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Characterization of amino-acid transport systems in guinea-pig intestinal brush-border membrane

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The amino-acid transport systems have been characterized in brush-border membrane vesicles prepared from guinea-pig small intestine. Uptake of all amino acids tested was measured at the initial velocity for 5 s. L-Proline, α-(methylamino)isobutyrate, glycine, L-alanine and L-methionine were transported dependent solely on an Na^+ gradient from the outside to the inside of the vesicles, and L-cysteine, L-phenylalanine and L-leucine were transported dependent largely on the Na^+ gradient with a small fraction of Na^+-independent transport. The transport of L-aspartic acid and L-lysine was independent of the Na^+ gradient and L-lysine transport was somewhat inhibited by the presence of cations, including Na^+, K^+ and Li^+. A cross-inhibition study of the uptake of these amino acids in the brush border of guinea-pig intestine revealed the presence of at least three Na^+-dependent and three Na^+-independent carrier-mediated systems. One Na^+-dependent system interacted mainly with imino acid. Another Na^+-dependent system interacted with neutral amino acids, while a third system was selective for glycine. One Na^+-independent system is for acidic amino acids, another is responsible for neutral amino acids and a third for cationic amino acids. These transport systems of amino acids in guinea-pig small intestine are compared with those in rabbit and mouse intestine.

Introduction

Amino-acid transport across the epithelial cells of the small intestine involves two processes: uptake from the gut across the brush-border membrane, and exit into the blood across the basal lateral membrane. In the first step, the solute is transported by three mechanisms: simple diffusion, an Na^+-dependent system and an Na^+-independent system. In Ehrlich ascites tumor cells [1], two distinct but overlapping transport systems for neutral amino acids, termed Systems A and L, were identified in 1963. Transport systems for neutral, anionic and cationic amino acids have been analyzed in various cell types [2], including rat hepatocyte [3] and rabbit kidney brush-border membrane vesicles [4]. Transport of L-proline in brush-border membrane vesicles of guinea-pig intestine [5] was shown to be supported mostly by the A system, and developmental change in this transport system has been reported [6]. Intestinal transport systems for amino acids have also been described in brush-border membrane vesicles of rabbit [7,8] and in everted sacs of mouse [9].

In human placental brush-border membrane vesicles, a cross-inhibition study has revealed the presence of at least three Na^+-dependent and two Na^+-independent carrier-mediated pathways [10]. Rabbit jejunal brush-border membrane vesicles were shown conclusively to contain multiple transport pathways [8], including passive diffusion, Na^+-dependent systems (NBB, IMINO and PHE) and Na^+-independent systems (y^+ and L).

In the present study, using brush-border membrane vesicles from the guinea-pig small intestine, transport systems for neutral, anionic and cationic amino acids were analyzed in detail. Simple diffusional, Na^+-dependent and Na^+-independent components were found to be present, which were characterized on the basis of the cross-inhibition profile for these amino acids.

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MeAIB, α-(methylamino)isobutyrate; transport systems NBB, IMINO, PHE, y^+ and L, see Ref. 8.

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Materials and Methods

Isolation of membrane vesicles. Membrane vesicles were prepared from guinea-pig intestine by the procedure of Fujita et al. [11] with slight modification as described previously [5]. Briefly, mucosal scrapings from

