

Peptide Carrier-Mediated Transport in Intestinal Brush Border Membrane Vesicles of Rats and Rabbits: Cephadrine Uptake and Inhibition

Hiroaki Yuasa,¹ Gordon L. Amidon,^{1,2} and David Fleisher¹

Received August 24, 1992; accepted September 16, 1992

The uptake kinetics of cephadrine, an amino- β -lactam antibiotic, were studied in rat and rabbit intestinal brush border membrane vesicles preparations using both the Ca^{2+} and the Mg^{2+} methods of preparation, in the presence of an inward proton gradient. The Ca^{2+} method demonstrated greater uptake of cephadrine in intestinal brush border vesicles prepared from both rat and rabbit and was used for these studies. The transport was observed to be of Michaelis-Menten carrier-mediated type with a passive transport component. The kinetic parameters obtained were as follows: for rat and rabbit, respectively, K_m , 1.6 and 1.9 mM; J_{max} , 1.7 and 20.7 nmol/mg/min; P_c ($= J_{\text{max}}/K_m$), 1.1 and 10.9 $\mu\text{L}/\text{mg}/\text{min}$; and P_m , 0.4 and 0.8 $\mu\text{L}/\text{mg}/\text{min}$. The kinetic parameters for the rat vesicles are consistent with those from our previous perfusion study using a conversion factor of 0.71 cm^2/mg protein. The rabbit vesicles exhibited a similar Michaelis constant and a 10-fold larger maximal transport velocity, suggesting a quantitative advantage for the study of carrier-mediated transport in the rabbit compared to rat vesicles from the intestine. Cephadrine uptake was inhibited by phenylpropionylproline, a proline derivative, and enalapril, an ACE inhibitor, which do not have an α -amino group, as well as dipeptides, tripeptides, and amino- β -lactam antibiotics in both rat and rabbit vesicles. These results support the suggestion that they share the same peptide carrier pathway for oral absorption and that the vesicles may be a useful tool in developing orally effective peptide-type drugs.

KEY WORDS: intestinal uptake; brush border membrane vesicles; rat; rabbit; peptide carrier; cephadrine; amino β -lactam antibiotics; ACE inhibitors; dipeptides; tripeptides; proline derivatives.

INTRODUCTION

The intestinal peptide carrier-mediated transport system required for dipeptide and tripeptide absorption, plays a substantial role in peptide-type drug absorption. The transport mechanism of di- and tripeptides has been attributed to a proton-dependent carrier-mediated transport system on the brush border membrane of the intestinal epithelial cell (1-6). Many cephalosporins, including several without an α -amino group, have also been shown to share the peptide carrier, as judged from mutual competition with peptides for uptake and the proton dependence of their transport (7-18). Perfusion studies suggested that some ACE inhibitors and proline derivatives, which lack an α -amino group, may also share the peptide carrier (19-24). A peptide-like structure appears

to be required for transport, but an α -amino group is not essential. However, the structural requirements for peptide transport are yet to be fully clarified.

We recently demonstrated nonlinear uptake and competition for transport in the perfused rat intestine for several compounds sharing the peptide carrier and determined the kinetic parameters (19-24). To investigate further the transport mechanism for peptide-like compounds and to clarify the structure-transport relationship, isolated brush border membrane vesicles were used. With cephadrine, an amino- β -lactam antibiotic, as a model compound, we determined its kinetic parameters and investigated competitive inhibition by other possible peptide carrier substrates. Two methods of preparation, Ca^{2+} and Mg^{2+} , and two animal species, rats and rabbits, are compared, and the results correlated with previous results from perfusion studies.

MATERIALS AND METHODS

Materials

Cephadrine (31-464-12179-617) was supplied by Squibb & Sons (Princeton, NJ), and enalapril maleate (L-154,739-001d076) was from Merck Sharp and Dohme Research Laboratories (Rahway, NJ). Phenylpropionylproline was synthesized (24). D-[1- $^3\text{H}(\text{N})$]Glucose (15.5 Ci/mmol) was purchased from DuPont-NEN Co. (Boston, MA). The other cephalosporins, dipeptides, tripeptides, and amino acids were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical or HPLC grade.

