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Multiple Transport Pathways for Neutral Amino Acids in Rabbit Jejunal Brush Border Vesicles

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Summary. Amino acids enter rabbit jejunal brush border membrane vesicles via three major transport systems: (1) simple passive diffusion; (2) Na-independent carriers; and (3) Na-dependent carriers. The passive permeability sequence of amino acids is very similar to that observed in other studies involving natural and artificial membranes. Based on uptake kinetics and cross-inhibition profiles, at least two Na-independent and three Na-dependent carrier-mediated pathways exist. One Na-independent pathway, similar to the classical L system, favors neutral amino acids, while the other pathway favors dibasic amino acids such as lysine. One Na-dependent pathway primarily serves neutral L-amino acids including 2-amino-2-norbornanecarboxylic acid hemihydrate (BCH), but not β-alanine or α-methylaminoisobutyric acid (MeAIB). Another Na-dependent route favors phenylalanine and methionine, while the third pathway is selective for imino acids and MeAIB. Li is unable to substitute for Na in these systems. Cross-inhibition profiles indicated that none of the Na-dependent systems conform to classical A or ACS paradigms. Other notable features of jejunal brush border vesicles include (1) no β-alanine carrier, and (2) no major proline/glycine interactions.

Key words amino acid transport · small intestinal transport · membrane vesicles · brush border transport · alanine transport · phenylalanine transport · proline transport

Introduction

Amino acids are transported across the small intestinal epithelium in two stages: uptake from the gut across the brush border membrane, and exit into the blood across the basal lateral membrane. Although mechanisms of intestinal amino acid transport have been extensively studied (*see* Schultz & Curran, 1970; Munck, 1981), there is still considerable confusion about the actual number of transport systems involved. In plasma membranes of nonpolar cells, such as Ehrlich cells and mouse fibroblasts, there is clear evidence of multiple transport pathways (*see* Christensen, 1975, 1979; Lever, 1980). These pathways include *i*) diffusion, *ii*) Na-independent carriers, e.g., the L system, and *iii*) Na-dependent carriers, e.g., the A and ASC systems. Mircheff, van Os and Wright (1980) have demonstrated similar transport pathways in intestinal basal lateral membranes. The present study elucidates the amino acid transport pathways in intestinal brush border membranes. We have used isolated membrane vesicles to explore and quantitate the diffusional, Na-independent, and Na-dependent pathways in rabbit jejunal brush borders. This study includes a comparison of our results with those for single cells, and for the rabbit renal brush border (Mircheff, Kippen, Hirayama & Wright, 1982).

Materials and Methods

Membrane Vesicle Preparation

The small intestine was removed from male New Zealand white rabbits, and flushed with ice cold buffer containing 300 mM D-mannitol in 10 mM HEPES/Tris, pH 7.5 ("300 MHT" buffer). Buffer containing 400 mM mannitol will be referred to as "400 MHT". Thirty cm of distal ileum and proximal duodenum were discarded, and the mucosa was scraped from the remaining jejunum. Scrapings from two to four animals were pooled, frozen on dry ice/methanol, and stored up to 1 week (−20° C) in 1-gm aliquots.

Brush border membranes were prepared in batches using a modification of Ca^{2+} precipitation methods (Schmitz et al., 1973; Kessler et al., 1978). All steps were carried out at 0–5° C. Briefly, 1-g aliquots of thawed mucosal scrapings were homogenized (8 ml of 300 MHT/g scrapings) using a Brinkman Polytron homogenizer (15 sec at #6 setting). One hundred mM $CaCl_2$ in 1 mM HEPES/Tris (pH 7.5) was added to the homogenate to produce a final concentration of 10 mM $CaCl_2$. After stirring for 20 min, the solution was centrifuged at $2,500 \times g$ for 5 min. The supernatant containing brush border material was centrifuged at $50,000 \times g$ for 30 min. The resulting pellet was resuspended in 400 MHT using a glass/Teflon homogenizer, and then recentrifuged at $50,000 \times g$ for 30 min. The final brush border membrane pellet was suspended in 400 MHT at a concentration of 10 to 15 mg protein/ml. Aliquots of the final suspension were stored in liquid nitrogen as described by Stevens et al. (1982). Transport studies involving blocks of com-

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parative data (e.g., the effects of variations unlabeled inhibitors on labeled substrate uptake) were performed using the same batch of stored membranes prepared from several rabbits. Similar results were obtained when the experimental blocks were repeated using different membrane batches. In the Results, membrane batch numbers accompany experimental blocks.

The brush border marker enzyme, alkaline phosphatase (*p*-nitrophenyl phosphatase E.C.3.1.3.1) was routinely assayed (Mircheff & Wright, 1976) before membranes were stored in liquid nitrogen. This enzyme was consistently enriched 20-fold in the final pellet compared to the crude homogenate. Protein was determined using the BioRad protein assay, with gamma globulin as the protein standard.

Transport Measurements

The simultaneous uptake of ^{14}C-labeled L-amino acids and ^{3}H-D-mannitol was measured utilizing a rapid mixing/filtration technique. All experiments were conducted at 23 °C.

For each uptake measurement, 10 µl of membrane vesicles and 20 µl of radioactive uptake buffer were placed separately at the bottom of a 12 × 100 cm polystyrene tube. The uptake buffer components were adjusted so that the final reaction mixture contained initial gradients of 100 mM NaCl or 100 mM KCl, and ^{14}C-labeled amino acids and ^{3}H-D-mannitol ranging in concentration from 0 to 100 mM. In addition, the final reaction mixture contained 0 to 100 mM inhibitors (adjusted to pH 7.5) and sufficient D-mannitol to maintain isosmotic conditions. An electronically controlled apparatus (*see* Stevens, 1980) initiated the reaction by rapidly vibrating the tube. After a prescribed reaction period (1 sec to 2 hr), 1 ml of ice cold 400 MHT was added to quench the reaction. The quenched mixture was then filtered using a prewetted and chilled 0.45 µm nitrocellulose filter (Sartorius SM11306). The membranes were washed once with 5 ml of 400 MHT, then dissolved in scintillation cocktail (Amersham PCS). The radioactivity of ^{3}H and ^{14}C trapped by the vesicles was measured by liquid scintillation counting. Values for nonspecific retention of radioactivity by the filters and vesicles were obtained from zero time uptakes, and were subtracted from total filter radioactivity. After appropriate radioisotope channel overlap corrections, the radioactivity was converted to units of pmol solute uptake/mg protein/time period.

Uptake was measured in the presence and absence of Na. Na-dependent carrier-mediated influx $J^2 = J^t(\mathrm{Na}) - J^t(\mathrm{K})$, where J^t is total influx measured in 100 mM NaCl or KCl. Na-independent carrier-mediated influx $J^1 = J^t(\mathrm{K}) - J^D$, where J^D is the diffusional component determined by measuring total influx of ^{14}C-substrate (J^t) in the presence of 100 mM unlabeled substrate.

Generally, the uptake time courses of 50 µM ^{14}C-substrate (in NaCl or KCl) were linear to 5 sec (*see* Fig. 1, Table 1), and therefore initial influxes were measured from 2 to 5 sec. However, due to the low specific activity of ^{14}C-MeAIB, its influx time was extended to 10 sec to allow sufficient accumulation of radioactivity.

Data were analyzed using a North Star computer (North Star, Inc., Berkeley, CA) and plotted on a Houston Instruments (Austin, TX) HiPlot DMP-4 digital plotter.

The symbols used throughout the paper are: PHE, phenylalanine; PRO, proline; ALA, alanine; GLY, glycine; MeAIB, N-methylaminoisobutyric acid; β-ALA, β-alanine; BCH, 2-amino-2-norbornanecarboxylic acid hemihydrate; LYS, lysine; MET, methionine, OH-L-PRO, 4-hydroxy-L-proline. All amino acids are the L-stereoisomer unless otherwise indicated. All unlabeled reagents were obtained from Sigma Chemical, St. Louis, MO, except 2-amino-2-norbornanecarboxylic acid hemihydrate (BCH) which was obtained from Aldrich Chemical Co., Milwaukee, WI.