guinea-pig intestine were taken up in 0.32 M sucrose containing 5 mM Tris-Hepes buffer (pH 7.5) and 0.5 mM neutralized EDTA (sucrose-EDTA buffer). This was then homogenized in a Dounce-type homogenizer by 50 up-and-down strokes without revolving the pestle, followed by centrifugation for 5 min at $270 \times g$. The pellet was taken up in sucrose-EDTA buffer, homogenized by 30 strokes and centrifuged for 5 min at $300 \times g$. The pellet was suspended in sucrose-EDTA buffer, homogenized by 20 strokes, then centrifuged for 5 min at $340 \times g$. The pellet was suspended in sucrose-EDTA buffer, homogenized by five strokes, then centrifuged for 5 min at $360 \times g$ and finally suspended in 5 mM Tris-Hepes buffer (pH 7.5) containing 0.5 mM EDTA. The suspension was homogenized in a Potter-Elvehjem-type homogenizer and then centrifuged for 30 min at $200\,000 \times g$. The resulting pellet was suspended in 5 mM Tris-Hepes buffer (pH 7.5) containing 0.5 mM EDTA and then homogenized in a Potter-Elvehjem-type homogenizer. The homogenate was applied to the top of a discontinuous sucrose gradient which was composed of 30%, 40%, 50% and 60% (w/v) sucrose solutions containing 5 mM Tris-Hepes buffer (pH 7.5) and then centrifuged for 120 min at $100\,000 \times g$. After centrifugation, the interface between the 40% and 50% sucrose layers was collected, diluted with 5 mM Tris-Hepes buffer (pH 7.5), and then centrifuged for 30 min at $200\,000 \times g$. The final pellet was suspended in 2 mM Tris-Hepes buffer (pH 7.5) containing 100 mM D-mannitol and 0.1 mM $MgSO_4$ to give a final protein concentration of approx. 3–4 mg/ml. The membrane vesicle preparation showed about 15-fold enrichment of alkaline phosphatase and sucrase over the starting homogenates, while Na^+/K^+-ATPase (ouabain-sensitive) was decreased one-fifth in specific activity.

Uptake method. All assays of transport activity were carried out with 20 μM substrate at 25°C. The membrane vesicles were preincubated in a medium containing 50 mM D-mannitol, 5 mM Tris-Hepes (pH 7.5) and 50 μM $MgSO_4$ at 25°C for 5 min; uptake was started by the addition of labeled substrate and incubation was continued at 25°C for the period described in the text. Other additions are described in the figure legends. The uptake of substrate was terminated by diluting an aliquot of the sample (approx. 100–200 μg membrane protein) with a 40-fold excess of ice-cold buffer composed of 150 mM NaCl, 50 mM $MgCl_2$, 30 mM D-mannitol and 10 mM Tris-Hepes buffer (pH 7.5). The diluted sample was filtered immediately through a Millipore cellulose filter (0.45 μm) and washed with 3 ml of the same ice-cold buffer. Radioactivity retained on the filter was counted using liquid scintillation [12].

Protein estimation. The protein concentration of the vesicle preparation was determined by the method of Lowry et al. [13] using bovine serum albumin as a standard.

Chemicals. All reagents were of the highest purity commercially available. All the labeled amino acids were purchased from New England Nuclear; α-(methylamino)isobutyrate was from Sigma.

Results

Uptake time-courses of amino acids

In order to analyze amino-acid transport systems in detail, time-courses of the uptake of representative amino acids were first investigated: L-proline for imino acids; L-phenylalanine, L-leucine and glycine for neutral amino acids; L-aspartic acid for acidic amino acids; L-lysine for cationic amino acids. The results are summarized in Fig. 1a–f. The uptake of these amino acids was measured in the presence of 100 mM NaCl or 100 mM KCl, except for L-lysine for which 100 mM KCl was replaced with 200 mM D-mannitol. The uptake of L-proline, L-phenylalanine, L-leucine and glycine was stimulated by the presence of an Na^+ gradient from the outside to the inside of the vesicles and reached an equilibrium 60 min after incubation, which was at approximately the same level in the presence of 100 mM NaCl and 100 mM KCl. The uptake of L-proline, L-phenylalanine and L-leucine showed a typical overshoot in the presence of an Na^+ gradient, although the extent of the uptake overshoot was marked in the order L-proline, L-phenylalanine and L-leucine (Fig. 1a, c, d). The uptake of α-(methylamino)isobutyrate, L-cysteine, L-alanine and L-methionine showed an overshoot in the presence of an Na^+ gradient (data not shown). Glycine transport was enhanced by the presence of an Na^+ gradient without displaying an overshoot (Fig. 1b). The uptake rate of L-aspartic acid was the same in the presence of 100 mM NaCl and of 100 mM KCl (Fig. 1e), showing independence of any Na^+ gradient. The uptake of L-lysine was lowered slightly in the presence of 100 mM NaCl (Fig. 1f) and of 100 mM KCl or 100 mM LiCl (data not shown). This is the reason why 200 mM D-mannitol was replaced with 100 mM KCl to measure the control rate of uptake. The uptake of L-aspartic acid was not inhibited by the presence of 100 mM NaCl and 100 mM KCl (data not shown).