Preparation of Brush Border Membrane Vesicles

Intestinal brush border membrane vesicles were prepared by the calcium precipitation method of Kessler *et al.* (25) with slight modifications. A male NZW rabbit (2-2.5 kg), 1 m jejunum, or eight male SD rats (250-350 g), 40 cm jejunum each, were used, and all procedures were performed on ice or under refrigeration. The intestinal lumen was washed with ice-cold saline, and the intestinal tissue was removed by squeezing. The tissue (approximately 13 g) was homogenized in 50 mM mannitol, 2 mM Hepes/Tris solution (pH 7.5) to make a 5% homogenate with a Waring blender for 2 min. After adding 1 M CaCl_2 solution to give a final concentration of 10 mM, the homogenate was stirred for 2 min and left standing for 18 min under refrigeration, then centrifuged at 3000g for 15 min. The supernatant was then centrifuged at 27,000g for 30 min. The resultant pellet was suspended in 50 mL of loading solution, 100 mM mannitol, 100 mM KCl, 10 mM Hepes/Tris (pH 7.5), and homogenized with a glass/Teflon Potter homogenizer at 1140 rpm and 10 strokes. Then the homogenate was centrifuged at 3000g for 15 min and the resultant supernatant was centrifuged at 27,000g for 30 min. The final pellet was resuspended with 0.6 mL of loading solution to give a brush border membrane vesicle preparation. Protein content was determined (see below) and verified with regard to a marker enzyme and transport activity (see Verification of Brush Border Membrane Vesicles, below), then used for uptake studies. The brush border membrane vesicles were kept on ice until use.

¹ College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109-1065.

² To whom correspondence should be addressed.

Uptake Experiments

Uptake experiments were conducted by a rapid filtration technique, using a Millipore filtration apparatus (Millipore Co. Bedford, MA). The uptake reaction was initiated by the addition of 80 μL of test solution containing a substrate to 20 μL of membrane vesicle solution (5 mg/mL for rabbit and 10 mg/mL for rat) at 25°C and stopped by adding 1 mL of ice-cold stop solution consisting of 150 mM NaCl and 10 mM Hepes/Tris (pH 7.5). The mixture was immediately applied on a prewetted Millipore membrane filter (PHWP; 0.30- μm pore, 25-mm diameter) for filtration under vacuum and washed twice with 2 mL of the ice-cold stop solution. The membrane filter was transferred into a counting vial and subjected to quantitation of the substrate taken up by the vesicles.

The vesicles were pre equilibrated in the loading solution, 100 mM mannitol, 100 mM KCl, 10 mM Hepes/Tris (pH 7.5) and the test solutions consisted of 100 mM KCl, 100 mM mannitol, and 10 mM Mes/Tris (pH 5.8), giving pH 6.0/7.5, for outside/inside, of the initial inward proton gradient in the mixture. Concentrations of cephradine, the substrate, and test compounds used as competitive inhibitors of cephradine uptake were set 25% higher in the test solution than those stated, to compensate for the dilution by the vesicles solution.

Quantitation by HPLC

Cephradine was extracted with 0.3 mL of distilled water adjusted to pH 3.0 with HCl by vortexing for 10 min and analyzed with a HPLC system: column, $\mu\text{Bondapak C}_{18}$, 3.9 mm \times 30 cm (Waters, Milford, MA); mobile phase, 13% acetonitrile in 0.02 M NaH_2PO_4 (pH 5.0); flow rate, 1.6 mL/min; and detector, UV 262 nm.

Data Treatment

The initial cephradine uptake was measured at 1 and 0.5 min, respectively, for rat and rabbit, corrected for the non-specific binding to the membrane filter, and expressed in terms of milligrams of protein. The nonspecific binding was typically 10 to 20% of uptake. The initial uptake rate was estimated as nanomoles per milligram of protein per minute, assuming the initial uptake is proportional to time, and the transport coefficient (permeability) was calculated as the initial uptake rate/concentration. The transport coefficient is expressed as P' ($\mu\text{L}/\text{mg}/\text{min}$ in this report), the product of the permeability coefficient (cm/min) and the surface area (cm^2/mg).

