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IMINO ACID TRANSPORT ACROSS THE BRUSH-BORDER MEMBRANE OF THE GUINEA-PIG SMALL INTESTINE

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The transport of imino and non-α-amino acids across the brush-border membrane of the guinea-pig small intestine has been examined. It was found that the guinea pig is without a transport system for non-α-amino acids. The transport of imino acids was characterized using methylaminoisobutyrate (MeAIB) as a substrate. This choice was validated by lack of kinetic evidence that more than one transport system was involved in the transport of MeAIB, by the identical values of the estimates of the passive permeability of MeAIB, the magnitude of its proline-resistant transport, and the permeability of mannitol. The transport system for MeAIB is moderately stereospecific. It does not accept cationic amino acids. It accepts α-amino-monocarboxylic acids but *N*-methylation increases the affinities of these amino acids by an order of magnitude. The length of the side-chain of the aliphatic imino acids seems of little importance for the affinity for the transport system, but the data on inhibition of the transport of MeAIB by proline and piperidine-2-carboxylic acid indicate that it is sharply increased by ring formation.

Introduction

In the rat, imino acids and non-α-amino acids are transported by a single transport mechanism, carrier, which also contributes to the transport of short-side-chain, neutral amino acids [1,2]. In the rabbit ileum imino acids are transported by a carrier which also acts as a low-affinity carrier of neutral amino acids [3,4]. In addition, the rabbit ileum possesses an amino acid carrier of very broad specificity, with high affinities for neutral and cationic amino acids and considerably lower affinities for imino and non-α-amino acids [3,4].

For the guinea-pig, intestinal transport of imino and non-α-amino acids has not been examined in any detail. Nevertheless, in general, descriptions of intestinal transport of amino acids must be based also on studies performed on this species [5,6]. Therefore, for a comprehensive view of the intestinal transport of amino acids it is of considerable interest to elucidate the cellular pathways of imino acids and non-α-amino acids also in the guinea pig. This is the aim of the present study.

Materials and Methods

Materials and experimental techniques were as described in the preceding paper [7]; ^{14}C-labelled β-alanine and MeAIB were purchased from the New England Nuclear Co. The K_i values of the various amino acids against J_{mc}^{MeAIB} are estimated from the paired data of Table I and Fig. 1 using the data of Eqn. 1 and assuming that, corrected for the diffusional contribution:

$$J_{mc}^{MeAIB} = \frac{[MeAIB]_m \cdot J_{max}}{[MeAIB]_m + K_t(1 + [I]_m/K_i)}$$

where I is inhibitor.

Abbreviation: MeAIB, methylaminoisobutyrate.

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Results

Influx of β-alanine across the brush-border membrane, $J_{mc}^{\beta\text{-Ala}}$

Measured in paired experiments at 1 mM β-alanine without or with 40 mM of the inhibitors MeAIB or Me-DLAla, $J_{mc}^{\beta\text{-Ala}}$ was respectively 0.04 ± 0.0008 ($n = 4$), 0.06 ± 0.01 ($n = 4$) and 0.05 ± 0.009 ($n = 4$) μmol/cm² per h. In a separate series of paired experiments at 1 mM β-alanine with or without 40 mM β-alanine as inhibitor, $J_{mc}^{\beta\text{-Ala}}$ was 0.07 ± 0.009 ($n = 8$) and 0.07 ± 0.009 ($n = 8$) μmol/cm² per h. Clearly, none of these inhibitors had any effect on $J_{mc}^{\beta\text{-Ala}}$. Particularly the lack of self-inhibition indicates that β-alanine passes the brush-border membrane only by diffusion.

Influx of MeAIB across the brush-border membrane, J_{mc}^{MeAIB}

The unidirectional influx J_{mc}^{MeAIB} was measured in paired experiments at concentrations between 0.5 and 80 mM. The results are described by Eqn. 1. This equation describes J_{mc}^{MeAIB} as the sum of one saturable process and free diffusion. By the chi-square test the fit between the results and Eqn. 1 is characterized by a P value of 0.92.

$$J_{mc}^{MeAIB} = (1.14 \pm 0.10)[MeAIB]_m/((1.16 \pm 0.23) + [MeAIB]_m) + (0.045 \pm 0.003)[MeAIB]_m \ \mu mol/cm^2 \text{ per h}$$

In the following, the K_t of 1.16 mM and the passive permeability of 0.045 μmol/cm² per h per mM will be used in estimating the K_i values of various amino acids against J_{mc}^{MeAIB}.

Stereospecificity. The stereospecificity of the transport system for MeAIB was examined in paired experiments using various inhibitors at 40 mM against 1 mM MeAIB.