In all cases of amino-acid uptake tested, the uptake for 5 s was linear and was used as the initial velocity to measure cross-inhibition by an excess of non-labeled amino acid.

Analysis of amino-acid transport system

Amino-acid transport systems existing in the brush-border membrane vesicles of guinea-pig small intestine were analyzed with ten labeled amino acids: L-cysteine, glycine, L-alanine, L-methionine, L-phenylalanine, L-leucine and α-(methylamino)isobutyrate for neutral

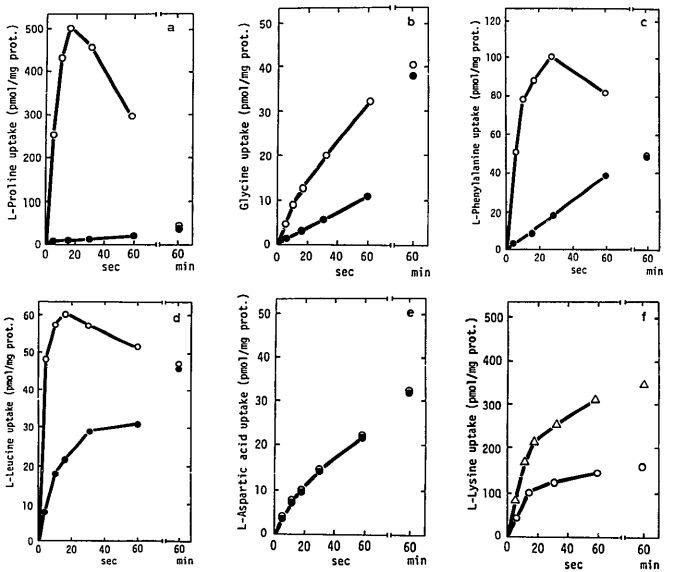

Fig. 1. Time-course of amino-acid uptake by brush-border membrane vesicles of guinea-pig intestine. Membrane vesicles were suspended in a medium containing 100 mM D-mannitol, 0.1 mM $MgSO_4$ and 2 mM Tris-Hepes (pH 7.5). The uptake of labeled amino acids, L-proline (a), glycine (b), L-phenylalanine (c), L-leucine (d), L-aspartic acid (e) and L-lysine (f), was initiated by adding 50 μl of membrane suspension (3–4 mg protein/ml) to 60 μl of incubation medium obtained by mixing 10 μl of 220 μM labeled amino acid with 50 μl of 110 mM D-mannitol, 110 μM $MgSO_4$, 11 mM Tris-Hepes (pH 7.5) and 220 mM NaCl (○) or 220 mM KCl (●) or 440 mM D-mannitol (△). Both the membrane suspension and the incubation medium were preincubated independently at 25 °C for 5 min before mixing, followed by further incubation at 25 °C. Each point is the mean of triplicate determinations.

amino acids; L-aspartic acid for acidic amino acids; L-lysine for cationic amino acids; L-proline for imino acids. The uptake by diffusion, Na^+-dependent and Na^+-independent components was first measured and cross-inhibition tests using these amino acids as both substrates and inhibitors were carried out [10].

Initial rate of amino-acid uptake

Table I summarizes the initial rate of amino-acid uptake measured under various conditions, as indicated in the legend. The Na^+-gradient-dependent uptake of given amino acid was expressed by subtracting the uptake in the presence of 100 mM KCl ($V_{(K)}$) from that in the presence of 100 mM NaCl ($V_{(Na)}$). The Na^+-gradient-independent and carrier-mediated uptake was expressed by subtracting the diffusional component (V_D) from the uptake in the presence of 100 mM KCl. The diffusional component of a substrate was determined by measuring the influx in the presence of 30 mM unlabeled substrate in addition to 100 mM KCl.

