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Transport of Imino Acids and Non-α-Amino Acids across the Brush-Border Membrane of the Rabbit Ileum

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Summary. The transport of β-alanine and MeAIB and their effects as inhibitors of the transport of alanine, leucine and lysine across the brush-border membrane of the intact epithelium from the rabbit's distal ileum has been examined. Two separate transport systems have been characterized: 1) A sodium-dependent, β-alanine-accepting system, which is a high-affinity transport system for α-amino-monocarboxylic acids (neutral a.a.) and for cationic a.a., accepts non-α-amino acids as well as non-α-imino acids, is moderately stereospecific, and for which the affinity of a neutral a.a. is greatly reduced by N-methylation. 2) A sodium-dependent transport system for imino acids, which is inaccessible to cationic amino acids and non-α-amino acids but accepts cyclic, non-α-imino acids, is moderately stereospecific, and for which neutral a.a. have much lower affinities than their N-methylated derivatives. On the basis of the observations of this and the preceding paper five transport systems for amino acids are ascribed to the rabbit ileum. Some discrepancies between the present results and those obtained with brush-border membrane microvesicles from the rabbit small intestine are discussed.

Key Words rabbit · small intestine · ileum · amino acid transport

Introduction

Transport of imino acids and non-α-amino acids by mammalian small intestine has been described mostly by experiments on the rat small intestine, which appears to possess only one system for the transport of these two groups of amino acids (Munck, 1981). The guinea pig, it now appears (Munck, 1983), possesses one system for the transport of imino acids but seems not to transport non-α-amino acids.

The question of the transport of these amino acids by the rabbit small intestine had not previously been studied. However, preliminary experiments (Munck, 1983) indicated that in the rabbit ileum at least two systems were involved in the transport of these amino acids. These transport systems have now been characterized; and, while these studies were completed, results obtained with microvesicles of the brush-border membrane from the total rabbit small intestine except the orally first and the anally last 30 cm have characterized a MeAIB transporting system (Stevens, Ross & Wright, 1982).

Abbreviations

α-, β-, or γ-ABA: α-, β-, or γ-amino-butyric acid; MeAIB: 2-(methylamino)-isobutyric acid; MeAla: N-methyl-alanine; MeGly: N-methyl-glycine (sarcosine); MeLeu: N-methyl-leucine; PIP: piperidine-2-carboxylic acid (pipecolic acid); NIP: piperidine-3-carboxylic acid (nipecotic acid); IsoNIP: piperidine 4-carboxylic acid (isonipecotic acid); Neutral a.a.: α-amino mono-carboxylic acids; a.a.: amino acids.

Materials and Methods

^{14}C-labeled β-alanine and MeAIB used in the present study were purchased from New England Nuclear Co.

In one series of experiments the segment of the small intestine located between 60 and 90 cm from the ileo-coecal junction was used. Otherwise the materials, methods, and analytical procedures were precisely as described in the preceding paper.

Results

The stage for the present study of transport of imino acids and non-α-amino acids was set by a series of experiments in which the possibility of mutual inhibition between β-alanine, MeAIB, leucine and lysine was examined. In addition MeGly (sarcosine) and Me-DL-alanine were used as inhibitors. In these experiments the transported species were present at 1 mM and the inhibitors at 40 mM. The results (Table 1) demonstrate that β-alanine and especially leucine and lysine are strong inhibitors of $J_{mc}^{\beta\text{-ala}}$, while

MeAIB and MeGly are only moderately effective as inhibitors of this transport. In contrast β-alanine and leucine are weak inhibitors of J_{mc}^{MeAIB}, which is strongly inhibited by MeAIB and Me-DL-ala but totally unaffected by lysine. Clearly at least two transport systems are involved in the transport of β-alanine and MeAIB. The data of Table 2 demonstrate that neither β-alanine nor MeAIB significantly affected J_{mc}^{Lys} as measured at 1 mM; at 1 mM leucine J_{mc}^{Leu} was unaffected by β-alanine and only moderately inhibited by MeAIB. These results show that the two transport systems involved in the transport of β-alanine and MeAIB cannot be identical to the major transport systems for neutral and cationic amino acids described in the preceding paper.

Kinetics of J_{mc}^{MeAIB} and $J_{mc}^{\beta\text{-ala}}$

In paired experiments J_{mc}^{MeAIB} was measured at eight different concentrations between 0.5 and 100 mM. As shown in Fig. 1 the results of these measurements did not indicate the involvement of more than one transport system for MeAIB. Analyzed by a nonlinear, least-square fitting to a model of one or two saturable processes plus diffusion the transport of MeAIB was best described as

$$J_{mc}^{MeAIB} = \frac{(4.7 \pm 0.2)[MeAIB]_m}{(3.6 \pm 0.2) + [MeAIB]_m} + (0.003 \pm 0.003)[MeAIB]_m. \quad (1)$$

By the chi-square test the fit between this equation and the experimental results is described by a P-value of 0.95. Similarly in paired experiments $J_{mc}^{\beta\text{-ala}}$ was measured at eight different concentrations between 0.5 and 100 mM. Analyzed in the same way as the data for J_{mc}^{MeAIB} the best fit to the experimental results was described as

$$J_{mc}^{\beta\text{-ala}} = \frac{(0.79 \pm 0.09)[\beta\text{-ala}]_m}{(2.0 \pm 0.4) + [\beta\text{-ala}]_m} + (0.018 \pm 0.004)[\beta\text{-ala}]_m. \quad (2)$$

By the chi-square test the fit between this equation and the experimental results (Fig. 2) is characterized by a P-value of 0.85.

