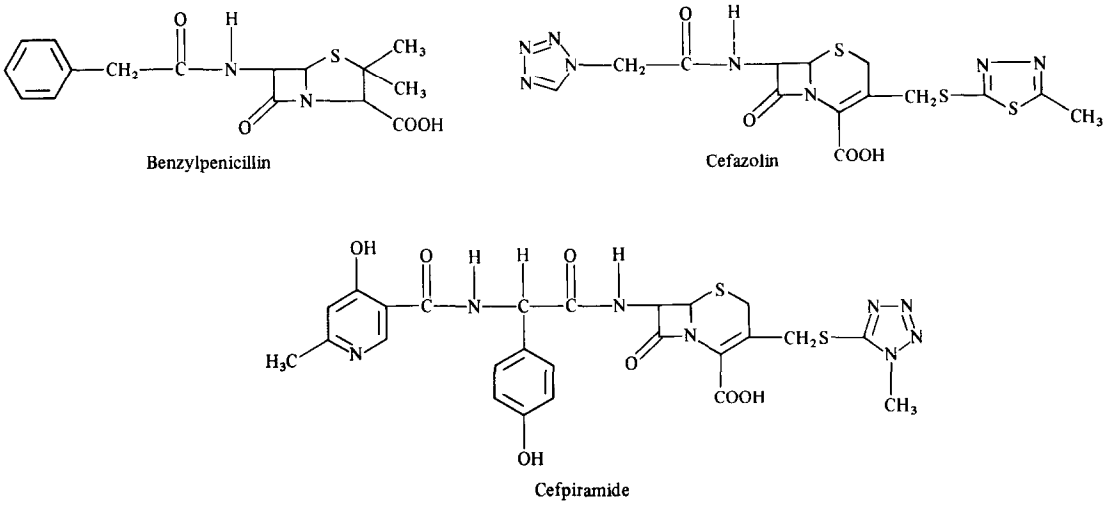
MATERIALS AND METHODS

Chemicals and radioisotopes. Benzylpenicillin and phenoxymethylpenicillin (Meiji Seika Kaisha, Tokyo, Japan), cefazolin and [^{14}C]cefazolin (0.93 μCi/mmole) (Fujisawa Pharmaceutical Co., Osaka, Japan), and cefpiramide and [^{14}C]cefpiramide (6.16 mCi/mmole) (Sumitomo Chemical and Industrial Co., Osaka, Japan, and Yamanouchi Pharmaceutical Co., Tokyo, Japan) were supplied from the cited companies. [^{14}C]Benzylpenicillin (54 mCi/mmole) and [^{14}C]inulin (5 mCi/mmole) were purchased from Amersham International Ltd., U.K. Collagenase (Clostridiopeptidase A) was obtained from Boehringer-Mannheim GmbH, Mannheim, F.R.G. All other reagents were of reagent grade and commercially available without the need for further purification.



Scheme 1. Structural formulae of β-lactam antibiotics.

Isolation of hepatocytes. Liver cells from male Wistar rats (Sankyo Laboratory Co., Ltd., Toyama, Japan) weighing 270–320 g, fed on standard chow, were isolated by the method of Moldeus *et al.* [20]. After washing with a washing medium (medium A) containing 2% bovine serum albumin, Fraction V (Sigma Chemical Co., St. Louis, MO, U.S.A.), 118 mM NaCl, 5.0 mM KCl, 1.2 mM $MgSO_4$ and 2.5 mM $CaCl_2$, buffered with 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 13 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) to pH 7.4, the cells were kept at 4° as a suspension in an incubation medium (medium B) composed of the above washing medium (medium A) containing, additionally, 10 mM glucose. The viability of each cell preparation was routinely checked by trypan blue staining and the lactate dehydrogenase latency test (LDH latency test) in the washing medium (medium A) at pH 7.4. Cells were used only if 0.4% trypan blue mixed 9:1 (v/v) with the cell suspension was excluded by more than 96% of the cells and if the cells exhibited a value greater than 92% for the LDH latency test [20].