As shown in Table I, the uptake of L-proline, α-(methylamino)isobutyrate, glycine, L-alanine and L-methionine was dependent almost solely on an Na^+ gradient suggesting the absence of transport of these amino acids catalyzed by an Na^+-independent carrier. The uptake of L-aspartic acid and L-lysine was not accelerated by an inwardly directed Na^+ gradient. The transport systems for L-cysteine, L-phenylalanine and L-leucine include the presence of both Na^+-dependent and Na^+-independent systems, in which the former

TABLE I

Initial rates of amino-acid uptake

The uptake levels of ten amino acids were measured. Membrane vesicles were suspended in a medium containing 100 mM D-mannitol, 0.1 mM $MgSO_4$ and 2 mM Tris-Hepes (pH 7.5). Amino-acid uptake was initiated by adding 50 μl of membrane suspension to 60 μl of incubation medium obtained by mixing 10 μl of 220 μM labeled substrate with 50 μl of 110 mM D-mannitol, 110 μM $MgSO_4$, 11 mM Tris-Hepes (pH 7.5) and 220 mM NaCl($V_{(Na)}$) or 220 mM KCl($V_{(K)}$). However, the incubation medium for L-lysine uptake was obtained by mixing 10 μl of 220 μM labeled substrate with 50 μl of 110 mM D-mannitol, 110 μM $MgSO_4$, 11 mM Tris-Hepes (pH 7.5) and 220 mM NaCl($V_{(Na)}$) or 440 mM D-mannitol. The diffusional component (V_D) was determined by measuring the total influx of labeled substrate in the presence of 30 mM unlabeled substrate. Incubation time for uptake measurements was 5 s. Values are means ± S.D. for five experiments. Na^+-dep., $V_{(Na)} - V_{(K)}$; Na^+-indep., $V_{(K)} - V_D$.

	Initial rates of uptake (pmol/5 s per mg protein)									
	Pro	MeAIB	Cys	Gly	Ala	Met	Phe	Leu	Asp	Lys
$V_{(Na)}$	118.3 ± 12.8	40.0 ± 14.6	45.6 ± 11.4	12.0 ± 2.2	19.7 ± 1.7	11.9 ± 4.5	21.7 ± 2.3	32.9 ± 6.7	4.6 ± 0.2	9.3 ± 1.3
$V_{(K)}$	2.0 ± 1.2	3.3 ± 0.2	12.0 ± 2.9	6.3 ± 0.8	3.0 ± 0.6	2.2 ± 0.9	7.9 ± 0.6	7.5 ± 0.6	5.6 ± 0.8	17.3 ± 1.0
V_D	0.0 ± 0.1	3.2 ± 0.2	3.8 ± 0.1	6.3 ± 0.4	2.0 ± 0.8	2.2 ± 0.5	4.3 ± 0.9	3.5 ± 0.9	0.0 ± 0.2	4.2 ± 0.4
Na^+-dep.	116.0 ± 12.1	37.6 ± 9.8	33.8 ± 8.8	6.2 ± 1.1	16.7 ± 1.3	9.8 ± 3.1	13.8 ± 1.6	25.4 ± 6.4	0.0 ± 0.1	0.0 ± 0.1
Na^+-indep.	2.0 ± 1.1	0.1 ± 0.1	8.2 ± 1.0	0.0 ± 0.1	1.0 ± 0.3	0.0 ± 0.1	3.7 ± 0.4	4.0 ± 0.5	5.6 ± 0.8	13.1 ± 1.4

transport contributes 75–85% and the latter, 15–25%. Uptake by passive diffusion of the amino acids tested was much smaller than carrier-mediated transport except for L-proline and L-aspartic acid, showing a negligible V_D, and for glycine, showing a V_D comparable to that by the Na^+-dependent uptake.