Characteristics of the Transport Mechanism for β-Alanine

Effects of N-Methylation

The effect of N-methylation on the affinity of an amino acid for the β-alanine-accepting transport

Table 1. Influx across the brush-border membrane of the distal rabbit ileum measured in paired experiments at 1 mM of β-alanine or MeAIB with and without 40 mM of the inhibitors[a]

Inhibitor	$J_{mc}^{\beta\text{-ala}}$ (μmol/cm² · hr ± SE)		Inhibitor	J_{mc}^{MeAIB} (μmol/cm² · hr ± SE)	
	Control	Inhibited		Control	Inhibited
β-alanine	0.44 ± 0.09 (6)	0.05 ± 0.01 (5)	β-alanine	1.16 ± 0.01 (4)	0.90 ± 0.10 (4)
MeAIB	0.23 ± 0.04 (4)	0.10 ± 0.01 (5)	MeAIB	1.16 ± 0.09 (4)	0.14 ± 0.01 (4)
Leucine	0.44 ± 0.09 (6)	0.03 ± 0.01 (5)	Leucine	0.85 ± 0.08 (5)	0.38 ± 0.01 (5)
Lysine	0.26 ± 0.04 (4)	0.04 ± 0.00 (4)	Lysine	1.16 ± 0.09 (4)	1.14 ± 0.10 (4)
MeGly[b]	0.26 ± 0.04 (4)	0.12 ± 0.01 (4)	Me-DL-Ala	0.85 ± 0.08 (5)	0.13 ± 0.01 (6)

[a] Number of observations in parentheses.
[b] Indicates that the K_i values listed in Table 4 are based on these observations.

Table 2. Effect of β-alanine and MeAIB as inhibitors of influx of leucine and lysine across the brush-border membrane of the distal rabbit ileum as measured in paired experiments at 1 mM leucine or 1 mM lysine with or without 40 mM β-alanine or 40 mM MeAIB[a]

Inhibitor	J_{mc}^{Leu} (μmol/cm² · hr ± SE)		J_{mc}^{Lys} (μmol/cm² · hr ± SE)	
	Control	Inhibited	Control	Inhibited
β-alanine	4.02 ± 0.18 (4)	4.15 ± 0.33 (4)	1.25 ± 0.19 (4)	1.38 ± 0.24 (4)
MeAIB	3.10 ± 0.23 (8)	2.45 ± 0.16 (8)	1.32 ± 0.03 (4)	1.45 ± 0.11 (4)

[a] Number of observations in parentheses.

site was examined by comparing the inhibitory effects of glycine and MeGly, alanine and MeAla (Me-D-ala and Me-DL-ala), serine and MeSer, and leucine and MeLeu. In these experiments β-alanine was used at 1 mM and the inhibitors at the concentrations stated in Tables 1 and 3, rows 1–5, and in Fig. 3, in which the results of these experiments are summarized. Using the data of Eq. (2) these results were used for estimates of the K_i of the various inhibitors against $J_{mc}^{\beta\text{-ala}}$. These estimates are summarized in Table 4. The results show that α-amino-mono-carboxylic acids, neutral a.a., have very high affinities for the β-alanine-accepting transport site and that N-methylation decreases the affinity by about two orders of magnitude. The affinities of glycine, alanine, serine and leucine do not differ much from each other, nor do those of MeGly, MeAla, MeSer and MeLeu. Nevertheless, the data on inhi-

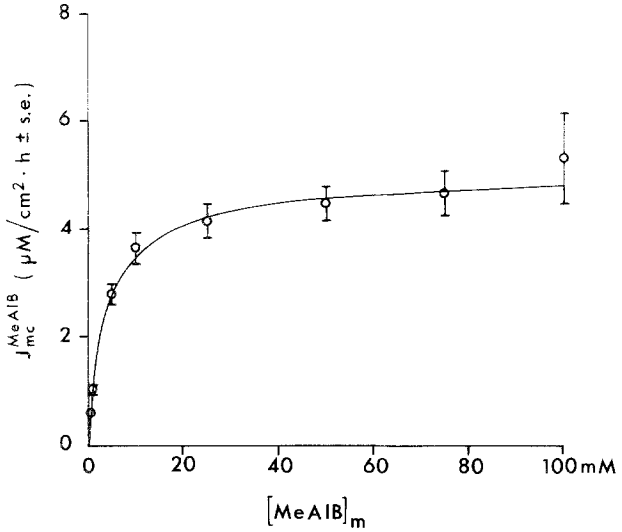

Fig. 1. Influx of MeAIB across the brush-border membrane of the distal 20- to 30-cm rabbit ileum. The data are means ± SE of eight measurements. The curve is described by Eq. (1)