Uptake experiment. The washed hepatocytes in aliquots of 1.0 ml (7.1 ± 2.1 mg protein/ml) were preincubated with shaking (130 cycles/min) at 37° for 5 min in the incubation medium (medium B). The medium was equilibrated with 95% O_2 and 5% CO_2. Uptake experiments were initiated by the addition of various concentrations of drugs dissolved in 1.0 ml of incubation medium without bovine serum albumin (medium C). Metabolic inhibitors (antimycin A, sodium cyanide, rotenone, 2,4-dinitrophenol, and ouabain) were simultaneously added at the start of preincubation. All β-lactam antibiotics tested for mutual inhibition effects were added to cell suspensions at the initiation of the uptake experiment. At appropriate time intervals, 0.2-ml aliquots of the cell suspension were removed and placed in 0.5-ml microcentrifuge tubes containing 100 μl of silicone oil ($d = 1.05$, Aldrich Chemical Co., Milwaukee, WI, U.S.A) and 50 μl of 3 M KOH. The uptake reaction was terminated by centrifugation at room temperature for 15 sec in a microfuge (centrifuge 5142, Eppendorf). After separation of hepatocytes from the medium, the pellet fraction containing KOH was neutralized with 50 μl of 3 M CH_3COOH, and then radioactivity in the cell precipitate was analyzed in a scintillation fluid. The remaining 50-μl samples obtained from the remainder of each cell suspension were also counted, and the amount of substrate within the pellet fraction was calculated based on the known concentration and radioactivity in the total cell suspension. The extracellular water content of hepatocytes was corrected from the values which were determined by the uptake of [^{14}C]inulin.

Albumin binding experiment. Binding of benzylpenicillin, cefpiramide, phenoxymethylpenicillin and cefazolin to the incubation medium containing 1% bovine serum albumin was determined by an equilibrium dialysis method using semimicro cells (Honda Science Co., Kanazawa, Japan) with a semipermeable membrane, Type 36/32 (Visking Co., Chicago, IL, U.S.A.), at 37° against the incubation medium without bovine serum albumin [10]. Drug concentrations on both sides of the dialysis chamber were determined by a high-performance liquid chromatograph (HPLC) assay. There should not have been any adsorption of the drug to the dialysis membrane or degradation of the drug during the equilibrium dialysis as the recovery of the amount of drug after equilibrium dialysis was almost 100%.

Analytical procedures. Cellular protein was determined by the method of Lowry *et al.* [21]. Radioactivity was determined in 10 ml of the scintillation fluid used (500 ml toluene, 500 ml Triton X-100, 6.0 g 2,5-diphenyloxazole, 75 mg 1,4-bis[2-(5-phenyloazolyl)]benzene) by means of an Aloka LSC-671 liquid scintillation counter (Aloka, Japan). Quenching was corrected by the external standard method. Concentrations of benzylpenicillin, cefpiramide, phenoxymethylpenicillin and cefazolin in the albumin binding experiment were determined by HPLC assay. After the following pretreatment of the incubation medium with and without bovine serum albumin, samples were applied to the HPLC system. Half-milliliter aliquots of the incubation medium

with bovine serum albumin, and 0.5 ml of methanol were put into a polyethylene tube (1.5 ml) and mixed vigorously. The mixture was cooled at 4° for 5 min and then centrifuged for 5 min in a microfuge (centrifuge 5142, Eppendorf). The supernatant fraction of the incubation medium without bovine serum albumin was filtered through a TM-2P membrane filter (Toyo Roshi Co., Ltd., Tokyo, Japan) and then applied to the HPLC. The solvent delivery system was a TRI ROTAR-II constant-flow pump (Japan Spectroscopic Co., Tokyo, Japan). The analytical column used was a reversed-phase column, μ-Bondapak C_{18} column (30 cm × 3.9 mm i.d., Waters Associates, Inc., Milford, MA, U.S.A.), packed in this laboratory. A guard column, C_{18}/CORASIL (Waters Associates, Inc.), was used between the injector and the analytical column. The mobile phase was acetonitrile–0.01 M ammonium acetate, (20:80 (v/v), for benzylpenicillin and phenoxymethylpenicillin; 18:82 for cefpiramide; and 10:90 for cefazolin). The solvent flow rates were 1.3 ml/min for benzylpenicillin and cefpiramide and 1.5 ml/min for phenoxymethylpenicillin and cefazolin. The column and solvent were kept at ambient temperature. The eluate was monitored continuously for absorbance at 220 nm for benzylpenicillin and phenoxymethylpenicillin, and 270 nm for cefpiramide and cefazolin, using a UVIDEC 100-III variable u.v. detector (Japan Spectroscopic Co.). The drug concentration was calculated from the calibration curve which was made by the same procedure to that of the respective sample.

Data analysis. Uptake of benzylpenicillin and cefpiramide was measured at 15, 30, 45 and 60 sec and that of cefazolin at 30, 60, 90 and 120 sec. Efflux was assumed to be negligible at these sampling points since a linear uptake relation was obtained for benzylpenicillin and cefpiramide until 60 sec and for cefazolin until 120 sec. The uptake rates were taken to be the slope of this linear regression line. The data were analyzed using a FACOM M-170F digital computer (Data Processing Center, Kanazawa University, Kanazawa, Japan). The kinetic parameters of uptake were estimated by a least squares regression analysis program, NONLIN [22].