Cross-inhibition of amino-acid uptake

The cross-inhibition study of uptake is usually used to analyze the existence of a given transport system. Amino-acid uptake at 20 μM was measured in the presence of amino-acid inhibitor at 30 mM, in the presence of Na^+ or K^+, which is referred to as $V_{i(Na)}$ or $V_{i(K)}$. The percentage inhibition of Na^+-dependent and Na^+-independent carrier-mediated uptake was calculated using the formulae $100 - 100([V_{i(Na)} - V_{i(K)}]/[V_{(Na)} - V_{(K)}])$ and $100 - 100([V_{i(K)} - V_D]/[V_{(K)} - V_D])$, respectively.

TABLE II

Cross-inhibition of Na^+-dependent amino-acid uptake

The uptake over a 5 s period was measured under the same conditions as those described in the text and the legend of Table I, except for addition of 30 mM unlabeled amino acid, and the osmolarity of the medium was adjusted to a constant value by addition of D-mannitol. Values are means ± S.D. of percentage inhibition calculated using the equation in the text for four experiments.

Substrate	Inhibitor							
	Pro	MeAIB	Gly	Cys	Ala	Leu	Met	Phe
Pro	100 ± 0.0	96.3 ± 1.5	30.8 ± 7.4	64.7 ± 11.4	50.3 ± 2.1	67.3 ± 3.9	80.0 ± 4.0	87.8 ± 7.9
MeAIB	99.3 ± 0.9	99.9 ± 0.2	31.8 ± 10.8	69.2 ± 7.5	64.1 ± 8.1	73.7 ± 2.9	80.1 ± 13.6	73.0 ± 10.4
Gly	2.3 ± 0.6	4.5 ± 1.7	90.9 ± 7.6	48.5 ± 7.2	40.5 ± 0.3	56.2 ± 9.4	0.0 ± 0.0	0.0 ± 0.0
Cys	55.4 ± 7.7	48.5 ± 5.6	71.9 ± 8.5	100 ± 0.0	93.9 ± 8.9	98.9 ± 0.2	84.9 ± 12.3	86.7 ± 7.7
Ala	46.6 ± 9.7	34.5 ± 2.5	58.1 ± 10.6	89.3 ± 10.3	93.9 ± 5.2	90.3 ± 6.3	95.2 ± 1.8	94.3 ± 1.4
Leu	63.8 ± 10.7	38.4 ± 3.7	73.3 ± 6.7	93.9 ± 5.7	81.8 ± 8.7	92.6 ± 4.2	91.3 ± 8.0	87.8 ± 9.6
Met	56.6 ± 10.3	47.9 ± 9.0	39.4 ± 9.5	97.5 ± 6.0	73.6 ± 6.2	82.8 ± 8.6	95.1 ± 5.4	71.4 ± 8.6
Phe	45.3 ± 2.8	36.9 ± 10.3	28.6 ± 3.6	84.7 ± 6.1	70.1 ± 10.9	91.6 ± 5.9	96.8 ± 5.7	92.1 ± 2.1

TABLE III

Cross-inhibition of Na^+-independent amino-acid uptake

The uptake over a 5 s period was measured under the same conditions as those described in the text and the legend of Table I, except for addition of 30 mM unlabeled amino acid, and the osmolarity of the medium was adjusted to a constant value by addition of D-mannitol. Values are means ± S.D. of percentage inhibition calculated using the equation in the text for four experiments.

Substrate	Inhibitor				
	Cys	Phe	Leu	Asp	Lys
Cys	100	78.8	83.0	0.0	48.3
	±0.9	±8.1	±7.1	±0.0	±17.6
Phe	94.6	100	84.1	0.0	60.9
	±0.2	±0.1	±7.9	±0.0	±10.1
Leu	84.9	100	100	0.0	50.2
	±8.7	±0.5	±0.1	±0.1	±10.5
Asp	0.0	0.0	0.0	100	0.0
	±0.1	±0.0	±0.0	±0.0	±0.1
Lys	39.0	53.6	28.8	0.0	100
	±10.8	±9.0	±11.9	±0.1	±0.1