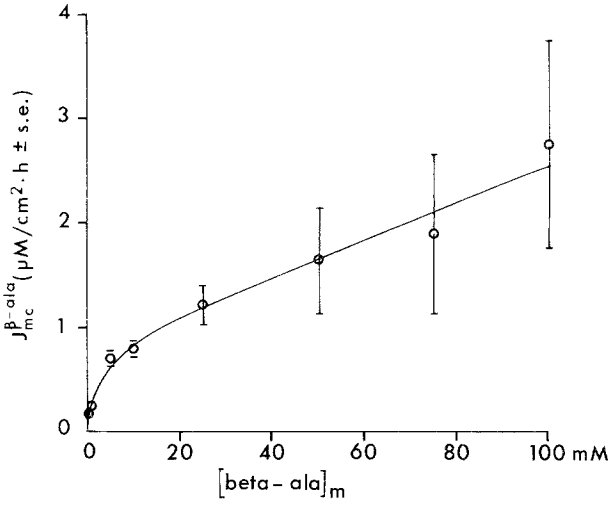

Fig. 2. Influx of β-alanine across the brush-border membrane of the distal 20- to 30-cm rabbit ileum. The data are means ± SE of six to eight measurements. The curve is described by Eq. (2)

Table 3. Influx of β-alanine (1 mM) across the brush-border membrane of the distal rabbit ileum[a]

	$J_{mc}^{\beta\text{-Ala}}$ (μmol/cm² · hr ± SE)				
1	control	0.25 mM AIB	0.5 mM AIB	1 mM AIB	2 mM AIB
	0.46 ± 0.04 (8)	0.46 ± 0.06 (6)	0.35 ± 0.04 (6)	0.27 ± 0.02 (6)	0.20 ± 0.01 (6)
2	control	20 mM MeAIB	40 mM MeAIB	80 mM MeAIB	
	0.23 ± 0.02 (6)	0.14 ± 0.02 (5)	0.11 ± 0.03 (5)	0.09 ± 0.02 (5)	
3	control	0.5 mM Gly	1 mM Gly		
	0.18 ± 0.01 (4)	0.08 ± 0.01 (4)	0.06 ± 0.01 (4)		
4	control	1 mM Ser*	10 mM Ser	10 mM DL-PIP	
	0.22 ± 0.05 (4)	0.07 ± 0.02 (4)	0.01 ± 0.01 (4)	0.02 ± 0.01 (4)	
5	control	10 mM MeSer*	10 mM Me-DL-Ala	10 mM Me-D-Ala	
	0.51 ± 0.13 (4)	0.29 ± 0.06 (4)	0.21 ± 0.05 (4)	0.29 ± 0.04 (4)	
6	control	40 mM α-ABA	40 mM β-ABA	40 mM γ-ABA	
	0.31 ± 0.08 (4)	0.02 ± 0.01 (4)	0.06 ± 0.01 (4)	0.10 ± 0.02 (4)	
7	control	40 mM DL-PIP	40 mM DL-NIP	40 mM Iso-NIP	
	0.23 ± 0.04 (4)	0.03 ± 0.01 (4)	0.11 ± 0.02 (4)	0.13 ± 0.02 (4)	
8	control	10 mM L-Pro*	10 mM D-Pro	10 mM HO-L-Pro*	10 mM HO-D-Pro
	0.25 ± 0.02 (5)	0.09 ± 0.0	0.16 ± 0.02 (5)	0.10 ± 0.02 (5)	0.25 ± 0.03 (5)

[a] Each row presents the results from paired experiments. The concentrations of the inhibitors are stated above the results, which are μmol/cm² · hr ± SE. The number of observations is in parentheses. The asterisks indicate values from which the similarly marked K_i estimates of Table 4 are made.

bition by serine and MeSer indicate an unfavorable effect of the HO-group.

The Effects of Changing the Amino/Imino Group from α- to β- or γ-Position

This aspect of the function of the β-alanine-accepting site was examined by the use of α-ABA, β-ABA, and γ-ABA as inhibitors of $J_{mc}^{\beta\text{-ala}}$ in one series of experiments, and by the use of piperidine-2-(PIP), -3-(NIP), and -4-(IsoNIP) carboxylic acids in another series of experiments. β-Alanine was 1 mM and all inhibitors 40 mM. β-ABA, PIP and NIP were all of DL configuration. All tested substances (Table 3, rows 6 & 7) did significantly inhibit $J_{mc}^{\beta\text{-ala}}$, but both for the amino and the imino acids the α-analogues were clearly the most efficient inhibitors.

The Stereospecificity of the β-Alanine-Accepting Transport System

This problem was examined using Me-DL-ala, and Me-D-ala, L- and D-proline, and HO-L- and HO-D-proline as inhibitors in paired experiments. The results (Table 3, rows 5 & 8) demonstrate a clear preference for the L-configuration, which in the case of HO-proline amounts to an absolute requirement. The data on the effects of Me-DL-ala and Me-D-ala (Table 3, row 5) were used to estimate the K_i of Me-L-ala (Table 4).