The following radioactive substrates were obtained from Amersham and New England Nuclear: (U-^{14}C)glycine, L-(U-^{14}C)alanine, β(U-^{14}C)alanine, L-(U-^{14}C)phenylalanine, L-(U-^{14}C)lysine, L-(U-^{14}C)proline, α(1-^{14}C)methylaminoisobutyric acid, and D-(1-^{3}H(N))mannitol.

Results

We investigated the differential uptakes of amino acids by brush border vesicles using uptake time course, kinetic, and cross-competition experiments. In each case, diffusional, Na-independent, and Na-dependent uptakes were measured.

Uptake Time Courses

Figure 1 shows the uptake of 50 µM ^{14}C-labeled PRO, PHE, ALA, GLY, and MeAIB measured in the presence of NaCl or KCl (initial gradient conditions). It is apparent that each substrate, except GLY, was rapidly accumulated in the vesicles against a chemical gradient (intravesicular/extravesicular) by a Na-stimulated mechanism. The peak accumulation ratios were: PRO, 7:1; PHE, 8:1; ALA, 1.4:1; MeAIB, 1.9:1. In the absence of Na, the initial influxes were reduced and no overshoot occurred. For GLY, although uptake did not occur against a chemical gradient, Na enhanced the initial rate of uptake. The uptake time courses of LYS and β-ALA (*not shown*) did not display overshoots, and were unaffected by Na (*see also* Table 1).

Initial Influxes

The ensuing kinetic data are based on initial influx values. Table 1 summarizes the initial influxes of amino acids measured under various conditions. As the legend indicates, uptake times were sufficiently short (e.g., 2 sec for PRO) to ensure measurement during the linear portion of the uptake time course. It is notable that sizable fractions of the total influxes (J^t) of PRO and PHE were Na-dependent (J^2), with smaller Na-dependent fractions (J^2) of ALA, MeAIB, and GLY influx. Substituting Na with K or Li reduced the total influxes of PHE, ALA, or GLY, although the total influxes in K or Li (J^t(K or Li)) were greater than diffusion (J^D). This suggested the presence of Na-independent carrier-mediated mechanisms (J^1) for these amino acids. PRO and MeAIB carrier-mediated uptakes, on the other hand, were strictly Na-dependent (J^2); when K or Li were substituted for Na, PRO or MeAIB total influxes (J^t) were reduced to passive diffusion (J^D).

The Na-independent LYS influx (J^1) exceeded diffusion (*see* Table 1). This suggested the presence of a carrier-facilitated pathway for this amino acid.

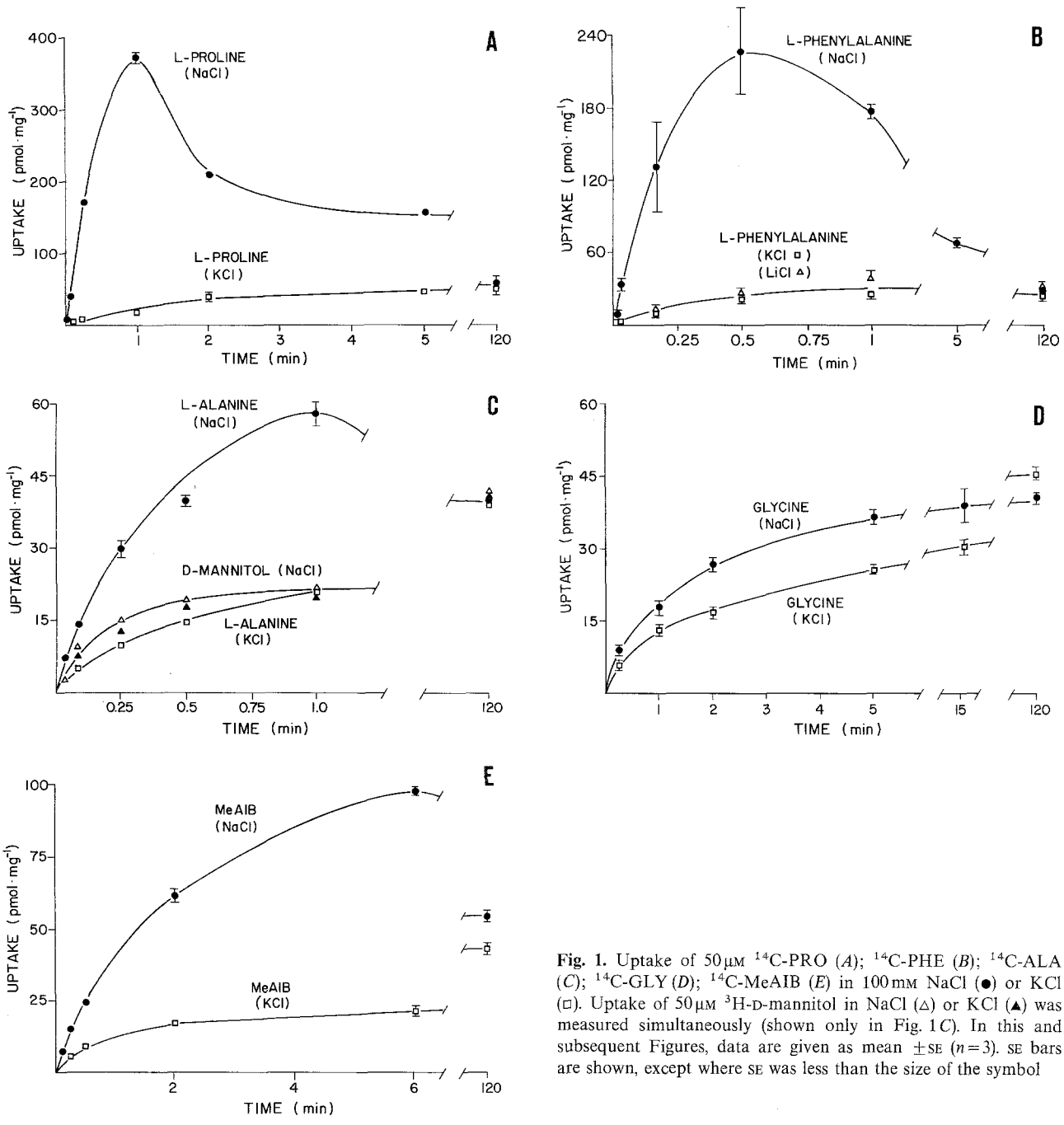


Fig. 1. Uptake of 50 μM ^{14}C-PRO (*A*); ^{14}C-PHE (*B*); ^{14}C-ALA (*C*); ^{14}C-GLY (*D*); ^{14}C-MeAIB (*E*) in 100 mM NaCl (●) or KCl (□). Uptake of 50 μM ^{3}H-D-mannitol in NaCl (△) or KCl (▲) was measured simultaneously (shown only in Fig. 1*C*). In this and subsequent Figures, data are given as mean ± SE ($n=3$). SE bars are shown, except where SE was less than the size of the symbol

Influx of β-ALA occurred primarily by diffusion with no apparent carrier mediation (i.e., $J^D = J^t$). The diffusion data in Table 1 yielded the following diffusion sequence for jejunal brush membrane vesicles: PHE > β-ALA > mannitol > ALA > MeAIB > PRO > GLY > LYS.

Kinetics of Neutral Amino Acid Transport (PRO, PHE, ALA)

Based on the results shown in Table 1, we investigated the Na-dependent uptake kinetics (J^2) of PRO. PHE and ALA uptakes apparently occurred via more than one carrier-mediated pathway (J^1 and J^2); therefore, the kinetics of these amino acids were investigated in an attempt to resolve the characteristics of these uptake pathways.