RESULTS

Time course of benzylpenicillin, cefpiramide and cefazolin uptake. The time course of benzylpenicillin uptake into isolated hepatocytes is illustrated in Fig. 1 at nine substrate concentrations ranging from 50 μM to 4 mM. These data show that the uptake rate of benzylpenicillin was linear over the 60-sec incubation period, and the uptake extrapolated to zero time failed to intersect the origin. A virtually similar result was obtained for the time course of cefpiramide uptake into hepatocytes, and the time course of cefazolin was linear over the 120-sec incubation period with "zero-time" uptake (results not shown). The uptake rates were esimated from the slope of this linear regression line and corrected for the "zero time" uptake.

Concentration dependence of benzylpenicillin, cefpiramide and cefazolin uptake. Figure 2a represents the relationship between the initial uptake rate of benzylpenicillin and the substrate concentration. The results suggest that uptake of benzylpenicillin consisted of two components, a saturable process evident at concentrations below about 1 mM and a nonsaturable process that was linearly related to the higher concentrations examined. Thus, the initial uptake rate (v) can be related to substrate concentration (s) as follows:

$$v = V_{max}s/(K_t + s) + k_d s \qquad (1)$$

where V_{max} is the maximum uptake rate for a carrier-mediated process, K_t is the apparent Michaelis constant, and k_d is the first-order rate constant. The relation between the initial uptake rate of cefpiramide and the substrate concentration is shown in Fig. 2b. The resulting plot demonstrates that the uptake rate of cefpiramide is also the sum of two rate components, the saturable and nonsaturable

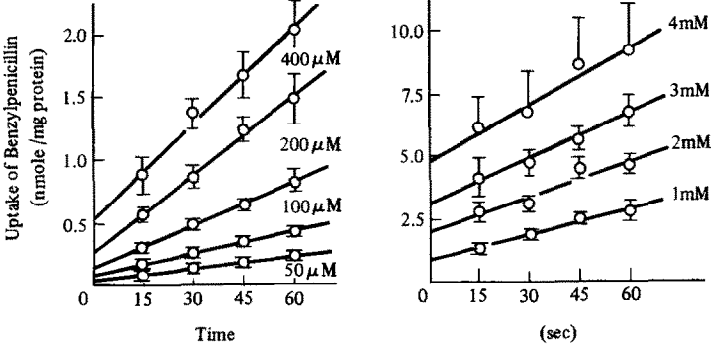


Fig. 1. Time course of benzylpenicillin uptake into isolated hepatocytes. After a 5-min preincubation of cell suspensions, the uptake was initiated by the addition of various concentrations of [^{14}C]benzylpenicillin. Benzylpenicillin uptake was measured at 37° and pH 7.4 over the following 60 sec in the incubation medium (for composition, see text) containing 1% bovine serum albumin. Each point represents the mean ± S.E.M. for three independent experiments. The solid lines were drawn by least-squares linear regression analysis. The value next to each line is the concentration of benzylpenicillin in the incubation medium.