In one series, 40 mM MeAIB was used as inhibitor together with Me-DLAla and Me-DAla (Table I, row 1). The data show that Me-DLAla is a statistically, significantly stronger inhibitor of J_{mc}^{MeAIB} than Me-DAla. The mean values of these observations correspond to a K_i of 3.7 mM for the former and 8.6 mM for the latter, these values give an estimate of 2.4 mM as the K_i of Me-LAla. The relative validity of these estimates is supported by the simultaneously measured inhibitory effect of 40 mM MeAIB, which evaluated in the same way corresponds to a K_i of 0.9 mM, a value reasonably close to the K_t stated in Eqn. 1.

In another series of experiments, the inhibitory

TABLE I

TRANSPORT OF MeAIB (1 mM MeAIB, 5 mM D-GLUCOSE) ACROSS THE BRUSH-BORDER MEMBRANE OF THE GUINEA PIG

Each row represents the results of paired experiments, the inhibitor concentrations are stated in the table, errors are S.E.; number of measurements are in parentheses. Pip, pipecolic acid; Nip, nipecotic acid.

1	0	40 Me-DLAla	40 Me-DAla	40 MeAIB	
	0.42 ± 0.02 (8)	0.10 ± 0.01 (8)	0.16 ± 0.028	0.06 ± 0.01 (7)	
2	0	40 LPro	40 DPro	40 OH-LPro	40 OH-DPro
	0.52 ± 0.04 (6)	0.06 ± 0.01 (5)	0.15 ± 0.03 (5)	0.06 ± 0.01 (5)	0.32 ± 0.04 (5)
3	0	40 α-ABA	40 β-ABA	40 γ-ABA	
	0.52 ± 0.02 (4)	0.30 ± 0.02 (4)	0.42 ± 0.01 (4)	0.51 ± 0.03 (4)	
4	0	40 DLPip	40 DLNip	40 IsoNip	
	0.46 ± 0.03 (4)	0.05 ± 0.02 (4)	0.30 ± 0.02 (4)	0.38 ± 0.15 (4)	
5	0	40 Ala			
	0.71 ± 0.04 (4)	0.47 ± 0.02 (4)			
6	0	40 β-Ala	40 Leu	40 Lys	
	0.59 ± 0.03 (8)	0.56 ± 0.01 (8)	0.33 ± 0.02 (8)	0.59 ± 0.06 (7)	
7	0	20 AIB	40 AIB	80 AIB	
	0.80 ± 0.05 (4)	0.64 ± 0.05 (4)	0.57 ± 0.07 (4)	0.46 ± 0.03 (4)	
8	0	10 Pro	40 Pro	80 Pro	160 Pro
	0.46 ± 0.03 (8)	0.09 ± 0.01 (6)	0.04 ± 0.01 (6)	0.06 ± 0.01 (6)	0.06 ± 0.01 (6)

effects of L-proline, D-proline, HO-L-proline, and HO-D-proline were compared (Table I, row 2); the results demonstrate a clear preference for L-proline over D-proline and an even more marked preference for the L-enantiomorph of HO-proline.

The importance of the position of the imino/amino group This was examined in paired experiments using the aminobutyric acids (Table I, row 3) and the piperidine-carboxylic acids (Table I, row 4) at concentrations of 40 mM against 1 mM MeAIB. The data of the table show that among the aminobutyric acids only the α-amino acid ($K_i = 32 \pm 11$ mM ($n = 4$)) significantly inhibited J_{mc}^{MeAIB}. Similarly (Table I), alanine (row 5), but not β-alanine (row 6), inhibited J_{mc}^{MeAIB}. These results indicate that the transport system for imino acids does not accept non-α-amino acids. In contrast (Table I, row 4), the data on the effects of the piperidine-carboxylic acids demonstrate that at least cyclic imino acids are accepted with the imino group in both the β- and the γ-positions, although the highest affinity is associated with the α-position.

Distinction between the imino acid transport system and those of cationic and neutral amino, acids. This distinction was investigated by comparing the inhibitory effects of 40 mM β-alanine, leucine, lysine and alanine on the influx of MeAIB at 1 mM concentration (Table I, rows 5 and 6). It is seen that neither β-alanine nor lysine inhibits the transport of MeAIB, and that both alanine and leucine are relatively poor inhibitors of J_{mc}^{MeAIB}. The inhibitory effect of alanine (Table I, row 6) corresponds to a K_i of 38 ± 5 mM ($n = 4$).