Pro Kinetics. The kinetics of PRO uptake were obtained in NaCl as shown in Fig. 2. The data of Fig. 2*A* indicate that uptake occurred via a saturable and a diffusional pathway. The Hofstee plot (Fig. 2*B*) was dissected into the appropriate Na-de-

pendent carrier-mediated component (J^2) plus diffusion (J^D) using a modification of the iterative stripping method of Spears, Sneyd and Loten (1971). Carrier-mediated influx can be described by the relationship:

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

Table 1. Initial rates of L-amino acid influxes[a]

	Initial influx (pmol mg^{-1} sec^{-1})						
	PRO	PHE	ALA	GLY	MeAIB	LYS	β-ALA
J^t(Na)	18.1	17.3	4.9	0.9	2.4	2.2	1.8
J^t(K)	1.0	3.9	1.7	0.7	0.8	2.3	1.9
J^t(Li)	0.9	2.8	1.8	-	0.7	-	-
J^2	17.0	13.5	3.2	0.13	1.6	0	0
J^D	0.8	2.0	1.0	0.6	0.9	0.5	1.8
J^1	0	2.0	0.7	0.22	0	1.5	0

[a] Influx values were obtained at a substrate concentration of 50 μM. The symbols used are: J^t, total influx of substrate in 100 mM NaCl, KCl or LiCl; J^2, Na-dependent carrier-mediated influx such that $J^2 = J^t(Na) - J^t(K)$; J^D, diffusional component of substrate influx determined by measuring total influx in the presence in 100 mM unlabeled substrate, the J^D for each substrate was the same in 100 mM NaCl or 100 mM KCL; J^1, Na-independent carrier-mediated influx such that $J^1 = J^t(K) - J^D$. Influx was measured after the following times, depending on the specific activity and linearity of time course: PRO, 2 sec; PHE, 2 sec; ALA, 2 sec; GLY, 5 sec; LYS, 5 sec; β-ALA, 5 sec; MeAIB, 10 sec. The J^D of mannitol measured between 2–10 sec was 1.5 ±0.1 pmol mg^{-1} sec^{-1}. Mean values are displayed ($n \geq 3$). The standard errors (*not shown*) were generally less than 10% with the following exceptions. J^2 of GLY was ±54%, J^D of PHE was ±16%, J^1 of PHE was ±17%, J^1 of ALA was ±13%, J^1 of GLY was ±36%. Membrane batch 126.

where J = carrier-mediated influx, J_{max} = maximal influx, $[S]$ = substrate concentration, and K_t = the transport constant such that $K_t = [S]$ when $J = J_{max}/2$. Simple diffusion of substrate (J^D) can be described by:

$$J^D = P[S] \quad (2)$$

where P = the permeability coefficient for the substrate, and is obtained from the abscissa intercept of the line approaching the vertical asymptote of the Hofstee plot. Total influx (J^t) of PRO is thus the sum of carrier-mediated + diffusional uptake: $J^t = J^2 + J^D$. The appropriate constants are presented in the legend of Fig. 2. Kinetics of PRO measured in KCl were the same as diffusion, indicating that carrier-mediated PRO uptake is strictly Na-dependent.

In Fig. 2*B* the computer plotted theoretical curve of the sum of Eq. (1) plus Eq. (2) (using the appropriate constants given in Fig. 2*B*) suggests that a carrier mechanism plus diffusion constitute the most conservative model of PRO uptake kinetics.

PHE Kinetics. The kinetics of PHE influx in NaCl and KCl are shown in Fig. 3. Uptake in KCl or NaCl (Fig. 3*A*) was greater than diffusion. This suggests the presence of Na-dependent and Na-independent carrier-mediated pathways in jejunal brush border. The Hofstee plots (Fig. 3*B*, *C*) bear out this observation.

In NaCl (Fig. 3*B*) PHE uptake occurred as a composite of two carrier-mediated pathways plus diffusion. Each carrier system, designated System 1 and System 2, fit the general Eq. (1). The computer-

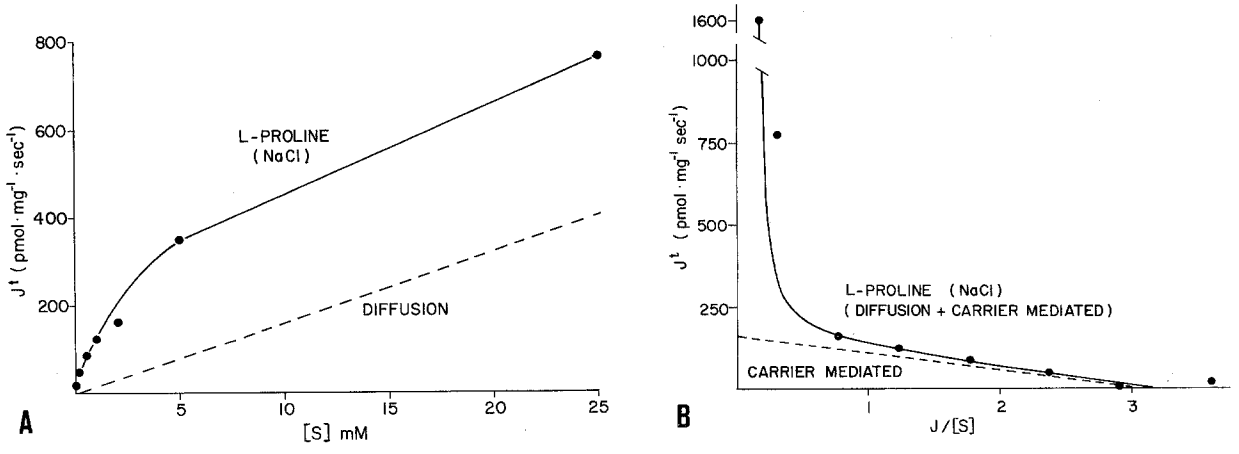


Fig. 2. Kinetics of PRO uptake. (*A*) Total influx of PRO (J^t) in 100 mM NaCl as a function of [PRO] (●). Passive diffusion of PRO (---) was estimated by measuring uptake of 50 μM ^{14}C-PRO in the presence of 100 mM unlabeled PRO. Influx time was 2 sec. (*B*) Hofstee plot of the total PRO influx in NaCl (J^t) *vs.* J^t/[PRO]. Abscissa units are 10^{-8} l mg^{-1} sec^{-1}. Kinetic constants for the Na-dependent carrier-mediated component (---) determined by nonlinear regression are $J_{max} = 166 \pm 10$ pmol mg^{-1} sec^{-1}, $K_t = 0.55 \pm 0.05$ mM. The total uptake of PRO (●) is the computer-plotted sum of the carrier-mediated influx plus diffusion (as explained in the text), where the permeability coefficient (abscissa intercept of vertical asymptote) $P = 1.6 \times 10^{-8}$ l mg^{-1} sec^{-1}. Membrane batch 421

plotted curve of this composite of PHE uptake (Fig. 3*B*) using the appropriate constants given in the legend suggests that $J^1+J^2+J^D$ appropriately describes the empirical data.

The kinetic data describing PHE uptake in KCl (Fig. 3*A*) were also converted to a Hofstee plot. In this case, System 1 plus diffusion account for Na-independent PHE uptake (Hofstee data *not shown*). To obtain a better estimate of the parameters J^1_{max} and K^1_t, the experiment was repeated using another batch of membrane vesicles (Fig. 3*C*). Uptake via system 1 is described by Eq. (1) (the appropriate constants are indicated in the legend of Fig. 3*C*). The computer-plotted curve (Fig. 3*C*) suggests that PHE uptake is reasonably described by J^1+J^D.

ALA Kinetics. ALA influx kinetics in NaCl are shown in Fig. 4. The Hofstee plot (Fig. 4*B*) was dissected into 3 components by iterative stripping: 2 carrier-mediated pathways plus passive diffusion, $J^t=J^1+J^2+J^D$. Each carrier-mediated system is described by the general Eq. (1), and passive diffusion by Eq. (2). The appropriate kinetic constants are indicated in the legend of Fig. 4. The computer-plotted curve (Fig. 4*B*) of the composite uptake in NaCl suggests this is a reasonable description of the empiricial data.