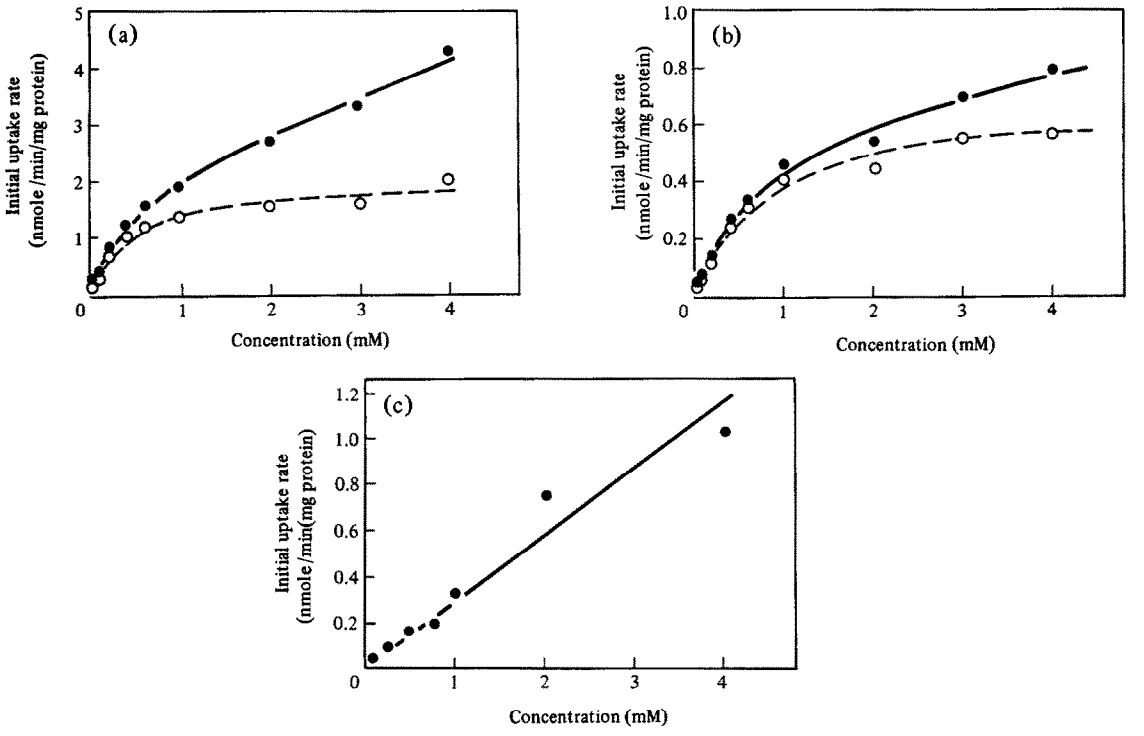


Fig. 2. Concentration dependence for transport of benzylpenicillin (a), cefpiramide (b) and cefazolin (c) into isolated hepatocytes. Closed circles represent the net transport process and open circles represent a saturable transport process. Each point represents the mean of three independent experiments. The solid lines of panels a and b were generated from equation 1, using the NONLIN-fitted parameters listed in Table 1. The solid line of panel c was drawn by linear regression analysis. The broken lines of panels a and b were generated from the Michaelis–Menten equation.

transport processes described by equation 1. Figure 2c illustrates the relationship between the initial uptake rate of cefazolin and the substrate concentration. In contrast to the results for benzylpenicillin and cefpiramide, the uptake of cefazolin exhibited only a nonsaturable transport process within the concentration range below 4 mM.

Table 1 lists the various kinetic parameters obtained from the NONLIN analysis for benzylpenicillin, cefpiramide and cefazolin transport. There was no significant difference in apparent K_t values between benzylpenicillin and cefpiramide, whereas the V_{max}/K_t value of benzylpenicillin was about five times greater than that of cefpiramide. At substrate concentrations well below the K_t concerning the saturable process, the uptake is apparently governed by first-order kinetics. Under linear conditions, the net uptake rates, $V_{max}/K_t + k_d$, for benzylpenicillin and cefpiramide were evaluated to be 4.85 and 0.873 nmoles/min/mg protein/mM, respectively, whereas the apparent first-order uptake rate of cefazolin was 0.289 nmoles/min/mg protein/mM.

Binding of benzylpenicillin, cefpiramide, cefazolin and phenoxymethylpenicillin to 1% bovine serum albumin. The binding of benzylpenicillin, cefpiramide, cefazolin and phenoxymethylpenicillin to the incubation medium containing 1% bovine serum albumin was measured at various drug concentrations. As illustrated in Fig 3, panels a–d, the binding values for all antibiotics examined were linearly related to the total antibiotic concentrations.

Table 1. Kinetic parameters of benzypenicillin, cefpiramide and cefazolin uptake by freshly prepared rat hepatocytes*

Antibiotics	V_{max} (nmoles/min/mg protein)	K_t (mM)	k_d (nmoles/min/mg protein/mM)
Benzylpenicillin	2.02 ± 0.48	0.473 ± 0.158	0.580 ± 0.012
Cefpiramide	0.70 ± 0.18	0.847 ± 0.254	0.047 ± 0.035
Cefazolin†			0.289

* Data are presented as the mean ± S.D. of three independent experiments.

† The value of cefazolin does not necessary show k_d only, but means the apparent first-order uptake rate.