The effect of methylation of the amino group. The effect of methylation of the amino group on the affinity for the imino acid carrier was examined by measurements of the inhibitory effects of leucine, methylleucine, and aminoisobutyric acid. For each inhibitor, paired measurements were made at inhibitor concentrations from 0 to 80 mM. The estimates of K_i for these amino acids are made from the mean values for J_{mc}^{MeAIB} shown in Table I and Fig. 1 using the estimates of Eqn. 1,

The data of Fig. 1 show that that at all concentrations leucine acts according to the same K_i, 21 ± 1 mM ($N = 4$); similarly, for methylleucine $K_i = 4.0 \pm 0.6$ mM ($n = 3$). Also for AIB, the estimates of K_i were independent of concentration (Table I, row 7); $K_i = 48 \pm 4$ ($n = 3$). The data of rows 2 and 4 of Table I demonstrate that the cyclic imino acids were the most effective inhibitors of J_{mc}^{MeAIB}. Partly to use this efficiency for an independent estimate of the passive permeability of MeAIB and partly to reach an estimate of the K_i of proline, this amino acid was used in paired experiments at 0–160 mM as inhibitor of J_{mc}^{MeAIB} measured at 1 mM MeAIB. The data on proline inhibition of J_{mc}^{MeAIB} (row 8) indicate that in these experiments 0.05 μmol/cm^2 per h per mM is a better estimate of the passive contribution to J_{mc}^{MeAIB}; using this value, the data for the inhibitory effect of 10 mM proline provide an estimate of K_i for proline of 0.63 ± 0.26 mM ($n = 6$). These data

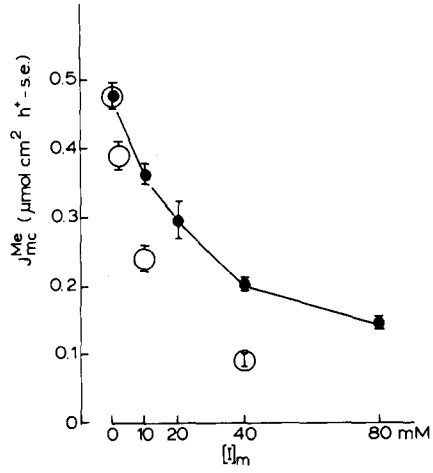

Fig. 1. Comparison of the efficiencies of leucine (●) and *N*-methylleucine (○) as inhibitors of J_{mc}^{MeAIB} as measured at 1 mM MeAIB + 5 mM D-glucose. The data for each inhibitor (I) represent paired experiments. The data are the means ± S.E. of 4–6 measurements.

TABLE II

K_i VALUES (mM) FOR VARIOUS L-AMINO ACIDS AS INHIBITORS OF J_{mc}^{MeAIB} MEASURED AT 1 mM MeAIB + 5 mM D-GLUCOSE

Errors are S.E., number of estimates in parentheses. ABA, aminobutyrate; AIB, aminoisobutyrate.

Ala	AIB	Leu	ABA
38 ± 5 (4)	48 ± 4 (3)	21 ± 1 (4)	32 ± 11 (4)
MeAla	MeAIB	MeLeu	Pro
2.4	1.2	4 ± 0.6 (3)	0.6 ± 0.3 (6)

together with the estimate of Eqn. 1 and the data of Table I, rows 1 and 5 demonstrate that *N*-methylation improves an amino acids affinity for the imino acid carrier by about an order of magnitude. In addition, the K_i value for proline indicates that ring formation further increases the affinity. The estimates of the K_i values are summarized in Table II.

Influx of leucine across the brush-border membrane

In the preceding paper [7], it was shown that leucine is transported by at least one of the carriers of lysine and by at least one lysine-insensitive carrier. By the concentration-independence of the K_i of leucine, it has now been demonstrated that leucine can completely inhibit the transport of MeAIB. This strongly indicates that leucine and MeAIB share the imino acid carrier.

In an attempt to resolve the influx of leucine into its (probably) at least three saturable components and diffusion, J_{mc} was measured at concentrations between 0.5 and 80 mM. However, the non-linear least-squares analysis provided as the best fit that:

$$J_{mc}^{Leu} = (2.9 \pm 0.6)[Leu]_m/((2.0 \pm 0.6) + [Leu]_m)) + (0.126 \pm 0.014)[Leu]_m$$

The fit between this description and the concentrations (Fig. 2) is characterized by a P value of 0.95.

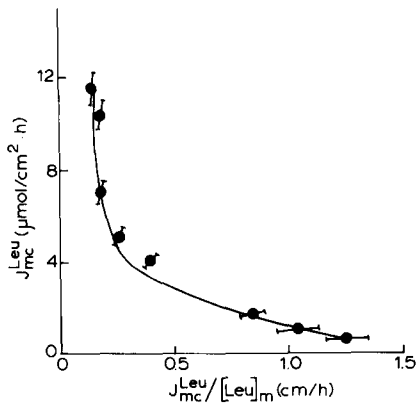

Fig. 2. Kinetics of influx of leucine across the brush-border membrane of guinea-pig mid-small intestine measured in the presence of 5 mM D-glucose. Each point represents the mean of 13 observations. The curve is described by Eqn. 2.