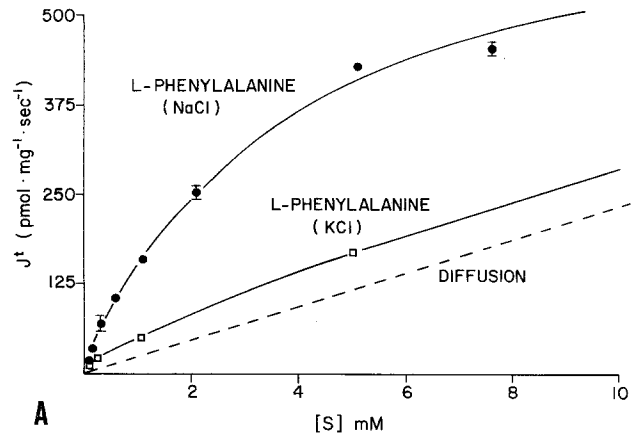
The kinetic constants for PRO, PHE, and ALA obtained from Figs. 2, 3 and 4 are summarized in Table 2.

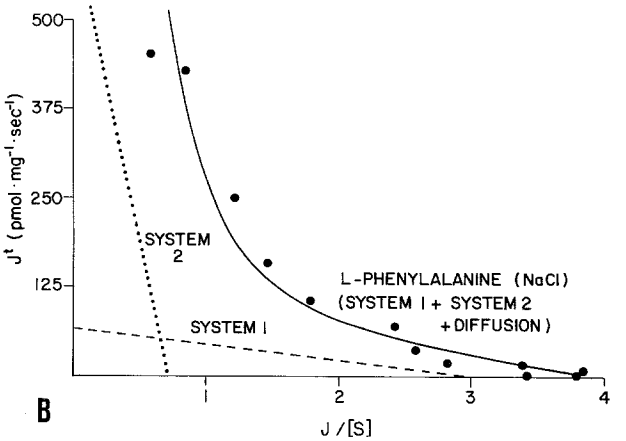
Competitive Inhibition

Unlabeled amino acids were tested for their effectiveness in competitively inhibiting Na-dependent and Na-independent uptake of ^{14}C-amino acids. The inhibition of Na-dependent carrier-mediated influx was determined by:

$$\% \text{ inhibition} = 100 - \left[\frac{J^t_i(\text{Na}) - J^t_i(\text{K})}{J^t(\text{Na}) - J^t(\text{K})}\right] \cdot 100 \qquad (3)$$

where J^t = total uninhibited (i.e., control) initial influx of 50 μM ^{14}C-amino acid substrate in





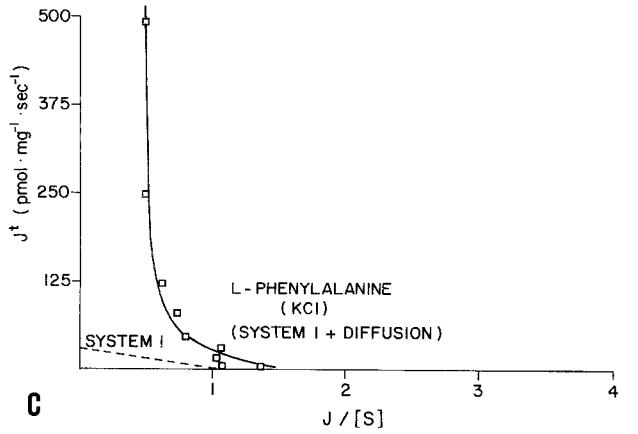


Fig. 3. Kinetics of PHE uptake. (*A*) Total influx of PHE (J^t) in 100 mM NaCl (●) or KCl (□) as a function of [PHE]. Passive diffusion of PHE (---) was estimated by measuring 50 μM ^{14}C-PHE uptake in the presence of 100 mM unlabeled PHE. The influx time was 2 sec. (*B*) Hofstee plot of the total PHE uptake in NaCl (J^t) *vs.* J^t/[PHE]. Abscissa units are 10^{-7} l mg^{-1} sec^{-1}. The total influx (●) in NaCl is the computer-plotted sum of two carrier-mediated systems (System 1 ---, System 2 ...) plus diffusion. The kinetic constants for System 1, determined by linear regression, are: $J^1_{max}=68\pm8$ pmol mg^{-1} sec^{-1}, $K^1_t=0.23\pm0.04$ mM. The constants for System 2 are $J^2_{max}=622\pm256$ pmol mg^{-1} sec^{-1}, $K^2_t=8.8\pm4.3$ mM. The permeability coefficient equals the abscissa intercept of the vertical diffusion component (*not drawn*): $P=2.4\times10^{-8}$ l mg^{-1} sec^{-1}. Membrane Batch 126. *C.* Hofstee plot of the total PHE influx in KCl (J^t) *vs.* J^t/[PHE]. Abscissa units are 10^{-7} l mg^{-1} sec^{-1}. The total influx (□) in KCl was the computer-plotted sum of a single carrier System 1 (---) plus diffusion (vertical line not indicated): $J^1_{max}=30\pm2$ pmol mg^{-1} sec^{-1}, $K^1_t=0.27\times0.02$ mM, and $P=4.5\times10^{-8}$ l mg^{-1} sec^{-1}. Membrane batch 219

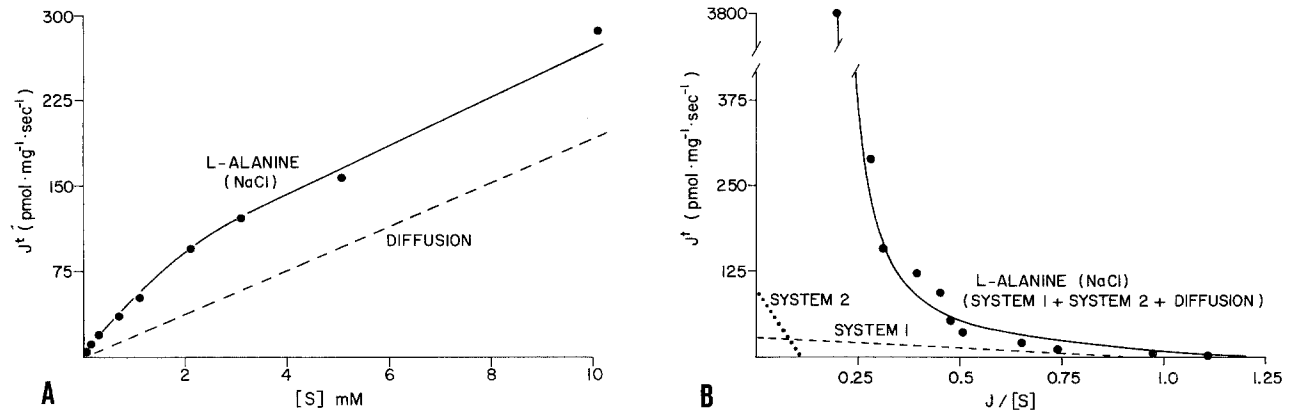


Fig. 4. Kinetics of ALA uptake (*A*) Total uptake of ALA (J^t) in 100 mM NaCl (●) as a function of [ALA]. Passive diffusion of ALA (---) was estimated by measuring 50 μM ^{14}C-ALA uptake in the presence of 100 mM unlabeled ALA. The influx time was 2 sec. (*B*) Hofstee plot of the total ALA influx in NaCl (J^t) *vs.* J^t/[ALA]. Abscissa units are 10^{-7} l mg^{-1} sec^{-1}. The total influx (●) in NaCl was the computer-plotted sum of two carrier-mediated systems (System 1 ---, System 2...) plus diffusion (*not shown*) as explained in the text. Kinetic constants were determined by curve fitting: $J^1_{max}=29$ pmol mg^{-1} sec^{-1}, $K^1_t=0.32$, $J^2_{max}=100$ pmol mg^{-1} sec^{-1}, $K^2_t=9$ mM, $P=1.9\times10^{-8}$ l mg^{-1} sec. Membrane batch 219

Table 2. Summary of transport kinetic parameters[a]

Amino acid	Measured in NaCl				
	P	K^1_t	J^1_{max}	K^2_t	J^2_{max}
PRO[b]	1.6	–	–	0.55	166
PHE[c]	2.4	0.23	68	8.8	622
ALA[d]	1.9	0.32	29	9	100
	Measured in KCl				
PHE[d]	4.5	0.27	30		

[a] Values were obtained as described in the text and Figs. 2, 3 and 4. The symbols represent: P, permeability coefficient, units of 10^{-8} l mg^{-1} sec^{-1}; K_t, transport constant of transport System 1 or System 2 for the appropriate substrate, units of mM; J_{max}, maximal influx rates for transport System 1 or System 2 for the appropriate substrate, units pmol mg^{-1} sec^{-1}.
[b] Membrane batch 421.
[c] Membrane batch 126.
[d] Membrane batch 219.