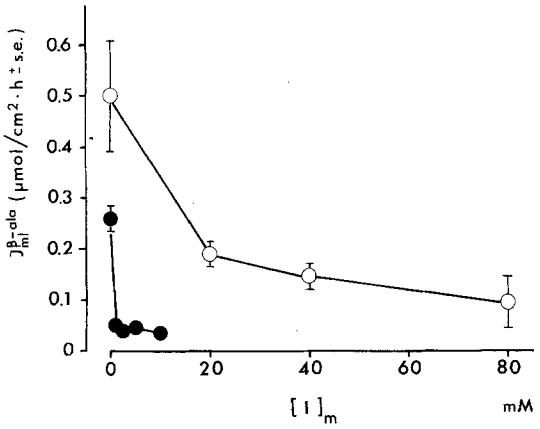

Fig. 3. Comparison of the efficiencies of leucine (●) and MeLeu (○) as inhibitors of the influx of β-alanine across the brush-border membrane of the distal 20- to 30-cm rabbit ileum. The data are means ± SE of six to eight measurements with leucine as inhibitor and four measurements with MeLeu as inhibitor

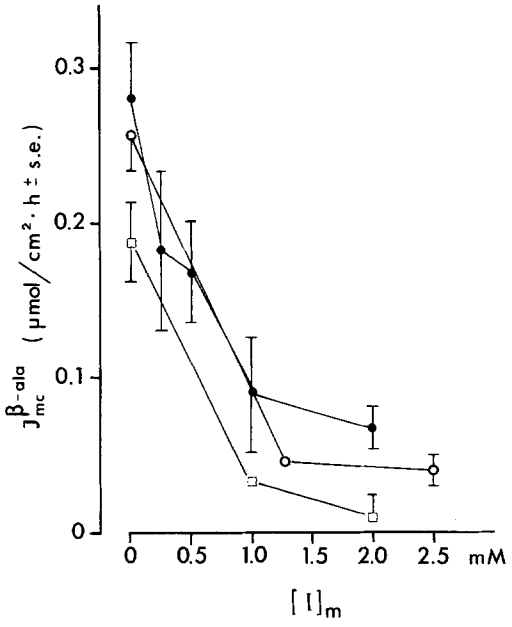

Fig. 4. Comparison of the efficiencies of alanine (□), leucine (○), and lysine (●) as inhibitors of the influx of β-alanine across the distal 20- to 30-cm rabbit ileum. The data are means ± SE of three to four measurements

Table 4. Estimates of K_i against $J_{mc}^{\beta\text{-Ala}}$ (mM ± SE)[a]

Gly	Ala	Ser*	AIB	Leu	Lys
0.20 ± 0.08 (3)	0.11 ± 0.01 (3)	0.30 ± 0.20 (4)	1.1 ± 0.1 (3)	0.10 ± 0.01 (3)	0.38 ± 0.06 (4)
MeGly*	MeAla	MeSer*	MeAIB	MeLeu	
17.8 ± 2.3 (4)	10.4	34.3 ± 8.3 (4)	22.1 ± 2.5 (3)	9.5 ± 0.9 (3)	
Pro*	HO-Pro*				
3.0 ± 0.3 (5)	3.4 ± 0.6 (5)				

[a] The estimates are based on the data of Eq. (2) and Tables 1 and 3. The values marked with an asterisk are based on the similarly marked data of Tables 1 and 3, on observations at only one inhibitor concentration; the number in parentheses indicates number of pairs of observations. Otherwise the estimates are based on the means of Tables 1 and 3 and the number in parentheses indicates the number of inhibitor concentrations.

Mutual Inhibition Between β-Alanine and Alanine and Lysine

The data of Table 1 demonstrated that both neutral and, exemplified by lysine, cationic a.a. are efficient inhibitors of $J_{mc}^{\beta\text{-ala}}$. The data of Table 3 and Fig. 3 characterized more precisely the neutral a.a. as inhibitors and indicated strongly that the β-alanine-accepting site might represent a carrier of broad specificity. To examine this point also lysine was used as inhibitor of $J_{mc}^{\beta\text{-ala}}$, the results (Fig. 4) establish lysine as a high-affinity inhibitor of $J_{mc}^{\beta\text{-ala}}$. Next β-alanine was examined as inhibitor of J_{mc}^{Ala} and J_{mc}^{Lys} at 0.1 mM of these amino acids. This low concentration was chosen to minimize the transport of alanine and lysine by the transport systems characterized in the preceding paper, and to compensate for the large difference between the K_t of β-alanine and the K_i of lysine and especially alanine. Among the neutral a.a. alanine was chosen because of its low affinities for the other known transport systems. The results (Fig. 5) show that J_{mc}^{Ala} was significantly inhibited by 40 and 80 mM β-alanine, and J_{mc}^{Lys} by 20 and 40 mM β-alanine. The concentration dependence of the inhibitory actions of β-alanine is characteristic for partial inhibition, demonstrating that even at 0.1 mM approximately two-thirds of J_{mc}^{Ala} and J_{mc}^{Lys} are by transport systems which do not accept β-alanine. The results of Fig. 5 together with those of Tables 2 and 3 and Fig. 3 indicate that the β-alanine-accepting carrier may be a carrier also of neutral and cationic a.a. as well as of non-α-amino acids and imino acids.