The Michaelis–Menten-type carrier-mediated transport model with a passive transport is expressed by either Eq. (1) or Eq. (2) as follows:

$$P' = J_{\max}'/(K_m + C) + P_m' \quad (1)$$

$$P' = P_c'/(1 + C/K_m) + P_m' \quad (2)$$

where P' is the aforementioned permeability–surface area product, C is the concentration, and J_{\max}' , K_m , P_m' , and P_c' are the maximal transport velocity, the Michaelis constant, the passive transport coefficient, and the carrier-mediated transport coefficient ($=J_{\max}'/K_m$), respectively. Equations

(1) and (2) were fitted to P' - C profiles separately to estimate J_{\max}' , K_m , P_m' , and P_c' , using a nonlinear regression program, NONLIN.

Protein Determination

Protein content was determined with a Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA) with bovine serum albumin as the standard. The protein concentration in the 5% homogenate was 4.3 and 5.2 mg/mL, respectively, for rat and rabbit. The yield of brush border membrane vesicles was 23 and 20 mg/preparation, respectively, for rat and rabbit.

Verification of Brush Border Membrane Vesicles

Alkaline phosphatase activity was determined with *p*-nitrophenylphosphate as the substrate. A diluted vesicle or homogenate sample, 0.2 mL, was mixed with 0.7 mL of a solution, consisting of 7.1 mM MgCl_2 , 0.36 mM CaCl_2 , 0.3 mM ZnCl_2 , and 71 mM Tris/maleate (pH 9.0), and 0.1 mL of 50 mM *p*-nitrophenylphosphate, and incubated at 37°C for 15 min. The reaction was stopped by the addition of 3 mL of 1 M NaOH and released *p*-nitrophenol was determined spectrophotometrically (410 nm).

D-Glucose uptake was measured in the same manner as described under Uptake Experiments, above. The test solution consisted of 125 mM NaCl, 50 mM mannitol, and 10 mM Hepes/Tris (pH 7.5) and contained 0.625 mM D-glucose with a tracer amount of ^3H -D-glucose, giving 100 mM/0 mM, for outside/inside, of the initial inward Na^+ gradient and 0.5 mM D-glucose in the mixture. D-Glucose was quantitated by radioactivity determination with a liquid scintillation counter, after adding 10 mL of scintillation cocktail (Cytoscint: ICN Biomedicals Inc., Cleveland, OH) to the membrane filter.

RESULTS

The activities of alkaline phosphatase, a brush border marker enzyme, and the Na^+ -dependent D-glucose uptake, a marker of transport function, were measured to verify the enrichment and transport capacity of the brush border membrane vesicle preparations (25). The Na^+ -dependent D-glucose transport system is located only at the brush border membrane, and D-glucose uptake by the brush border membrane vesicles also shows overshoot, i.e., the initial and temporary accumulation over the equilibrium uptake, in the presence of Na^+ .

Alkaline phosphatase activity was enriched in the brush border membrane vesicle preparation compared with the starting 5% homogenate as follows: for rat and rabbit, respectively, the enzyme activity in the vesicles was 11 and 0.78 U/mg; that in the homogenate was 1 and 0.07 U/mg; giving enrichment factors of 11 and 12, respectively. D-Glucose uptake showed overshoot as follows: for rat and rabbit, respectively, the maximum uptake (at 0.5 min for rat and 1 min for rabbit) was 4.3 and 13 nmol/mg, while the equilibrium uptake (at 60 min) was 0.53 and 1.2 nmol/mg; giving maximum-to-equilibrium uptake ratios of 8.0 and 11, respectively. These results indicate that the brush border mem-