100 mM NaCl or KCl media, J^t_i = the corresponding influx in the presence of amino acid inhibitor. The inhibition of Na-independent carrier-mediated influx was thus:

$$\% \text{ inhibition} = 100 - \left[\frac{J^t_i(K) - J^D}{J^t(K) - J^D}\right] \cdot 100 \qquad (4)$$

where J^t and J^t_i were described above, and J^D is the diffusional influx of substrate amino acid determined by measuring total influx of 50 μM ^{14}C-substrate in the presence of 100 mM unlabeled substrate (KCl).

Inhibition of Na-Dependent Transport

The inhibition of Na-dependent transport of several neutral amino acids is shown in Table 3. In general, PHE inhibited carrier-facilitated uptake of all sub-

Table 3. Inhibition of Na-dependent transport of neutral amino acids[a]

Substrate	J^2 (pmol mg^{-1} sec^{-1})	Percent inhibition by 100 mM inhibitor					
		PRO	ALA	PHE	MeAIB	GLY	β-ALA
PRO	17.0 ±1.2	100	49	94	96	21	0
PHE	13.5 ±0.3	33	77	100	4	32	11
ALA	3.2 ±0.1	58	100	100	6	73	22
MeAIB	1.6 ±0.02	100	76	100	100	15	+8[b]
GLY	0.13±0.07	15	81	100	+42[b]	59	10

[a] Initial Na-dependent influx of 50 μM ^{14}C-substrate (J^2) was measured as described in the text and Table 1. The percent inhibition was calculated based on triplicate measurements of influx in the presence and absence of unlabeled inhibitor. There was negligible inhibition of Na-dependent neutral amino acid influx by 100 mM LYS. β-ALA influx was not inhibited by any amino acid indicated. Membrane batch 126.
[b] Stimulation compared to control influx.

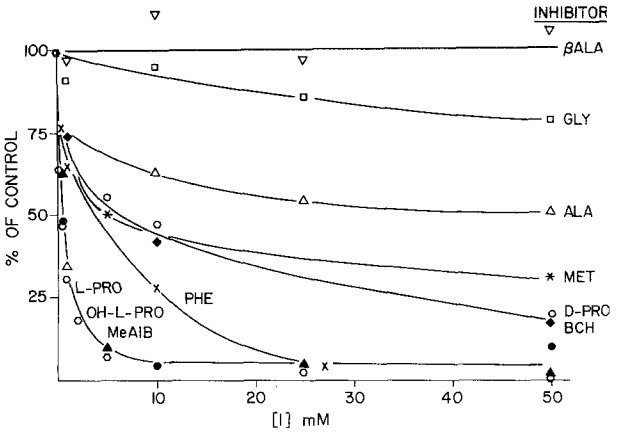


Fig. 5. Inhibition of 50 μM ^{14}C-PRO Na-dependent influx by unlabeled amino acids. The influx times were 2 sec. The values were determined by: % control = % inhibition [*see* Eq. (3)] − 100%. The symbols are: β-ALA (▽), GLY (□), ALA (△), MET (*), D-PRO (○), BCH (◆), PHE (×), L-PRO (○), OH-L-PRO (●), MeAIB (▲). Membrane batch 424

strates, whereas β-ALA affected all substrates only to a minor extent. PRO and MeAIB were mutually inhibitable, but displayed differential inhibition patterns with other amino acids. Notably, PHE and ALA uptakes were virtually insensitive to MeAIB, but not to PRO. Many of the inhibitions were partial (e.g., PRO inhibition of PHE, ALA and GLY; ALA inhibition of PRO, PHE, and MeAIB) which suggests the presence of overlapping pathways. β-ALA influx was not inhibited by any inhibitor tested. None of the substrates was appreciably inhibited by 100 mM LYS. In addition to the inhibitors of Table 3, PRO was also inhibited 100% by 100 mM OH-L-PRO and PHE was inhibited 100% by 100 mM MET. The differential patterns of inhibition were investigated in more detail with additional inhibitors for the substrates PRO and PHE.

Inhibition of PRO. Figure 5 depicts inhibition of Na-dependent PRO influx by neutral amino acids. GLY, ALA, and MET inhibited only a fraction of the carrier-mediated PRO pathways, as shown by their incomplete inhibition of PRO uptake. PRO, OH-L-PRO, and MeAIB, however, produced complete inhibition, with similar inhibition curves. PHE also completely inhibited PRO influx, but at a higher concentration. Inspection of the inhibition curves for BCH and D-PRO suggests that these amino acids should produce complete inhibition at concentrations greater than 50 mM.

The apparent inhibitor constants (K_i) for Na-dependent PRO transport were calculated for the various test inhibitor amino acids (Hajjar & Curran, 1970; Preston, Schaffer & Curran, 1974), and are shown in Table 4. Apparent K_i values were calculated assuming a single Na-dependent carrier-mediated PRO pathway exists in jejunal brush border. The apparent K_i's for MET, ALA and GLY were estimated from the fraction of PRO transport inhibitable by these amino acids (*see* Table 4).

Table 4. Apparent K_i values for Na-dependent proline transport[a]

Inhibitor [I]	K_i (mM)
OH-L-PRO	0.43
MeAIB	0.78
PHE	1.5
MET	3[b]
ALA	3[b]
D-PRO	6
BCH	7
GLY	12[b]

[a] The apparent K_i was calculated using:

$$K_i = \left[\frac{J_i^2}{J^2 - J_i^2}\right]\left[\frac{K_t[I]}{[\mathrm{PRO}] + K_t}\right]$$

where J^2 = total uninhibited Na-dependent ^{14}C-PRO influx at [PRO] = 50 μM (J^2 = total PRO influx in 100 mM NaCl minus total PRO influx in KCl). J_i^2 involves analogous influx measurements in the presence of unlabeled inhibitor. The concentrations of inhibitor used were (in mM): 0.5 OH-L-PRO, 0.5 MeAIB, 0.5 PHE, 5 MET, 10 ALA, 5 D-PRO, 10 BCH and 25 GLY. K_t = 0.55 mM PRO (*see* Table 2). J^2 and J_i^2 were measured in triplicate. Membrane batch 424.

[b] Data were adjusted to the fraction of Na-dependent ^{14}C-PRO transport inhibitable by these amino acids (*see* Fig. 5). For example, based on the data of Fig. 5, the apparent K_i for ALA was determined assuming that the maximum inhibition (i.e., asymptote) at [I] = 50 mM was 100% instead of 49% (i.e., 0% of control instead of 51% of control influx).

The wide range of apparent K_i's indicates varying degrees of affinity for the PRO carrier(s). The K_i's of OH-L-PRO, MeAIB, and PHE were similar to the K_t for PRO, which suggests that these these substrates possibly share a common carrier. The higher apparent K_i's for MET, ALA, D-PRO, BCH, and GLY indicate that these amino acids may interact with the PRO carrier, but not as its primary substrates. The moderately low K_i for D-PRO suggests a weak stereospecificity of this system.