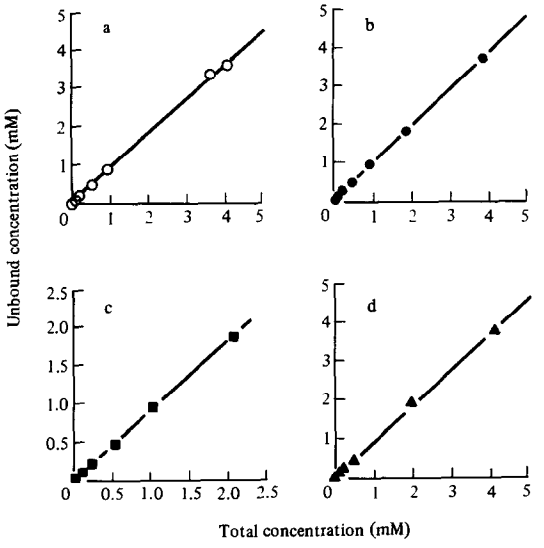


Fig. 3. Extent of binding to 1% bovine serum albumin as a function of β-lactam antibiotic concentration. Binding experiments were carried out in incubation medium containing 1% bovine serum albumin, pH 7.4, at 37° by the equilibrium dialysis method. Key: benzylpenicillin (○); cefpiramide (●); phenoxymethylpenicillin (■); and cefazolin (▲).

The unbound fractions for benzylpenicillin, cefpiramide, cefazolin and phenoxymethylpenicillin were 0.908, 0.951, 0.930 and 0.898 respectively.

Zero-time uptake of benzylpenicillin, cefpiramide and cefazolin. The linear concentration-dependence was obtained for the relationship between the zero-time intersects of benzylpenicillin, cefpiramide and cefazolin and their corresponding substrate concentrations. The result demonstrates that zero-time uptake of the three antibiotics is due to nonsaturable binding to the plasma membrane of hepatocytes. The values for apparent nonsaturable binding of benzylpenicillin, cefpiramide and cefazolin obtained from the slope of the lines were 1.16, 0.174 and 0.0267 nmoles/mg protein/mM respectively.

Temperature dependence of initial uptake rate of benzylpenicillin. Uptake of 200 μM benzylpenicillin into hepatocytes was determined at four different temperatures, 22°, 27°, 32° and 37°. The uptake rate was dependent on the temperature with an activation energy of 16.8 kcal/mole.

Effects of metabolic inhibitors on the initial uptake rate of benzylpenicillin. Figure 4 illustrates the effects of metabolic inhibitors on the initial uptake rate of 200 μM benzylpenicillin. Addition of the respiratory chain inhibitors antimycin A (10 μM), sodium cyanide (1 mM), and rotenone (1 mM), or the uncoupler 2,4-dinitrophenol (1 mM), diminished benzylpenicillin uptake into hepatocytes significantly ($P < 0.05$). In contrast to these results for metabolic inhibitors, there was no significant effect of the Na^+,K^+-dependent ATPase, ouabain (1 mM), on the uptake of benzylpenicillin.

Effects of cefpiramide, cefazolin and phenoxymethylpenicillin on the initial uptake rate of benzylpenicillin. The effect of cefpiramide on the uptake of benzylpenicillin was examined. The Dixon plot of benzylpenicillin showing inhibition by cefpiramide is illustrated in Fig. 5a. The result demonstrates that cefpiramide competitively inhibited the uptake of benzylpenicillin. The inhibition constant, K_i, calculated from the intercept on the baseline of the Dixon plot was 0.583 mM. Figure 5b represents the inhibitory effect of cefazolin on the uptake of benzylpenicillin at various concentrations, demonstrating that cefazolin also competitively inhibited the uptake of benzylpenicillin. The inhibition constant, K_i, of cefazolin was evaluated to be 11.7 mM, relatively larger than the Michaelis constant of benzylpenicillin and cefpiramide. Figure 5c also demonstrates that phenoxymethylpenicillin competitively inhibited the uptake of benzylpenicillin, and the K_i value obtained for this structural analogue was 0.680 mM.

Effect of benzylpenicillin on the initial uptake rate of cefpiramide. Figure 6 represents the Dixon plot of cefpiramide uptake showing inhibition by benzylpenicillin. The result indicates that cefpiramide uptake was competitively inhibited by benzylpenicillin, and the K_i value of benzylpenicillin obtained was 0.655 mM.

DISCUSSION

Recently, we successfully clarified the mechanisms of tissue distribution [10, 23] and renal excretion [10] after intravenous administration and the mechanism

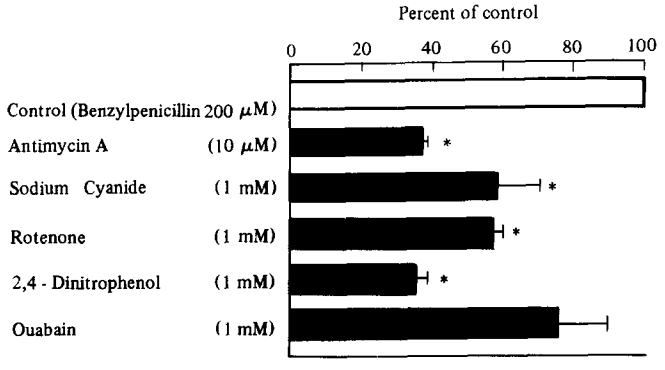


Fig. 4. Effects of metabolic inhibitors on benzylpenicillin uptake. Each bar represents the mean and S.E.M. of three independent experiments. Level of significance was set at $P < 0.05$.