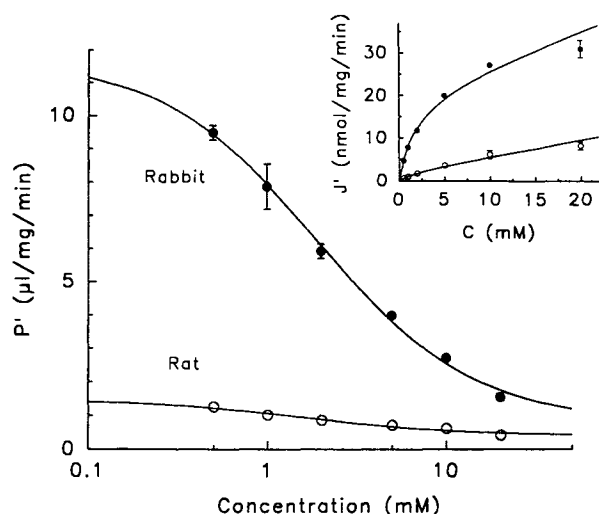


Fig. 1. Concentration dependency of cephradine uptake in the presence of an inward proton gradient pH 6.0/7.5 (outside/inside). Data are represented as mean \pm SE ($n = 3$). The curves show the results of nonlinear regression fitting.

brane vesicle preparations were well enriched and that the transport function was intact.

The concentration dependence of cephradine uptake was tested in the presence of an inward proton gradient, pH 6.0/7.5 (outside/inside). The cephradine uptake exhibited the typical concentration dependency in both rat and rabbit, indicating the presence of carrier-mediated transport (Fig. 1). The faster initial uptake rate, or the larger P' , for rabbit than rat was attributed to the larger J_{\max}' for rabbit (Table I). The effect of various compounds (10 mM) on the cephradine (1 mM) uptake was tested in the presence of an inward proton gradient, pH 6.0/7.5 (Table II). In both rat and rabbit, cephradine uptake was not inhibited by glycine and proline, but it was significantly inhibited by other substrates of the peptide carrier, enalapril, an ACE inhibitor, and phenylpropionylproline (Phenylpropionyl-Pro), a proline derivative, which does not have an α -amino group, as well as cephalixin and cefadroxil, amino- β -lactam antibiotics, glycylglycine (Gly-Gly) and glycylproline (GlyPro), dipeptides, and glycylglycylproline (GlyGlyPro) and glycylglycylphenylalanine (GlyGlyPhe).

DISCUSSION

The Uptake Kinetics of Cephradine

For a simple and efficient divalent cation precipitation

method, the choice of divalent cation, Ca^{2+} or Mg^{2+} , has been an issue of discussion (26); hence, we compared vesicles prepared by the Ca^{2+} and Mg^{2+} precipitation methods. For comparison, a pooled homogenate was divided into two portions and subjected to the Ca^{2+} or the Mg^{2+} methods. Calcium vesicles showed faster initial cephradine uptake than Mg^{2+} vesicles by 20% in rats and 60% in rabbits. We also observed comparable initial D-glucose uptake in Ca^{2+} and Mg^{2+} vesicles in both rats and rabbits, contrary to the observation in renal brush border membrane vesicles (27). Thus, we chose the Ca^{2+} precipitation method in this project.

The cephradine uptake showed nonlinear kinetics and could be described by the Michaelis-Menten-type carrier-mediated transport model with parallel passive transport, suggesting the contribution of carrier-mediated transport, probably via the proton dependent peptide carriers as discussed elsewhere (13-15). This suggestion is further supported by the inhibition of cephradine uptake by dipeptides and tripeptides. Cephradine uptake was accelerated by the inward-directed proton gradient (Fig. 2). Inhibition of cephradine uptake by other amino- β -lactam antibiotics supports the suggestion that they generally share the peptide carrier (7-9,17,18,23). Inhibition of cephradine uptake by enalapril and phenylpropionylproline suggests that they also bind to the peptide carrier, consistent with our findings in perfusion studies that their absorption showed nonlinear kinetics and was inhibited by peptides and amino- β -lactam antibiotics (21,24). These results further support the suggestion that an α -amino group is not an absolute requirement for peptide carrier substrates, though a peptide bond-like structure seems to be necessary (20-22,24).