Characteristics of the Transport System for MeAIB

The Effect of N-Methylation

The effects of N-methylation on the affinity of an amino acid for the transport system for MeAIB was examined by comparing the inhibitory effect of AIB of J_{mc}^{MeAIB} with that of self-inhibition by MeAIB as

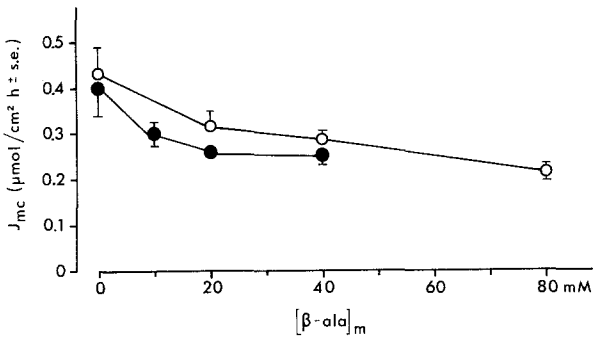

Fig. 5. β-alanine as inhibitor of the influx of alanine (0.1 mM) (○), and lysine (0.1 mM) (●), across the brush-border membrane of the distal 20- to 30-cm rabbit ileum. The data are means ± SE of eight measurements

Table 5. Influx of MeAIB across the brush-border membrane of the distal rabbit ileum[a]

	J_{mc}^{MeAIB} (μmol/cm² · hr ± SE)				
1	control	2 mM MeGly	10 mM MeGly	20 mM MeGly	100 mM MeGly
	1.13 ± 0.16 (4)	1.01 ± 0.11 (3)	0.73 ± 0.08 (3)	0.45 ± 0.05 (3)	0.18 ± 0.01 (3)
2	control	50 mM Ala	100 mM Ala	200 mM Ala	300 mM Ala
	0.81 ± 0.05 (8)	0.66 ± 0.03 (6)	0.53 ± 0.05 (6)	0.39 ± 0.02 (6)	0.35 ± 0.02 (6)
3	control	20 mM Me-D-Ala*	20 mM Me-DL-Ala*	20 mM MeSER	
	1.26 ± 0.07 (8)	0.78 ± 0.06 (8)	0.38 ± 0.04 (8)	0.66 ± 0.05 (8)	
4	control	10 mM HO-L-Pro*	100 mM Ser	200 mM Ser*	
	1.10 ± 0.10 (8)	0.15 ± 0.01 (8)	0.96 ± 0.15 (8)	0.75 ± 0.03 (8)	
5	control	20 mM AIB	40 mM AIB	80 mM AIB	
	1.31 ± 0.16 (4)	0.94 ± 0.06 (3)	1.14 ± 0.15 (4)	0.76 ± 0.02 (4)	
6	control	2 mM Pro	10 mM Pro*	100 mM Pro	200 mM Pro
	0.98 ± 0.03 (8)	0.42 ± 0.02 (6)	0.11 ± 0.01 (6)	0.01 ± 0.003 (5)	0.03 ± 0.01 (6)
7	control	40 mM α-ABA	40 mM DL-β-ABA	40 mM λ-ABA	
	1.15 ± 0.05 (4)	0.80 ± 0.11 (4)	0.97 ± 0.05 (4)	1.06 ± 0.11 (4)	
8	control	40 mM DL-PIP	40 mM DL-NIP	40 mM Iso-NIP	
	0.79 ± 0.07 (8)	0.02 ± 0.005 (6)	0.40 ± 0.03 (7)	0.55 ± 0.05 (7)	
9	control	40 mM L-Pro	40 mM D-Pro	40 mM HO-L-Pro	40 mM HO-D-Pro
	1.10 ± 0.23 (4)	0.05 ± 0.02 (3)	0.24 ± 0.01 (3)	0.05 ± 0.01 (3)	0.50 ± 0.01 (3)

[a] Details as described for Table 3.

described by Eq. (1), and by comparing the effects on J_{mc}^{MeAIB} of alanine and MeAla, leucine and MeLeu, and of serine and MeSer. In addition, the inhibitory effects of MeGly, proline and HO-proline were examined. These experiments were carried out at 1 mM MeAIB using a range of inhibitor concentrations as stated in Table 5 and Fig. 6. The results show for all these inhibitors that the estimates of their K_i values against J_{mc}^{MeAIB} are independent of the concentrations used. This is clearly demonstrated by the data of Fig. 6, which by directly comparing the inhibitory effects of leucine and MeLeu also demonstrate the large increase in affinity induced by N-methylation.

The Effects of Changing the Position of the Amino/Imino Group from α- Through β- to γ-Position

The role of the position of the amino/imino group was examined by comparing the inhibitory effects of the aminobutyric acids and the piperidine-carboxylic acids on J_{mc}^{MeAIB} as measured at 1 mM MeAIB with the inhibitors at 40 mM. The results (Table 5, rows 7 & 8) demonstrate that for both amino and imino acids the highest affinity is associated with an α-position of the amino/imino group; only for the piperidine-carboxylic acids were the β- and γ-position acceptable for the MeAIB transport system.