Inhibition of PHE. Figure 6 shows that MeAIB did not inhibit PHE transport, while PHE and MET produced complete inhibition. As in the case of PRO, several amino acids inhibited only a fraction of the Na-dependent PHE pathways. Inspection of the D-PHE inhibition curve suggests that this stereoisomer inhibition should eventually completely inhibit PHE transport.

The apparent K_i values for Na-dependent PHE transport are given in Table 5. As mentioned above

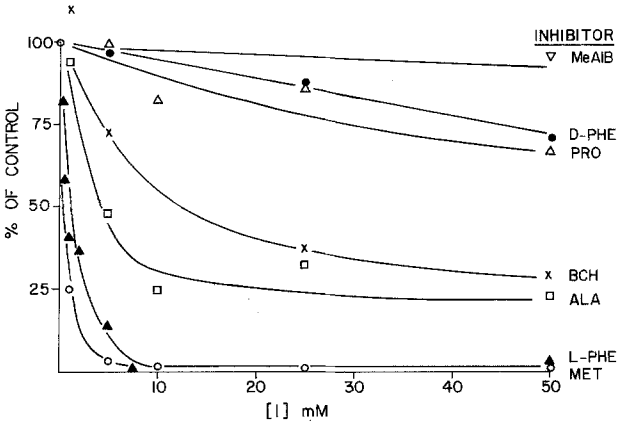


Fig. 6. Inhibition of 50 μM ^{14}C-PHE Na-dependent influx by unlabeled amino acids. Influx times were 2 sec. The % control values were determined as explained in Fig. 5. The symbols are: MeAIB (▽), D-PHE (●), PRO (△), BCH (×), ALA (□), L-PHE (▲), MET (○). Membrane batch 424

Table 5. Apparent K_i values for Na-dependent phenylalanine transport[a]

Inhibitor [I]	K_i (mM)
MET	0.3
ALA	2[b]
BCH	8[b]
PRO	32[b]
D-PHE	122

[a] The apparent K_i was calculated using:

$$K_i = \left[\frac{J_i^2}{J^2 - J_i^2}\right]\left[\frac{K_t[I]}{[\text{PHE}]+K_t}\right]$$

where J^2 = total uninhibited Na-dependent ^{14}C-PHE influx at [PHE] = 50 μM (J^2 = total PHE influx in 100 mM NaCl minus total PHE influx in KCl). J_i^2 involves analogous influx measurements in the presence of unlabeled inhibitor. The concentrations of inhibitor used were (in mM): 1 MET, 5 ALA, 5 BCH, 10 PRO, 50 D-PHE. K_t = 8.8 mM PHE (*see* Table 2). Membrane batch 424.

[b] Data were adjusted to the fraction of Na-dependent ^{14}C-PHE transport inhibitable by these amino acids (*see* Fig. 6). For example, based on the data of Fig. 6, the apparent K_i for ALA was determined assuming that the maximum inhibition (i.e., asymptote) at [I] = 50 mM was 100% instead of 75% (i.e., 0% of control 25% of control influx).

for PRO transport, the apparent K_i's were cautiously determined by assuming that inhibition occurred in a single PHE Na-dependent pathway. The low apparent K_i of MET (0.3 mM) suggests that it is possibly a preferred substrate of this carrier. The relatively low K_i's of ALA (2 mM) and BCH (8 mM) suggests that these amino acids also interact preferentially with a PHE carrier. However, PRO is a relatively poor substrate for this system. The high apparent K_i for D-PHE indicates a high degree of stereoselectivity.

Inhibition of Na-Independent Transport

Neutral Amino Acids. Jejunal brush border membranes possess Na-independent carrier-mediated pathway(s), as suggested by the uptake time courses (Fig. 1), initial influxes (Table 1), and uptake kinetics (Fig. 3). Candidate substrates of Na-independent transport route(s) were tested for their cross-inhibition as shown in Table 6. PHE uptake was inhibited partially by ALA, GLY, and PRO, and completely inhibited only by itself. ALA uptake was totally inhibited by PHE, ALA, GLY, and partially by LYS. ALA and GLY uptakes were mutually totally inhibitable, and GLY was totally inhibited only by ALA and GLY. PHE inhibited only a fraction of GLY influx. The substrates PRO, MeAIB, and β-ALA were omitted from Table 6 because $J^t = J^D$, and they were not inhibited by any test amino acid. However, PRO, MeAIB, and β-ALA served as inhibitors as shown in Table 6.

Using ^{14}C-PHE as substrate, we determined apparent K_i values for various amino acids sharing the Na-independent pathway (Table 7), assuming uptake occurs via a single pathway. The range of K_i's was from 0.27 to 1 mM. The low K_i for D-PHE implies a low degree of stereospecificity for this system.

Basic Amino Acid Transport. The data of Table 1 imply the presence of Na-independent carrier-me-

Table 6. Inhibition of Na-independent transport[a]

Substrate	J^1 (pmol mg^{-1} sec^{-1})	Percent inhibition by 100 mM inhibitor						
		PHE	ALA	GLY	PRO	MeAIB	β-ALA	LYS
PHE	2.0±0.3	100	77	47	35	15	11	10
ALA	0.7±0.1	100	100	100	26	+34[b]	29	44
GLY	0.2±0.08	38	94	100	46	28	47	85

[a] Initial Na-independent influx of 50 μM ^{14}C-substrate (J^1) was measured as described in the text and Table 1. The percent inhibition was calculated based on triplicate measurements of influx in the presence and absence of unlabeled inhibitor. Substrates PRO, MeAIB, and β-ALA were omitted because their uptakes in KCl were equivalent to passive diffusion. Membrane batch 126.

[b] Stimulation compared to control influx.

diated pathway(s) for LYS uptake. Preliminary kinetic experiments gave an approximate K_t = 1 mM, and J_{max} = 30 pmol mg^{-1} sec^{-1}. The apparent K_i's for LYS transport are shown in Table 8. The similarity of K_i's for the test dibasic amino acids and homoserine suggested that these substrates share a dibasic amino acid carrier site with LYS. The higher K_i's for MET and LEU suggested a smaller degree of interaction of these substrates with the LYS carrier. PHE, GLY, β-ALA, and ALA inhibited LYS influx, but only to a minor extent. We were unable to show inhibition of LYS influx by PRO, MeAIB, or BCH. The moderately low apparent K_i for D-LYS implies a relatively small degree of stereoselectivity in this system. A more comprehensive treatment of dibasic amino acid transport is currently under investigation.

Discussion

Utilizing liquid nitrogen-stored membrane vesicles, we have attempted to delineate some of the pathways by which amino acids enter the brush border of rabbit jejunum. A summary of the amino acid pathways deduced in the present study appears in Table 9. Three gross categories of amino acid transport systems are readily apparent: 1) simple passive diffusion, 2) Na-independent carriers, and 3) Na-dependent carriers.

Simple Passive Diffusion

The extent to which each test amino acid enters the brush border by passive diffusion (Table 1) depends on the substrate structure in accordance with the principles discussed by Diamond and Wright (1969). The permeability sequence for at least five of the solutes - PHE, mannitol, ALA, GLY, and LYS - is very similar to that obtained for red blood cells (*see* Young & Ellory, 1977), and liposomes (Klein, Moore & Smith, 1971; Wilson & Wheeler, 1973).

β-ALA uptake insensitivity to Na, and to competitive inhibition indicates that β-ALA uptake is solely by passive diffusion with no carrier mediation (Tables 1, 3, 6, 9). The β-ALA/ALA permeability ratio of 1.8 was expected in view of the fact that at pH 7.5 more β-ALA is in the uncharged form than ALA; the isoelectric points for β-ALA and ALA are pH 6.9 and 6 (*see also* Christensen, 1979). Our findings agree with earlier work by Lin, Hagihira and Wilson (1962), and Spencer, Bow and Markulis (1962) who found that β-ALA was not actively transported by hamster intestine, and failed to interact with L-ALA uptake. The absence of a β-ALA carrier reported here for jejunal brush border appears to be unique among tissues investigated for multiple transport pathways (Christensen, 1964, 1969; Mircheff et al., 1981).