The kinetic parameters for the rat vesicles are consistent with those from our previous perfusion study (19) with a similar K_m and P_c' -to- P_m' ratio, suggesting a comparable contribution of the carrier-mediated and passive transport pathways. The rabbit vesicles exhibited a 10-fold larger J_{\max}' than rat vesicles but similar K_m , suggesting a similar substrate affinity but a different number of carriers, and hence, a quantitative advantage for transport studies in rabbit compared to rat vesicles.

Comparable values of K_m and J_{\max}' of cephradine uptake with those in this report were reported in intestinal brush border membrane vesicles: 9.4 mM and 5.7 nmol/mg/min in rats (13) and 5.0 mM and 38 nmol/mg/min in rabbits (15). Inui *et al.* (15) have further suggested the existence of two different transport systems, a neutral pH-prefering system (Type I) and an acidic pH-prefering system (Type II), but also suggested that the Type I system was

Table I. Kinetic Parameters of Cephradine Uptake^a

Animal	J_{\max}' , vesicles (nmol/mg/min)	J_{\max}' , perfusion (nmol/cm ² /min) ^b	K_m		P_c' , vesicles ($\mu\text{L}/\text{mg}/\text{min}$)	P_c , perfusion ($\mu\text{L}/\text{cm}^2/\text{min}$) ^b	P_m' , vesicles ($\mu\text{L}/\text{mg}/\text{min}$)	P_m , perfusion ($\mu\text{L}/\text{cm}^2/\text{min}$) ^b
			Vesicles (mM)	Perfusion (mM) ^b				
Rat	1.7 \pm 1.0	2.4 \pm 1.2	1.6 \pm 1.1	1.5 \pm 0.8	1.1 \pm 0.2	1.7 \pm 0.2	0.4 \pm 0.1	0.5 \pm 0.2
Rabbit	20.7 \pm 4.6		1.9 \pm 0.5		10.9 \pm 0.6		0.8 \pm 0.4	

^a Values are represented as parameter \pm SE.

^b Data from our previous report (19), parameter \pm SD.

Table II. Effect of Various Compounds on Cephadrine Uptake^{a,*}

Compound	Rat		Rabbit	
	Uptake (nmol/mg/min)	% inhibited	Uptake (nmol/mg/30 sec)	% inhibited
None (control)	1.01 ± 0.07		3.93 ± 0.34	
Cephalexin	0.77 ± 0.09 ^c	24	1.33 ± 0.14 ^a	66
Cefadroxil	0.82 ± 0.04 ^c	18	1.21 ± 0.19 ^a	69
Enalapril	0.38 ± 0.05 ^a	62	0.47 ± 0.24 ^a	88
Phenylpropionyl-Pro	0.63 ± 0.11 ^b	38	1.19 ± 0.07 ^a	70
GlyGly	0.27 ± 0.06 ^a	74	0.51 ± 0.19 ^a	87
GlyPro	0.82 ± 0.06 ^c	19	1.62 ± 0.10 ^a	59
GlyGlyPhe	0.59 ± 0.03 ^a	42	1.32 ± 0.18 ^a	66
TyrGlyGly	0.77 ± 0.08 ^c	24	1.63 ± 0.10 ^a	59
Glycine	1.07 ± 0.02	0	3.33 ± 0.03	15
Proline	1.02 ± 0.05	0	3.47 ± 0.11	12

^a Values of uptake are represented as mean ± SE ($n = 3$). The experiments were conducted at 1 mM cephradine, 10 mM test compound and in the presence of an inward proton gradient, pH 6.0/7.5 (outside/inside).

* Significance levels of difference against control (Student's t test) are as follows: a, $P < 0.01$; b, $P < 0.05$; c, $P < 0.1$.

the major system at pH 6.0 and the Type II system contributed minimally, consistent with our analysis assuming a single transport system.