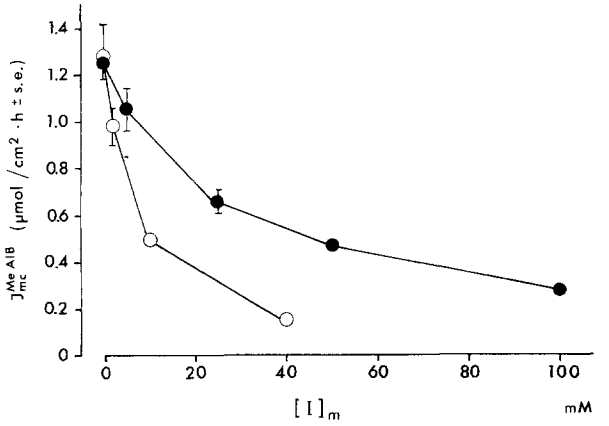

Fig. 6. A comparison of the efficiencies of leucine (●) and MeLeu (○) as inhibitors of the influx of MeAIB across the brush-border membrane of the distal 20- to 30-cm rabbit ileum. The data are means ± SE of six to seven measurements with leucine as inhibitor and four measurements with MeLeu as inhibitor

The Stereospecificity of the MeAIB-Accepting Transport System

The stereospecificity of the transport system for MeAIB was examined by comparing the efficiencies of L- and D-proline, HO-L- and HO-D-proline, and Me-DL- and Me-D-alanine as inhibitors of J_{mc}^{MeAIB} as measured at 1 mM MeAIB with 40 mM of the inhibitors. The results (Table 5, rows 3, 8 & 9) demonstrate a strong stereospecificity for all three imino acids. On this background the data on DL-PIP as inhibitor of J_{mc}^{MeAIB} indicate that of the amino acids tested L-PIP has the highest affinity for the transport system for MeAIB.

The data on the inhibitory effects of alanine and serine, MeAla and MeSer, and proline and HO-proline indicate that an HO-group on the side chain reduces the affinity for the MeAIB site. The relative affinities of the amino/imino acids for the MeAIB transport system are summarized in Table 6.

Sodium-Dependence of the Transport of β-Alanine and MeAIB

Sodium-independence of the transport of β-alanine and/or MeAIB would affect the interpretations of part of the foregoing study. This question was therefore examined by measuring $J_{mc}^{\beta\text{-ala}}$ and J_{mc}^{MeAIB} under sodium-free conditions at 1 mM of these amino acids in the absence or presence of 40 mM of

Table 6. Estimates of K_i against J_{mc}^{MeAIB} [a]

K_i against J_{mc}^{MeAIB} (mM ± SE)					
	Ala	Ser*	α-ABA	AIB	Leu
—	158 ± 7 (4)	257 ± 25 (8)	86 ± 21 (4)	71 ± 15 (3)	23 ± 1 (4)
MeGly	MeAla	MeSer		MeAIB	MeLeu
13.2 ± 1.0 (4)	4.8	17.4 ± 3.1 (7)	—	3.6	4.7 ± 0.3 (4)
Pro*	HO-Pro*	Me-D-Ala*	Me-DL-Ala*		
0.8 ± 0.1 (6)	1.3 ± 0.1 (8)	25 ± 4 (7)	8.0 ± 0.4 (8)		

[a] The estimates are based on the data of Eq. (1) and Tables 1 and 5. Details as described for Table 4.

the most efficient inhibitors of these transports. The results (Table 7), particularly the absence of self-inhibition, indicate that mediated transport of β-alanine and MeAIB does not take place under sodium-free conditions.

Transport of β-Alanine in the Rabbit Jejunum

The data from experiments performed on microvesicles of the brush-border membrane of the whole rabbit small intestine excluding 30 cm from each end indicated that the rabbit small intestine was not equipped with a β-alanine-accepting system (Stevens et al., 1982). In order to determine whether this absence was specific for the microvesicle technique, $J_{mc}^{\beta\text{-ala}}$ was measured using the section of the intestine between 60 and 90 cm from the ileo-coecal junction. In paired experiments $J_{mc}^{\beta\text{-ala}}$ was measured at 1 mM β-alanine without or with 40 mM α-ABA, DL-PIP or β-alanine. The results (Table 8) show that $J_{mc}^{\beta\text{-ala}}$, although significantly higher than the estimated diffusive permeability in the distal ileum [Eq. (2)], is much lower than the total distal ileal transport. However, the statistically significant inhibition of $J_{mc}^{\beta\text{-ala}}$ both by β-alanine and by α-ABA and DL-PIP demonstrates beyond any doubt that mediated transport of β-alanine can be accomplished by a rather large part of the section of the small intestine studied by the microvesicle technique.

Discussion

The data of Tables 1 through 4 and 7 and Figs. 2 through 4 indicate that the β-alanine-accepting carrier may be a sodium-dependent, high-affinity transport system for neutral a.a. and of cationic amino acids, which, with a loss of affinity, tolerates N-methylation and displacement of the amino/imino group to the β- and γ-position for which the cyclic imino acids have much higher affinities than the aliphatic imino acids, which is moderately stereospecific, and for which the affinities are reduced by an HO-group on the side chain.