Cross-inhibition of Na^+-dependent amino-acid uptake. The inhibition of Na^+-dependent transport of eight amino acids is shown in Table II. Transport of L-proline and α-(methylamino)isobutyrate was inhibited totally by the acids themselves, and partially by with glycine (30–32%), and other neutral amino acids (50–88%). Glycine transport was inhibited totally by glycine itself and inhibited partially (50%) by L-cysteine, L-alanine and L-leucine, but not by L-proline, α-(methylamino) isobutyrate, L-methionine and L-phenylalanine. Transport of L-cysteine, L-alanine, L-leucine, L-methionine and L-phenylalanine showed complete mutual inhibition, and was inhibited partially (30–73%) by L-proline, α-(methylamino)isobutyrate and glycine. These results suggest that: (1) a fraction of amino-acid transport is mediated by the imino-acid-specific system, which was unable to interact with the other amino acids tested; (2) glycine is mediated by the glycine-specific system which interacts with several neutral amino acids; (3) a common pathway is present for neutral amino acids. L-Lysine and L-aspartic acid, which were transported independently of an Na^+ gradient, were not effective in inhibiting Na^+-dependent uptake for any of the tested substrates (data not shown).

Cross-inhibition of Na^+-independent amino-acid uptake. As shown in Table III, five amino acids which were transported abundantly by Na^+-independent systems were selected. Transport of L-cysteine, L-phenylalanine and L-leucine showed complete mutual inhibition, suggesting the presence of a common Na^+-independent neutral amino-acid transport pathway, which was inhibited partially (50–60%) by L-lysine. Transport of L-lysine was inhibited completely by L-lysine itself and partially (30–50%) by neutral amino acids, which suggests the presence of an L-lysine-specific system interacting partially with neutral amino acids. L-Aspartic acid transport was inhibited completely only by L-aspartic acid, which inhibited the uptake of no other substrates, and conversely uptake of this acidic amino acid was not inhibited by any other amino acid. These results suggest that a fraction of the L-aspartic-acid-specific Na^+-independent system is involved, which does not interact with any other amino acid tested.

Discussion

The present study investigated the transport pathways for amino acids in guinea-pig small-intestinal brush-border membrane vesicles. The brush-border membrane vesicles of guinea-pig intestine used in this study have high refinement for amino-acid transport activity, as shown for L-proline (Fig. 1a). The results indicate the presence of: (1) a diffusional component; (2) at least three Na^+-dependent pathways, including the imino, glycine and neutral systems; (3) at least three Na^+-independent pathways, including the neutral, acidic and cationic systems, as summarized in Table IV, which is a summary of data from Tables I–III. However, this classification is not perfect, and is perhaps the most conservative suggestion for the amino-acid transport pathways.

(1) *Simple diffusion.* This is passive non-saturable transport, which contributes at variable levels to the transport of all amino acids.

(2) *Na^+-dependent pathways.* There are at least three Na^+-dependent carrier-mediated amino-acid transport pathways. First, L-proline and α-(methylamino)isobutyrate showed total mutual inhibition, suggesting the

TABLE IV

Amino-acid transport pathways in brush-border membrane of guinea-pig intestine

This summary is taken from Tables I–III.

1. Simple diffusion

2. Na^+-dependent pathways

Primary substrates	Inhibitors		
	total	partial	minimal
Imino (Pro, MeAIB)	Pro, MeAIB	Cys, Ala, Leu Met, Phe, Gly	none
Gly	Gly	Cys, Ala, Leu	none
Neutral (Cys, Ala, Leu, Met, Phe)	Cys, Ala, Leu Met, Phe	Pro, MeAIB, Gly	none

3. Na^+-independent pathways

Primary substrates	Inhibitors		
	total	partial	minimal
Neutral (Cys, Phe, Leu)	Cys, Phe, Leu	Lys	none
Acidic (Asp)	Asp	none	none
Cationic (Lys)	Lys	Cys, Phe, Leu	none

presence of the imino system, which partially shares the transport of all neutral amino acids. Second, glycine uptake is not inhibited by L-proline, α-(methylamino) isobutyrate, L-methionine and L-phenylalanine, and is inhibited totally only by itself. This suggests the presence of a specific Na^+-dependent pathway for glycine, which partially shares the transport of neutral amino acids. Third, L-cysteine, L-alanine, L-leucine, L-methionine and L-phenylalanine showed complete mutual inhibition, suggesting the presence of a common pathway for a large proportion of the neutral amino acids. This partially shares the transport of substrates for the imino and the glycine systems.