The carrier-mediated transport accounts for 60% (rat) and 90% (rabbit) of the total cephradine uptake at 1 mM, based on the estimated kinetic parameters as $[J_{\max}'/(K_m + C)]/P'$. Enalapril and glycylglycine appeared to inhibit the carrier-mediated cephradine uptake completely in both rat and rabbit. The complete inhibition by enalapril is reasonable, considering its lower K_m , 0.070 mM, compared to that of cephradine, 1.5 mM, in our rat perfusion studies (19,21). The smaller inhibition by other amino- β -lactam antibiotics seems to be reasonable, since the reported K_m 's of 7.2 mM for cephalexin and 5.9 mM for cefadroxil in our rat perfusion study are two orders of magnitude larger than that of enalapril and close to that of cephradine (19). Compared to the K_m 's of cephradine in this report, the K_m 's reported for gly-

lyglycine in rabbit vesicles are lower at 0.25 and 0.43 mM, depending on the proton gradient (1). Phenylpropionylproline, $K_m = 0.045$ mM, close to that of enalapril (24), showed a larger inhibition than the amino- β -lactam antibiotics but a smaller inhibition than enalapril in both rat and rabbit. For glycylproline, which least inhibited the cephradine uptake in both rat and rabbit, the highest reported K_m 's were 30.8 mM in mouse vesicles (4) and 17.4 mM in rat vesicles (5), though a relatively low K_m of 0.9 mM is also reported in rabbit vesicles (2). Kinetic parameters for the tripeptides are not available. Thus the inhibition data are similar in both rat and rabbit and appear to be reasonable, considering the reported K_m 's and assuming a simple competitive mechanism.

Comparison Between Vesicles and Perfusion: Vesicle's Surface Area Estimation

Vesicles data are expressed in terms of milligrams of membrane protein since the effective surface area for uptake is unknown. On the other hand, perfusion data are generally normalized by the geometrical surface area of the perfused intestinal segment. From comparison of the kinetic parameters, J_{\max}' from vesicles to J_{\max} from perfusion, an estimate of the equivalent surface area, S_e , of the brush border membrane vesicles as an operational factor is 0.71 cm²/mg (1.7/2.4 from Table I). However, the geometric vesicle surface area (S_v) is estimated to be about 376 cm²/mg, or about 500 times larger. This estimate is based on a total vesicular volume of 0.805 μ L/mg from the equilibrium uptake of D-glucose and an average diameter of 0.127 μ m from electron microscopic observation, which gives a surface area-to-volume ratio of 4.7×10^5 cm⁻¹ (28). The S_e of 0.71 cm²/mg implies that vesicle uptake data gives the same flux as perfusion if 1 mg of membrane protein represents 0.71 cm², or alternatively the flux in a perfusion experiment could be predicted by simply dividing the uptake rate in vesicles by S_e . The larger S_v than S_e values suggest a significantly smaller flux per unit area in vesicles than in perfusion.

There are two possible explanations for the difference. First, the surface area used in the calculation of the perme-

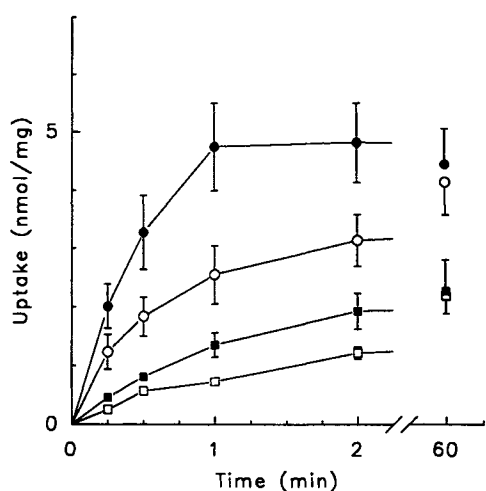


Fig. 2. pH-dependent cephradine uptake, rat (squares) and rabbit (circles), in the presence of pH 6.0/7.5 (outside/inside) of an inward proton gradient (filled symbols) and the absence of the proton gradient, pH 7.5/7.5 (open symbols). Data are represented as mean ± SE from three preparations.

ability from perfusion studies is the minimum (geometric) estimate based on the assumption of cylindrical geometry. The anatomical surface area could be about 30 times larger than the cylindrical surface area, lowering the flux per unit area estimates from the perfusion method. Second, the transport function might be damaged in vesicles by the preparation procedure. Finally, a difference in kinetic approaches, steady-state analysis in perfusion versus initial-rate analysis in vesicles, is another significant factor. It is possible that membrane binding could slow the initial uptake rate and hence lead to a lower permeability compared to the steady-state permeability measured in perfusion.