The data of Tables 1, 2, 5, 7 and Figs. 1 and 5 define the principal carrier of MeAIB as a sodium-dependent, high-affinity transport system for imino acids and, probably, a low-affinity system for neutral a.a., which does not accept cationic a.a. For the neutral a.a. an α-position of the amino group is required, although for the cyclic imino acids both the β- and the γ-position are tolerated. This transport system is more stereospecific, and, as for the β-alanine-accepting system, an HO-group on the side chain reduced the affinity.

The mutual inhibition between β-alanine and MeAIB (Tables 1 & 3) indicates that the data of Fig. 1 include contributions by the β-alanine-accepting transport system. Then Eq. (1) presents a partly erroneous description of the principal transport system for MeAIB. Assuming that the β-alanine-accepting transport system contributes to J_{mc}^{MeAIB} with

Table 7. Influx of β-alanine or MeAIB across the brush-border membrane of the distal rabbit ileum measured under sodium-free conditions[a]

J_{mc} (μmol/cm² · hr ± SE)				
	control	40 mM DL-PIP	40 mM Lys	40 mM β-Ala
β-Ala (1 mM)	0.029 ± 0.010 (4)	0.032 ± 0.010 (4)	0.048 ± 0.020 (4)	0.037 ± 0.008 (4)
	control	40 mM MeAIB	40 mM Pro	40 mM Leu
MeAIB (1 mM)	0.033 ± 0.017 (4)	0.066 ± 0.010 (4)	0.038 ± 0.015 (4)	0.045 ± 0.017 (4)

[a] Number of observations in parentheses.

Table 8. Influx of β-alanine (1 mM) measured in paired experiments with or without the inhibitors (40 mM) indicated in the table[a]

$J_{mc}^{\beta\text{-ala}}$ (μmol/cm² · hr ± SE)			
0	20 mM α-ABA	40 mM DL-PIP	40 mM β-Ala
0.074 ± 0.014 (12)	0.032 ± 0.006 (12)	0.015 ± 0.006 (12)	0.033 ± 0.006 (12)

[a] The section of the small intestine between 60 and 90 cm from the ileo-coecal junction was used. Number of observations in parentheses.

a J_{max} of 0.8 μmol/cm^2 · hr and a $K_t = K_i$ against $J_{mc}^{\beta\text{-ala}} = 22$ mM (Table 3), this error can be judged by subtracting this contribution from the data of Fig. 1 and plotting the resulting J_{mc}^{MeAIB} against $J_{mc}^{MeAIB}/[MeAIB]_m$. Such a plot gives a J_{max} of 4.1 μmol/cm^2 · hr and a K_t of 3.1. Thus this error has not significantly affected the estimates of K_i summarized in Table 6.

Comparisons with the Intestinal Transport Systems for Imino Acids in Other Species

The β-alanine-accepting transport system described here appears unique for the rabbit small intestine, as it differs from the β-alanine-accepting transport system of the rat (Munck, 1981) and chicken (Lerner & Karcher, 1978) small intestine in having high affinities for neutral and cationic amino acids. In its general characteristics the MeAIB system appears similar to the imino acid transport systems of the hamster and guinea pig small intestine, which also do not accept β-alanine. In having increasing affinities for neutral a.a. with increasing length of the side chain, the MeAIB system of the rabbit ileum and the guinea pig small intestine differ from the β-alanine-accepting imino acid transport mechanism of the rat, which accepts glycine and alanine but is barely inhibited by α-ABA and not significantly inhibited by valine or leucine (Munck, 1981). The MeAIB system of the rabbit jejunum appears to be only partially inhibitable by neutral a.a. (Stevens et al., 1982). This difference from the rabbit ileum is intriguing. It may depend on the use of different techniques; or in some restricted section of the small intestine an imino acid transport system may exist which is totally insensitive to neutral a.a.

Comparison with other Studies of Amino Acid Transport by the Rabbit Ileum

This and the accompanying paper define a total of five systems for transport of cationic and neutral amino/imino acids.

1) A high-affinity, sodium-dependent carrier of neutral a.a. which does not accept cationic a.a., non-α-amino acids or imino acids (Munck, preceding paper).

2) A high-affinity, sodium-dependent carrier of neutral and cationic a.a., which accepts non-α-amino acids and imino acids.

3) A high-affinity, sodium-independent carrier of neutral and cationic a.a., which does not accept non-α-amino acids (Munck, preceding paper).

4) A low-affinity, sodium-dependent carrier of neutral and cationic a.a., which does not accept non-α-amino acids or imino acids (Munck, preceding paper).

5) An intermediate affinity, sodium-dependent carrier of imino acids, which is also a low-affinity carrier of neutral a.a., but does not accept cationic a.a. or non-α-amino acids.