In previous studies of amino acid uptake by intestine (*see* Schultz & Curran, 1970; Preston et al., 1974), the diffusional component was considered unimportant. In the present study, it is apparent that at low concentrations of most amino acids diffusion offers a significant pathway for transmembrane movement in vesicles. For example, at 5 mM diffusion accounts for 25% of the total PHE uptake in NaCl at 23° C.

Table 7. Apparent K_i values for the Na-independent transport system[a]

Inhibitor [I]	K_i (mM)
L-PHE	0.27
LEU	0.20
ALA	0.37
BCH	0.41
MET	0.44
D-PHE	1

[a] The apparent K_i was calculated using:

$$K_i = \left[\frac{J_i^1}{J^1 - J_i^1}\right]\left[\frac{K_t[I]}{[\text{PHE}] + K_t}\right]$$

where J^1 = total uninhibited ^{14}C-PHE influx in KCl minus J^D at [PHE] = 50 μM (J^D = the diffusional component of PHE influx determined by measuring total influx in the presence of 100 mM PHE). J_i^1 involves analogous PHE influx measurements in the presence of inhibitor [I] = 0.5 mM. K_t = 0.27 mM PHE (*see* Table 2). All influx values were measured in triplicate. Membrane batch 219.

Table 8. Inhibition of lysine transport[a]

Inhibitor [I]	K_i (mM)
Arginine	0.7
Homoserine	0.7
Histidine	0.9
Ornithine	1
D-LYS	2
MET	6
LEU	6

[a] The apparent K_i was calculated using:

$$K_i = \left[\frac{J_i^{Ly}}{J^{Ly} - J_i^{Ly}}\right]\left[\frac{K_t[I]}{[\text{LYS}] + K_t}\right]$$

where J^{Ly} = total unhibited LYS influx in KCl minus J^D at [LYS] = 50 μM (J^D = the diffusional component of LYS influx determined by measuring total influx in the presence of 100 mM LYS). J_i^{Ly} involves analogous LYS influx measurements in the presence of inhibitor [I] = 1 mM. J^{Ly} = 1.5 ± 0.1 pmol mg^{-1} sec^{-1} (n = 3, ± SE), and K_t = 1 mM (*see text*). Lysine influx was not inhibited by PRO, MeAIB, or BCH. Membrane batch 219.

Table 9. Amino acid pathways in rabbit jejunum brush border[a]

A. Simple diffusion

B. Na-Independent pathways

Primary substrates	Inhibitors		
	Total	Partial	Minimal
PHE, ALA, GLY	LEU BCH MET	PRO	MeAIB PRO β-ALA LYS
Dibasic amino acids	-	-	-

C. Na-Dependent pathways

Primary substrates	Inhibitors		
	Total	Partial	Minimal
Neutral amino acids (PHE, PRO, ALA, GLY)	-	-	MeAIB β-ALA LYS D-PHE
PHE	MET	-	MeAIB PRO BCH ALA D-PHE
PRO, MeAIB	OH-PRO PHE D-PRO	MET ALA	β-ALA GLY LYS

[a] This represents the most conservative delineation of routes available to amino acids, with additional possibilities explained in the text. The dashed line (-) indicates incomplete results.

Na-Independent Carrier-Mediated Pathways

Neutral Amino Acids. The time courses in Fig. 1, and the initial influxes of Table 1 suggest the presence of Na-independent carrier-mediated pathways in jejunal brush border vesicles. Analysis of PHE kinetics measured in NaCl and KCl revealed that a portion of the PHE carrier-mediation (i.e., J^1) was operational in the presence or absence of Na (*see* Fig. 3, Table 2): uptake in Na masked the existence of J^1 due to the low J^1_{max} ($<10\%$ of J^2_{max}). Further investigation (Table 7) revealed that L-PHE, LEU, ALA, BCH, MET and D-PHE share this carrier with varying affinities. Although Na-independent kinetics of ALA were not explicitly measured, it is probable that the J^1 for ALA is equivalent to the Na-independent PHE route. The bases for this conclusion are: 1) ALA's apparent K_i for PHE uptake (0.37 mM) is similar to the K_t^1's of ALA (0.32 mM) and PHE (0.27 mM), 2) the K_t^1 and J^1_{max} values for PHE and ALA (Table 2) are virtually the same in membranes of the same batch (batch 219) and are similar between batches 126 and 219, and 3) ALA and PHE display cross-inhibition patterns (Table 6). It should be noted that a fraction of the Na-independent PHE influx is not sensitive to any of the inhibitor amino acids (Table 6). This suggests the presence of multiple Na-independent PHE pathways. The single pathway shared by PHE, ALA, and GLY listed in Table 9 reflects our most conservative description of Na-independent neutral amino acid uptake.

Using PHE as the primary substrate, the data of Table 7 suggest that the Na-independent neutral amino acid pathway is not strictly stereoselective (ratio of inverse K_i's was about 3:1, L-PHE/D-PHE), and can be inhibited by BCH and MET. Substrates that are not transported by this pathway include MeAIB, PRO, and β-ALA.

The Na-independent pathway in jejunal brush border (Table 9) is reminiscent of the "L" (the so-called "LEU preferring") system originally described for the Ehrlich ascites tumor cell by Christensen's laboratory (Oxender & Christensen, 1963). Key characteristics of the classical L system are its Na-independence, and preference for hydrophobic amino acids such as LEU, PHE, MET, and especially BCH (Christensen, 1969, 1975; Christensen et al., 1969). Nonetheless, virtually all natural amino acids, including GLY and ALA, are transported by the L system even when they show reactivity with other Na-dependent pathways (Christensen, 1969, 1975; Oxender & Christensen, 1963; Christensen, Liang & Archer, 1967; Jacquez, Sherman & Terris, 1970). Another notable similarity between the classical L system and the Na-independent system reported here is that MeAIB shows no reactivity (Christensen, Oxender, Liang & Vatz, 1965). The bicyclo amino acid BCH has been purported to be a model substrate for the L system (Christensen et al., 1969; Tager & Christensen, 1971). However, recent retrospect analyses of the transport of the isomers of BCH by various membranes indicate that BCH is not an exclusive substrate for the L system (McClellan & Schafer, 1973; Ullrich, Runrich & Kloss, 1974; Christensen, 1975, 1979). In the present study BCH reacted with Na-sensitive and Na-insensitive pathways (Figs. 5 and 6; Tables 4 and 5).

Dibasic Amino Acids. Our preliminary data indicated that LYS uptake occurs by a Na-independent pathway(s) in rabbit jejunal brush border vesicles (Table 1). The apparent K_i's (Table 8). are approximately the same for all the dibasic amino acids tested, and the system exhibits weak stereoselectivity. The neutral amino acids inhibited Na-inde-

pendent LYS transport, but had substantially greater K_i's, with the exception of homoserine. The high affinity of homoserine for cationic amino acid carriers has been observed in other tissues (Christensen & Antonioli, 1969; Munck, 1980). Our observation that PRO, MeAIB, and BCH failed to inhibit LYS uptake is in conflict with earlier studies using intact rabbit ileum (Munck & Schultz, 1969; Munck, 1980). We are currently studying dibasic amino acid transport in more detail.