In summary, the vesicle and perfusion studies of the peptide transport of drugs are in excellent qualitative agreement. The main quantitative difference resides in the maximal transport flux. Thus vesicle systems can be used to study mechanism and rank order drugs regarding their carrier transport parameters. Extrapolating the results to extent of absorption in man requires correlation with more *in vivo* systems such as the perfused intestinal segment. Future research should establish a more quantitative relationship between these two approaches to studying drug absorption.

ACKNOWLEDGMENTS

This work was supported in part by NIGMS Grant R01-GM37188 and NS Grant 24616.

REFERENCES

1. N. Takuwa, T. Shimada, H. Matsumoto, M. Himukai, and T. Hoshi. Effect of hydrogen ion-gradient on carrier-mediated transport of glycylglycine across brush border membrane vesicles from rabbit small intestine. *Jpn. J. Physiol.* 35:629-642 (1985).
2. V. Ganapathy, J. F. Mednicino, and F. Leibach. Transport of glycyl-L-proline into intestinal and renal brush border vesicles from rabbit. *J. Biol. Chem.* 256:118-124 (1981).
3. V. Ganapathy and F. Leibach. Is intestinal peptide transport energized by a proton gradient? *Am. J. Physiol.* 249:G153-G160 (1985).
4. V. M. Rajendran, A. Berteloot, and K. Ramaswamy. Transport of glycyl-L-proline by mouse intestinal brush-border membrane vesicles. *Am. J. Physiol.* 248:G682-G686 (1985).
5. H. Said, F. K. Ghishan, and R. Redha. Transport of glycyl-L-proline in intestinal brush-border membrane vesicles of the suckling rat: Characteristics and maturation. *Biochim. Biophys. Acta* 941:232-240 (1988).
6. K. Iseki, M. Sugawara, H. Saitoh, K. Miyazaki, and T. Arita. Comparison of transport characteristics of amino β -lactam antibiotics and dipeptides across rat intestinal brush border membrane. *J. Pharm. Pharmacol.* 41:628-632 (1988).
7. E. Nakashima, A. Tsuji, S. Kagatani, and T. Yamana. Intestinal absorption mechanism of amino- β -lactam antibiotics. III. Kinetics of carrier-mediated transport across the rat small intestine in situ. *J. Pharmacobio-Dyn.* 7:452-464 (1984).
8. E. Nakashima, A. Tsuji, H. Mizuo, and T. Yamana. Kinetics and mechanism of *in vitro* uptake of amino- β -lactam antibiotics by rat small intestine and relation to the intact-peptide transport system. *Biochem. Pharmacol.* 33:3345-3352 (1984).
9. E. Nakashima and A. Tsuji. Mutual effects of amino- β -lactam antibiotics and glycylglycine on the transmural potential difference in the small intestinal epithelium of rats. *J. Pharmacobio-Dyn.* 8:623-632 (1985).
10. A. Tsuji, T. Terasaki, I. Tamai, and H. Hirooka. H^+ gradient-dependent and carrier-mediated transport of cefixime, a new cephalosporin antibiotic, across brush border membrane vesicles from rat small intestine. *J. Pharmacol. Exp. Ther.* 241:594-601 (1987).
11. T. Yoshikawa, N. Muranushi, M. Yoshida, T. Oguma, K. Hirano, and H. Yamada. Transport characteristics of cefbuten (7432-S), a new oral cephem, in rat intestinal brush-border membrane vesicles: Proton-coupled and stereoselective transport of cefbuten. *Pharm. Res.* 6:302-307 (1989).
12. N. Muranushi, T. Yoshikawa, M. Yoshida, T. Oguma, K. Hirano, and H. Yamada. Transport characteristics of cefbuten, a new oral cephem, in rat intestinal brush-border membrane vesicles: Relationship to oligopeptide and amino β -lactam transport. *Pharm. Res.* 6:308-312 (1989).