(3) *Na^+-independent pathways.* The data in Table I suggest the presence of Na^+-independent carrier-mediated amino-acid transport pathways in the guinea-pig intestine. There are at least three pathways. One system is the neutral amino-acid transport pathway, which shares partial transport of L-lysine. The second is the specific system for L-aspartic acid. The third is the cationic amino-acid pathway, which shares partial transport of neutral amino acids.

The results concerning properties of amino-acid transport in guinea-pig intestinal brush-border membrane vesicles were especially interesting. The uptake of glycine in the presence of an inward Na^+ gradient showed a stimulated initial velocity of uptake without showing 'overshoot' (Fig. 1b). L-Lysine transport is not dependent on an Na^+-gradient, but is inhibited by several cations, including Na^+ (Fig. 1f), K^+ and Li^+. L-Aspartic acid is transported by one specific Na^+-independent system without contribution of Na^+-dependent and diffusional components (Fig. 1e and Table I). The mechanisms for such transport properties described should be explored in the light of energy coupling to the transport.

It is important to explore the mechanism of transport of nutrients across the brush-border membrane of intestinal epithelial cells, since this is the first step in nutrient absorption and one of the regulatory steps for its intracellular metabolism. The systematic analysis of amino-acid transport systems was started with the Ehrlich cell [1], in which Christensen classified the amino-acid transport systems of a wide variety of non-epithelial cell types into six fundamental categories: A, ASC, Gly, β, y^+ and L transport systems [14]. However, amino-acid transport systems characterized in the rabbit jejunal [7,8], rabbit kidney [4] and human placental [10,15] brush-border membranes appear to be distinct from the previously described Na^+-dependent transport systems found in non-epithelial cells and in the basal lateral plasma membranes of intestinal epithelial cells [16]. Stevens et al. [8] reported that rabbit jejunal brush-border membrane transports amino acids via at least three Na^+-dependent carrier systems (the NBB, PHE and IMINO systems), and two Na^+-independent carrier systems (y^+ and L systems).

From the results described in this paper together with other results reported [7,8], it is evident that the imino acid transport pathway (IMINO system) exists both in the guinea-pig intestine (Table II) and in rabbit jejunum, but the PHE system does not exist in the guinea-pig intestine. Other examples of the difference in amino-acid transport systems between rabbit and guinea-pig are for L-leucine and glycine: L-leucine is transported by an Na^+-independent mechanism in the former [8] and by an Na^+-dependent on in the latter (Fig. 1d); the glycine system is found only in guinea-pig intestine. Cationic amino-acid transport is Na^+-independent in both guinea-pig (Table III) and rabbit (y^+ system [8,17]). However, the system in guinea-pig intestine has the unusual property of an inhibitory effect of Na^+ (Fig. 1f). The anionic amino-acid transport system is not well characterized in rabbit jejunum [8], but rat intestine has the anionic amino-acid system which is dependent on an Na^+ gradient [18]. Recent studies of amino-acid transport systems in animals have been reviewed from the viewpoints of biochemistry and genetics [19].

As described above, the intestinal transport systems for amino acids differ among animal species, though there are some similar pathways. It is important to analyze possible changes in the characteristics and discrimination of amino-acid transport systems in relation to any metabolic disorder causing to the absorption processes. It is also interesting to investigate whether the variety of transport systems in different animals is due to evolutionary events for the species and whether possible changes in transport activity can be ascribed only to a specific system or to all transport systems for amino acids. This is the reason why the amino-acid transport systems have been analyzed in detail in this study.

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