Of these 1, 2 and 3 must have contributed to the previously described high-affinity carrier of neutral a.a. (Munck & Schultz, 1969*b*; Preston, Schaeffer & Curran, 1974; Sepúlveda & Smith, 1978; Paterson, Sepúlveda & Smith, 1979); 2 and 3 contributed to the previously described high-affinity carrier of cationic a.a. (Munck & Schultz, 1969*a*; Paterson, Sepúlveda & Smith, 1981); 4 and 5 were responsible for the low-affinity transport of neutral a.a. (Munck & Schultz, 1969*b*; Sepúlveda & Smith, 1978; Paterson, Sepúlveda & Smith, 1980); 4 was recognized as a low-affinity sodium-dependent carrier of cationic a.a. (Munck & Schultz, 1969*a*).

Sepúlveda and Smith (1978) and Paterson et al. (1980) have described a low-affinity, sodium-independent transport system for neutral a.a. The present studies have not provided convincing evidence in favor of this proposal, and it seems possible that the impression of such a system could have been created by the sodium-independent, high-affinity carrier of neutral and cationic a.a. together with an insufficient correction for diffusive transport.

A transport system for the anionic amino acids glutamic and aspartic acid has been described in the rabbit ileum (Schultz et al., 1970). It remains to be determined whether this is a sixth transport system in the rabbit ileum or whether it is included in one of the five systems described here. Also, only additional studies can determine whether the brush-border membrane of the rabbit ileum possesses equivalents to the N and ASC systems of the hepatocyte (Kilberg, Handlogten & Christensen, 1980, 1981). The crucial procedure in the present resolution of the several transport systems described here has been the use of β-alanine and MeAIB, both as inhibitors of the transport of cationic and neutral a.a. and as transported substances. In addition, as pointed out by Sepúlveda and Smith (1978) the use of a wider range of concentrations has been a contributing factor.

Regional Differences in the Rabbit Small Intestine

In contrast to the results obtained with brush-border membrane microvesicles (Stevens et al., 1982) the accompanying paper presented conclusive evidence of mutual inhibition between neutral and cationic amino acids and confirmed previously pub-

lished results (Munck & Schultz, 1969*b*); and in the present paper it was demonstrated that mediated transport of β-alanine is not confined to the most distal ileum but is present in sections of the intestine used for the preparation of brush-border membrane microvesicles (Stevens et al., 1982). These differences may indicate that both these transport qualities gradually disappear as the distance to the ileo-coecal junction increases. In this way in brush-border membrane preparations from the whole intestine, except the most distal 30 cm, these transport qualities could be diluted beyond recognition. However, it is also possible that during the preparation of the microvesicles the cell membranes undergo changes which prevent the expression of the transport processes. This interpretation gains some credibility from studies on microvesicles prepared from brush-border membranes from the rat small intestine, in which several characteristics of the transport of lysine across the luminal membrane could not be reproduced (Cassano, Leszczynska & Murer, 1983).

Systematizing the Intestinal Transport of Amino Acids

From imaginative studies of amino acid transport by unicellular systems, especially the Ehrlich ascites tumor cell a picture of several transport systems has been developed (the A & L systems, Oxender & Christensen, 1963; the β-alanine system, Christensen, 1964; the ASC system, Christensen, Liang & Archer, 1967; the Ly^+ system (now y^+), Christensen, Handlogten & Thomas, 1969). These studies have been an important source of inspiration also for studies of intestinal transport of amino acids, and several attempts have been made to apply the developed terminology on the intestine. The imino acid transport system of the luminal membrane of the rat small intestine is in many respects (Munck, 1966; 1981) similar to the A system (Oxender & Christensen, 1963); and the low-affinity transport system for lysine is, as described in the preceding paper similar to the Ly^+ system described for the Ehrlich ascites tumor cell (Christensen et al., 1969). However, with these two exceptions the several amino acid transport systems of the brush-border membranes of the guinea pig, rabbit and rat small intestine (Munck, 1981, 1983; Stevens et al., 1982) do not fit into the framework of systems described for unicellular amino acid transport. Therefore it presently seems more rewarding to stress the difference in purpose of the intestinal (and kidney proximal tubule) brush-border membrane and the cell membrane of the ascites tumor cell, the erythrocyte, or hepatocyte; that of the former being to take care of the needs of the whole animal and that of the latter to serve only one organ. In agreement with such dichotomy in cellular transport of amino acids, transport by brush-border membrane vesicles prepared from the rabbit kidney (Mircheff et al., 1982) fails to fall in line with the A-ASC systematics, whereas the basolateral membrane from the small intestine, which must also serve organ-specific needs, in many respects transports amino acids according to the unicellular systems (Mircheff, Os & Wright, 1980).

The specificity of amino acid transport by microvesicles of rabbit kidney brush-border membranes (Mircheff et al., 1982) is in many respects very similar to the present description of the specificity of the rabbit ileum. Mircheff et al. (1982) found it necessary to invoke the operation of eight transport systems, and none of these are quite like the five described in this paper. However, in order not to take effects of gradient dissipation for evidence of competitive inhibition the more crucial experiments on the kidney microvesicles compare the inhibitory effects of 100 mM of the amino acids with that of 100 mM D-glucose, and only degrees of inhibition in excess of that of glucose are seen as resulting from competition for shared transport systems. This analysis is correct only in the rather unlikely event that for all these substrates the gradient-dissipating effects are the same.

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