13. T. Okano, K. Inui, M. Takano, and R. Hori. H^+ gradient-dependent transport of aminocephalosporins in rat intestinal brush-border membrane vesicles. *Biochem. Pharmacol.* 35:1781-1786 (1986).
14. T. Okano, K. Inui, H. Maegawa, M. Takano, and R. Hori. H^+ coupled uphill transport of aminocephalosporins via the dipeptide transport system in rabbit intestinal brush-border membranes. *J. Biol. Chem.* 30:14130-14134 (1986).
15. K. Inui, T. Okano, H. Maegawa, M. Kato, M. Takano, and R. Hori. H^+ coupled transport of p.o. cephalosporins via dipeptide carriers in rabbit intestinal brush-border membranes: Difference of transport characteristics between cefixime and cephadrine. *J. Pharmacol. Exp. Ther.* 247:235-241 (1988).
16. M. Kato, H. Maegawa, T. Okano, K. Inui, and R. Hori. Effect of various chemical modifiers on H^+ coupled transport of cephadrine via dipeptide carriers in rabbit intestinal brush-border membranes: Role of histidine residues. *J. Pharmacol. Exp. Ther.* 251:745-749 (1989).
17. W. Kramer, F. Girbig, I. Leipe, and E. Petzoldt. Direct photoaffinity labelling of binding proteins for β -lactam antibiotics in rabbit intestinal brush border membranes with (3H)benzylpenicillin. *Biochem. Pharmacol.* 37:2427-2435 (1988).
18. W. Kramer, F. Girbig, E. Petzoldt, and I. Leipe. Inactivation of the intestinal uptake system for β -lactam antibiotics by diethylpyrocarbonate. *Biochim. Biophys. Acta* 943:288-296 (1988).
19. P. J. Sinko and G. L. Amidon. Characterization of the oral absorption of β -lactam antibiotics. I. Cephalosporins: Determination of intrinsic membrane absorption parameters in the rat intestine in situ. *Pharm. Res.* 5:645-650 (1988).
20. M. Hu and G. L. Amidon. Passive and carrier-mediated intestinal absorption components of captopril. *J. Pharm. Sci.* 77:1007-1011 (1988).
21. D. I. Friedman and G. L. Amidon. Passive and carrier-mediated intestinal absorption components of two angiotensin converting enzyme (ACE) inhibitor prodrugs in rats: Enalapril and fosinopril. *Pharm. Res.* 6:1043-1047 (1989).
22. D. I. Friedman and G. L. Amidon. Intestinal absorption mechanism of dipeptide angiotensin converting enzyme inhibitors of the lysyl-proline type: lisinopril and SQ 29,852. *J. Pharm. Sci.* 78:995-998 (1989).
23. P. J. Sinko and G. L. Amidon. Characterization of the oral absorption of β -lactam antibiotics. II. Competitive absorption and peptide carrier specificity (submitted for publication).
24. P.-F. Bai, P. Subramania, H. H. Mosberg, and G. L. Amidon. The structural requirements for the intestinal mucosal cell peptide transporter: The need for N-terminal α -amino group (submitted for publication).
25. M. Kessler, O. Acuto, C. Storelli, H. Murer, M. Muller, and G. Semenza. A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. *Biochim. Biophys. Acta* 506:136-154 (1978).
26. A. Berteloot and G. Semenza. Advantages and limitations of vesicles for the characterization and the kinetic analysis of transport systems. *Methods Enzymol.* 192:409-437 (1990).
27. J. Biber, B. Steiger, W. Haase, and H. Murer. A high yield preparation for rat kidney brush border membranes. *Biochim. Biophys. Acta* 647:169-176 (1981).
28. K. Sagara, K. Higaki, A. Yamazaki, M. Hashida, and H. Sezaki. Effect of medium-chain glycerides on the membrane transport of D-glucose and sulfanilic acid in the intestinal brush-border membrane vesicles. *J. Pharmacobio-Dyn.* 13:57-63 (1990).