Thus, it was not possible by this procedure to confirm the results of the inhibition studies. But the necessity of a very high estimate of the passive permeability of leucine may well disguise a contribution by one or more low-affinity transport system.

J_{mc}^{Leu} was also measured at 25 mM leucine in paired experiments with or without 40 mM MeAIB as inhibitor. In these experiments MeAIB did only statistically, insignificantly reduce J_{mc}^{Leu} from 6.07 $\pm$ 0.28 ($n = 12$) to 5.77 $\pm$ 0.21 ($n = 12$) μmol/cm² per h.

Discussion

In the present study, it has been shown that the guinea-pig small intestine possesses a transport system for imino acids but is without a specific transport system for β-alanine or, more general, for non-α-amino acids. In this respect it differs from the rabbit ileum [3,4] and the small intestine of the rat [2] and hen [8].

The imino acid transport system has here been characterized using MeAIB as a representative substrate for its transport function. This choice is warranted by the lack of kinetic evidence that more than one transport system should be contributing to J_{mc}^{MeAIB} (Eqn. 1). The validity of the interpretation of J_{mc}^{MeAIB} expressed by Eqn. 1 is supported by the agreement between the estimated passive permeability of MeAIB, the measured permeability of mannitol [7] and the magnitude of the proline resistant fraction of J_{mc}^{MeAIB} (Table I, row 8). The observation (Table I, row 7; Fig. 1) of concentration-independent values of K_i against J_{mc}^{MeAIB} for leucine, methylleucine, and aminoisobutyrate also provides support for the conclusion that only one transport system contributes to J_{mc}^{MeAIB}, and it lends credit to the estimates of K_i for alanine, Me-DLAla, Me-DAla, aminobutyrate and proline, which were based on measurements at a single inhibitor concentration.

From a comparison (Table II) of the inhibitory effects of alanine and methylalanine, aminoisobutyric acid and MeAIB, and leucine and methylleucine is seen that *N*-methylation increases the affinity by an order of magnitude. The data for alanine, aminoisobutyrate and leucine fit into the scheme described for the rabbit ileum [9] where

elongation of the side-chain increases the affinity for the transport system, whereas increasing its size by branching is without effect on the affinity. This indicates that for the α-amino-monocarboxylic acids hydrophobic interactions with the cell membrane play a role. Contrarily, the very similar K_i values for MeAla, MeAIB, and MeLeu indicate that interactions between the imino acids' side-chain and the membrane are much less important. Similar results have been obtained for the rabbit ileum [3]. The exceptionally low K_i for proline might be interpreted as caused by a more rigid positioning of the imino group with respect to the carboxylic group.

The data (Table I, rows 1 and 2) on the inhibitory effects of Me-DL-alanine and Me-D-alanine, L-proline and D-proline, and of HO-L-proline and HO-D-proline demonstrate a significant stereospecificity similar to that observed for the rabbit ileum [3] but more pronounced than for the rat small intestine [2].

Both for the aminobutyric acids and the piperidine carboxylic acids it was observed (Table I, rows 3 and 4) that increasing the distance between the carboxylic and the amino/imino group reduced the affinity for the MeAIB carrier. But an α-position appears to be an absolute requirement only for the amino acids.

The inability (Table I, row 5) of lysine to inhibit J_{mc}^{MeAIB} demonstrates that imino acids and cationic amino acids are transported by separate transport systems. The data on J_{mc}^{Leu} show that a dominant fraction of this transport must take place in a process with a much lower K_t than that characterizing the interaction of leucine with the MeAIB carrier. The data of the preceding paper demonstrated that leucine was transported by both at least one lysine-resistant carrier and by at least one lysine carrier. Therefore the present observations (Fig. 1) indicate that leucine is transported by at least three transport systems. In the present study it was not possible by inhibition with MeAIB to demonstrate that leucine is transported by the carrier of MeAIB; but considering the relatively low J_{max} for the MeAIB carrier this is not surprising. Consequently, the agreement between the present statistical analysis of the concentration-dependence of J_{mc}^{Leu} and the interpretation of the concentration-dependence of leucine uptake by rings of the guinea-pig small intestine [6] must be taken as evidence of common limitations to the power of these techniques to resolve the transport of leucine into its several components. That this is the case is supported by the very high estimate of the passive contribution to J_{mc}^{Leu} shown in Eqn. 2.

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