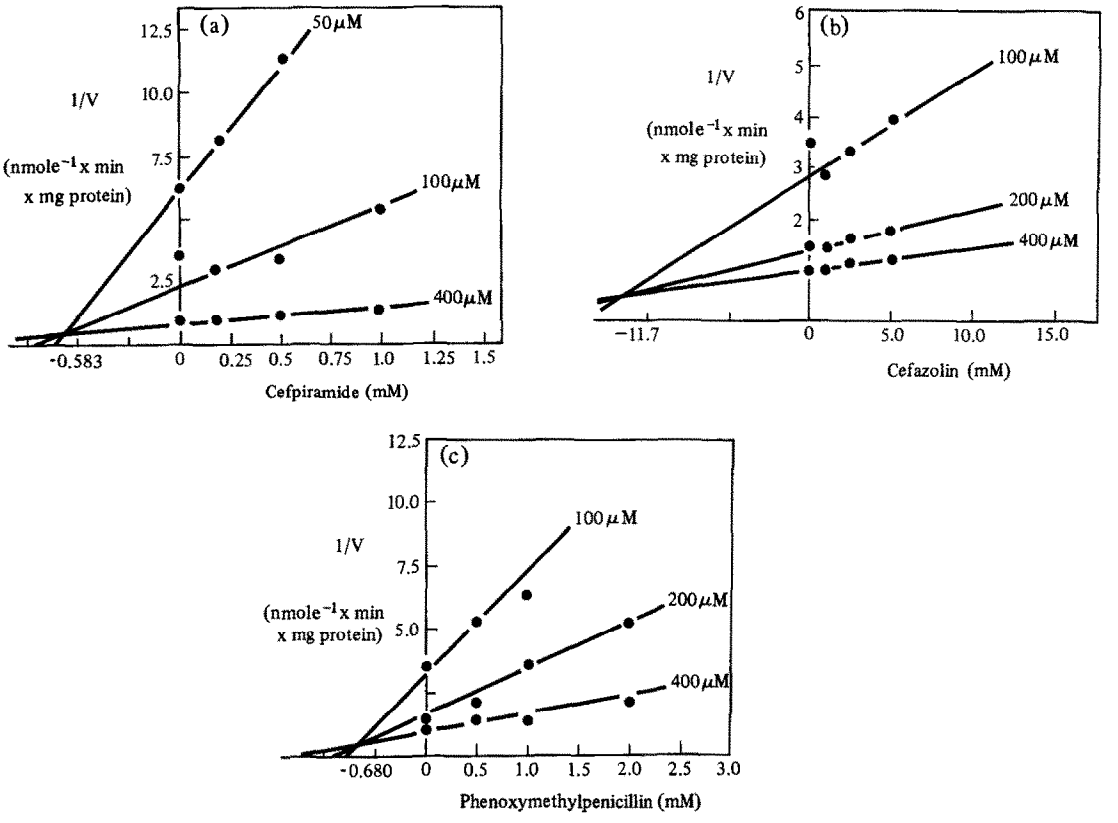


Fig. 5. Dixon plots of benzylpenicillin uptake showing inhibition of cefpiramide (a), cefazolin (b) and phenoxymethylpenicillin (c). The inhibitor was simultaneously added at the initiation of benzylpenicillin uptake. The plots were obtained from three independent experiments. The value next to each line is the concentration of benzylpenicillin in the incubation medium. Values of the inhibitor constant, K_i, calculated from the intercept on the baseline of the Dixon plot, were 0.583 mM for cefpiramide, 11.7 mM for cefazolin, and 0.680 mM for phenoxymethylpenicillin.