Na-Dependent Pathways

Common Neutral Amino Acid Pathway. There are at least three Na-dependent carrier-facilitated pathways in jejunal brush border vesicles (Table 9). In contrast to rat hepatocytes (Kilberg, Christensen & Handlogten 1979), Li cannot substitute for Na in jejunal brush border. The time courses (Fig. 1) suggest that the accumulation of neutral amino acids against a chemical gradient is in accordance with the well-documented "Na-gradient hypothesis" (Crane, 1960, 1962; Schultz & Curran, 1970). Although a portion of GLY uptake apparently was Na-dependent (Fig. 1, Tables 1, 3 and 4), the relatively slow influx rate (J^2) possibly prevented GLY from accumulating against a chemical gradient before dissipation of the Na electrochemical gradient occurred across the vesicle membrane. GLY transport against a chemical gradient has been reported in intact hamster ileum (Spencer et al., 1962).

The cross-inhibitions shown in Table 3 suggest that neutral L-amino acids share a common Na-dependent carrier, with two exceptions: 1) β-ALA, whose uptake was apparently not carrier-mediated, and 2) MeAIB, which failed to inhibit PHE, ALA, or GLY uptake (Table 3). Closer inspection of the data of Table 3 and Fig. 6 reveals that several of the cross-inhibitions are only partial. For example, mutual partial inhibitions occur between the pairs PRO/ALA, PRO/GLY, and ALA/GLY. Other features of the Na-dependent uptake mode include a lack of inhibition by LYS, and a high degree of stereoselectivity for PHE (ratio of inverse apparent K_i's $> 10:1$, L-PHE/D-PHE). These observations, taken with the detailed inhibition profiles of Figs. 5 and 6, and the K_i's of Tables 4 and 5 suggest the presence of an additional Na-dependent pathway serving PRO and MeAIB, and another serving PHE.

PRO/MeAIB Pathway. A striking observation of Table 3 is that MeAIB can completely inhibit transport of only PRO or MeAIB. Conversely, PRO can totally inhibit only PRO and MeAIB transport. The time courses (Fig. 1) and data of Table 1 indicate that carrier-mediated uptake of these two amino acids is strictly Na-dependent. The kinetics of Fig. 2 taken with the data of Fig. 5 and the apparent K_i's of Table 4 suggest that OH-L-PRO, PHE, D-PRO, and BCH share this pathway. It is unlikely that this pathway serves as a major carrier for PHE, because the maximal influx (J^2_{max}) is only about 20% that of Na-dependent PHE transport (*see* Table 2). The partial inhibition of PRO uptake by the amino acids indicated in Tables 3 and 4, and Fig. 5 is probably due to inhibition of the fraction of PRO transport handled by the common neutral amino acid carrier.

Na-Dependent PHE Pathway. PHE uptake was incompletely inhibited by all test amino acids, with the exception of PHE and MET (Tables 3 and 5, Fig. 6). The complete inhibition by PHE and MET was due to blocking of the additional Na-dependent PHE pathway that was inaccessible to other test amino acids. This additional PHE pathway is not resolvable from the Hofstee plot of Fig. 3*B*, because multiple components are confidently resolved from a Hofstee plot only when the kinetic constants differ greatly (Segal, 1975).

Comparison with Previous Intestinal Studies

Early work in rat, hamster, and rabbit offered conflicting conclusions regarding the multiplicity of amino acid transport pathways in intact *in vitro* small intestine. For instance, Hagihira, Wilson and Lin (1962) suggested the presence of a specific imino acid transport system in addition to other neutral amino acid pathways. Newey and Smyth (1964) postulated a PRO/GLY system in hamster intestine in addition to a general system which could transport PRO, GLY, and MET. Conflicting with Newey and Smyth, Munck (1966) implicated an imino acid pathway in rat intestine that could be shared by LEU, ALA, and sarcosine. The work of Akedo and Christensen (1962), and Schultz and Markscheid-Kaspi (1971) suggested the presence of at least two neutral amino acid carriers. A neutral amino acid inhibition study by Preston et al. (1974) found major discrepancies between K_i and K_t values, which suggested multiple pathways. However, Preston et al. agreed with Schultz and Curran (1970) that a single carrier offered the most conservative explanation of their data. Sepulveda and Smith (1978) suggested the plausibility of two neutral pathways in rabbit. Paterson, Sepulveda and Smith (1979) determined that one of these systems had a high affinity (K_t) but low capacity (J_{max}), and the other was low affinity, high capacity. Utilizing SER, ALA, and

MET. Smith and Sepulveda (1979) and Paterson, Smith and Sepulveda (1980) speculated that only the high affinity, low capacity system was Na-dependent.

In the elegant kinetic studies of Schultz, Curran and co-workers (*see* Schultz & Curran, 1970) it was assumed that there was only a single carrier for neutral amino acids. It is now clear, however, that multiple amino acid pathways do exist in the small intestinal mucosa. The resolution of the brush border pathways in intact epithelia has been confounded by the problems of substrate metabolism, paracellular pathways, intercellular spaces, and unstirred layers. The problem is compounded by variations in transport among species and anatomical regions of small intestine. For example, GLY and VAL were maximally absorbed to the same extent in chicken jejunum and ileum, but MET was maximally absorbed in jejunum at twice the rate of ileum (Mitchell & Levin, 1981).

The basal lateral membrane pathways responsible for exit of amino acids from the epithelium to the blood are different from the brush border entry pathways. Mircheff et al. (1980) showed that an A-like, an ACS-like, and a dominant L-like amino acid pathway exists in rat jejunal basal lateral membrane vesicles. The Na-independent L-like system apparently also exists in brush border membrane (*see* Table 9). However, the inability of MeAIB to inhibit Na-dependent GLY, ALA, or PHE uptakes precludes the existence of a brush border A system. The cross-inhibitions of Na-dependent (Li-insensitive) PHE, ALA, PRO, and GLY uptakes, taken with the exclusive PRO/MeAIB pathway prevent the conclusion that a strictly classical ASC system exists in brush border membranes

Comparative Renal Studies

Based on ultrastructural, biochemical, and transport studies, the epithelia of mammalian small intestine and renal proximal tubule perform the same basic functions of removing nutrient solutes from the lumen of the respective structure.

Early clinical observations of renal function lead to the acceptance of multiple transport systems in kidney (*see* Silbernagel, Foulkes & Deetjen, 1975). Recent work utilizing *in vitro* techniques including perfusions and purified membrane vesicles (*cf.* Silverman & Turner, 1980; Mircheff et al., 1981) have confirmed and extended the clinical observations. There are four main classes of amino acid transport carriers which have been elucidated by studies of renal malabsorption syndromes (associated diseases shown in parentheses): 1) PRO, OH-L-PRO, GLY (iminoglycinuria); 2) dicarboxylic amino acids; 3) dibasic amino acids including cystine (classical cystinuria); and 4) neutral amino acids (Hartnup's disease).

Neutral amino acid transport can be further dissected. Mircheff et al. (1981) have resolved six amino acid transport systems in rabbit renal brush border membranes. The renal pathways are similar to the intestinal pathways (Table 9), with some notable exceptions. The similarities include a distinct PRO/MeAIB pathway, and pathways that transport PHE and ALA. The most notable disparity is that in kidney β-ALA uptake occurs via a Na-stimulated carrier that can interact with PRO, MeAIB, and taurine, whereas in jejunal brush border vesicles there is no detectable carrier-facilitated β-ALA uptake. Also, PHE and LYS share a transport carrier in kidney, as also reported for the rabbit reticulocyte (Christensen & Antonioli, 1969), the Ehrlich cell (Christensen & Liang, 1966), and intact rabbit ileum (Munck, 1980). In the present study LYS transport is exclusively Na-independent, and relatively insensitive to neutral amino acids. In kidney vesicles, and jejunal brush border vesicles MeAIB fails to inhibit uptake of any neutral amino acid, other than PRO or MeAIB itself. This is in contrast to the behavior predicted by the classical A system of the Ehrlich cell (Christensen, 1975).

We conclude that both kidney and jejunal brush border membranes transport neutral amino acids via similar pathways that do not conform to the classical A or ASC pathways of the Ehrlich cell (Christensen, 1975), or intestinal basal lateral membrane (Mircheff et al., 1980).

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