of intestinal absorption [24–26] of β-lactam antibiotics. Whether the hepatic intake of β-lactam antibiotics is a carrier-mediated transport process or a passive diffusion process is an interesting problem which needs to be resolved. Previous studies concerning the hepatic elimination process have focused on the determination of the excreted biliary amount of β-lactam antibiotics [12–16] and the hepatic metabolism of β-lactam antibiotics by using *in situ* perfused liver [11]. However, the uptake mechanism of β-lactam antibiotics through the hepatic plasma membrane has not yet been examined. We have determined the initial uptake rate over a wide concentration range (0.05 to 4 mM) and its kinetic parameters for benzylpenicillin, cefpiramide and cefazolin in freshly prepared rat hepatocytes to elucidate the transport mechanism of β-lactam antibiotics, as well as the difference of the substrate specificity to the transport carrier. Although several methodologies have been used in studies of the hepatic transport mechanism of drugs [17, 18, 27–30], we used the freshly prepared rat hepatocyte system since the use of isolated hepatocytes has the advantage of permitting examination of the criteria of a carrier-mediated transport mechanism, e.g. substrate concentration dependence, energy requirement, sodium ion dependence, temperature dependence and mutual inhibition among structural analogues in the uptake process. Based on the following reasons, we selected the above three antibiotics (Scheme 1) for the present studies; benzylpenicillin was used as a fundamental model drug, cefpiramide as a specifically biliary-excreted drug and cefazolin as a poorly biliary-excreted drug.

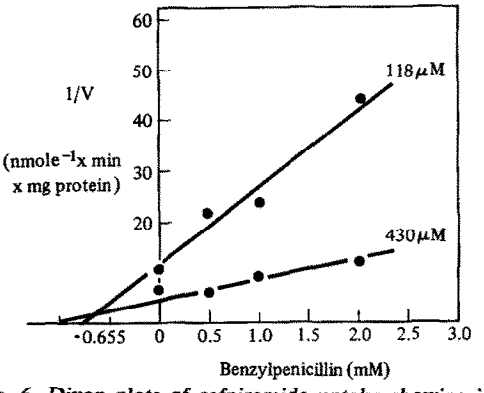


Fig. 6. Dixon plots of cefpiramide uptake showing inhibition by benzylpenicillin. Benzylpenicillin was simultaneously added at the initiation of cefpiramide uptake. The plots were obtained from three independent experiments. The value next to each line is the concentration of cefpiramide in the incubation medium. The value of the inhibitor constant, K_i, for benzylpenicillin was 0.655 mM.

To determine whether only unbound β-lactam antibiotic in the medium or both unbound and antibiotic bound to serum albumin are transported through the hepatic plasma membrane, the extent of binding of each drug to 1% bovine serum albumin was measured. The results indicate that the bound form of benzylpenicillin, cefpiramide, cefazolin or phenoxymethylpenicillin was less than 10% of the total drug concentration in the medium. Because of this small amount of binding, it is not clear whether β-lactam antibiotic bound to serum albumin can be transported. Therefore, the apparent kinetic parameters were evaluated for the uptake system without any correction for binding to bovine serum albumin (Table 1).

Values for zero-time uptake of the three antibiotics examined in this study were determined by extrapolating the linear regression line between 15 and 60 sec for benzylpenicillin and cefpiramide and 30 and 120 sec for cefazolin. Positive zero-time uptake values for these three antibiotics were several times smaller than those of bile acids [18] reported for isolated hepatocytes. Nonspecific binding of β-lactam antibiotics to the plasma membrane rather than their uptake into hepatocytes is suggested for the zero-time uptake since the result of zero-time uptake showed linear concentration-dependence.

As the uptake rates of benzylpenicillin and cefpiramide consisted of combination rates for both saturable and nonsaturable processes (Fig. 2a and Fig. 2b), these two antibiotics are suggested to be transported into rat hepatocytes by at least one carrier-mediated system. The apparent nonsaturable uptake process observed for cefazolin (Fig. 2c) could be explained by a low-affinity transport mechanism operating far below the K_t for cefazolin and/or a mechanism by which cefazolin enters the cell by passive diffusion. Plasma concentrations of β-lactam antibiotics after intravenous administration at a dose of 20 mg/kg have been reported to be smaller than 100 μM [6, 10]. According to the kinetic parameters listed in Table 1, a carrier-mediated transport system is suggested to be the dominant process for the hepatic intake process of β-lactam antibiotics at concentrations below 200 μM, since the $(V_{max}/K_t)s$ value is ten times or more greater than the value of k_d s for benzylpenicillin and cefpiramide. It is noteworthy that the V_{max}/K_t value of cefpiramide was much smaller than that of benzylpenicillin (Table 1), whereas cefpiramide was more rapidly and substantially eliminated into bile from the body through the liver than benzylpenicillin [6, 13]. From this difference between *in vivo* and *in vitro* characteristics, it would be possible to assume that the hepatic uptake process is not a rate-limiting step for biliary excretion of β-lactam antibiotics. The estimated K_t value determined for hepatic uptake of benzylpenicillin and cefpiramide (Table 1) is smaller than the K_t value of the amino β-lactam antibiotics, cyclacillin (1.15 ± 0.23 mM) and cephalexin (6.85 ± 1.54 mM) in a small intestinal transport process which has been determined previously by *in vitro* experiments [26]. Moreover, the intestinal carrier system restricts the transport of other such β-lactam antibiotics without an α-amino group in their side chain [25, 26]. Recent findings by Inui *et al.* [7, 8] have indicated the existence of a carrier-mediated transport system for amino-cephalosporins in renal brush border membrane which may be a part of the mechanism of tubular reabsorption of these antibiotics. The characteristics of the carrier system sensitive to amino β-lactam antibiotics in the kidney are very similar to those in the intestine. By contrast, liver cells have a carrier-mediated system highly sensitive to β-lactam antibiotics lacking an α-amino group. These differences of affinity to the carrier and the character suggest that the hepatic transport system is different from that of either the small intestine or the kidney.

To confirm the nature of the carrier-mediated transport system of β-lactam antibiotics in hepatocytes, we investigated both temperature dependence and metabolic energy dependence (Fig. 4). As the extent of the ratio of the saturable transport process to the total intake process of benzylpenicillin is greater than 90% at a concentration of 200 μM in the incubation medium (Fig. 2a and Table 1), 200 μM benzylpenicillin was used in this experiment. Reduction of the incubation temperature from 37° to 22° decreased the initial uptake rate by 75.6%, yielding an activation energy of 16.8 kcal/mole. This temperature dependence is characteristic of a carrier-mediated transport system. As shown in Fig. 4, an energy requirement for the transport of benzylpenicillin is obvious since all metabolic inhibitors studied (antimycin A, NaCN, rotenone, 2,4-dinitrophenol), except ouabain, significantly reduced the initial uptake velocity of benzylpenicillin ($P < 0.05$). Although ouabain did not inhibit significantly the uptake of benzylpenicillin (Fig. 4), the result would be indistinct for the sodium independence of the transport system. More definitive experiments with sodium substitutes are needed to determine the detailed mechanism of the uptake process.

The study of mutual inhibition among structural analogues, which is one of the important criteria for a carrier-mediated transport mechanism, was also carried out to clarify the hepatic carrier-mediated transport system. Since the structure of phenoxymethylpenicillin is very close to that of benzylpenicillin, inhibition experiments involving phenoxymethylpenicillin and the uptake of benzylpenicillin were carried out. The result of competitive inhibition of phenoxymethylpenicillin on the uptake of benzylpenicillin (Fig. 5c) supports the existence of a carrier-mediated hepatic transport system for penicillin derivatives. An experiment to test the inhibition of cefpiramide on the uptake of benzylpenicillin was also carried out (Fig. 5a). The competitive inhibition observed suggests a common carrier-mediated system for penicillin and cephalosporin derivatives, though the structural similarities are β-lactam, a carboxyl group and an aromatic group of 6- or 7-side chain, and a D-alanyl-alanine conformation. The fact that cefazolin competitively inhibited the uptake of benzylpenicillin (Fig. 5b) and the magnitude of the inhibition constant, K_i, for cefazolin (11.7 mM) suggest that cefazolin is transported by a low-affinity, carrier-mediated process. The result obtained for cefazolin, shown in Fig. 5b, was in accordance with the apparent nonsaturable concentration dependence of the initial uptake rate of cefazolin (Fig. 2c).

In addition to the inhibition study of the hepatic uptake of benzylpenicillin, an inhibition study of benzylpenicillin on the uptake of cefpiramide was also performed (Fig. 6). A common carrier-mediated transport system was confirmed for the uptake process of both antibiotics since cefpiramide was competitively inhibited by benzylpenicillin (Fig. 6) and, conversely, benzylpenicillin was also competitively inhibited by cefpiramide (Fig. 5a). However, the possibility still remains of nonspecific inhibition of the formation of the substrate–carrier complex since our preliminary study failed to demonstrate either a counter-transport effect of cefpiramide on the uptake of benzylpenicillin or a trans-stimulation effect of benzylpenicillin.

In conclusion, the overall results obtained in this study indicate that a carrier-mediated transport system for β-lactam antibiotics exists in the first process of elimination of β-lactam antibiotics from the liver. Furthermore, it is also suggested that this hepatic transport system requires metabolic energy and that the system is common to at least the four β-lactam antibiotics studied, benzylpenicillin, phenoxymethylpenicillin, cefpiramide and cefazolin.

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