Phenylalanine transport in rabbit small intestine

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1. The proposal that rabbit small intestine possesses a separate, sodium-dependent carrier of phenylalanine has been examined by measurements of the unidirectional influx of amino acids across the brush-border membrane of the intact epithelium of the rabbit small intestine.

2. We demonstrate that, like alanine, glycine and leucine, phenylalanine shares sodium-dependent as well as sodium-independent transport with lysine.

3. Using the distal ileum we applied the A (phenylalanine)–B (leucine)–C (alanine) test on the sodium-dependent, lysine-resistant transport of phenylalanine. For phenylalanine, $K_{½}$ (concentration required for half-maximal transport) was 3·1 ± 0·2 mM ($n = 7$) and K_i (inhibitor constant) against leucine transport was 3·1 ± 0·2 mM ($n = 4$). For leucine, $K_{½}$ was 1·1 ± 0·1 mM ($n = 4$) and K_i against transport of phenylalanine was 1·1 ± 0·1 mM ($n = 4$). For alanine, $K_{½}$ was 12·6 ± 1·1 mM ($n = 3$), K_i against phenylalanine was 13·1 ± 1·8 mM ($n = 4$) and K_i against leucine was 11·0 ± 0·4 mM ($n = 4$).

4. Using the jejunum we applied the A (phenylalanine)–B (alanine)–C (methionine) test on the lysine-resistant, sodium-dependent transport of phenylalanine. For phenylalanine, $K_{½}$ was 4·7 ± 0·2 mM ($n = 7$) and K_i against alanine was 4·8 ± 0·2 mM ($n = 4$). For alanine, $K_{½}$ was 15·6 ± 0·8 mM ($n = 7$) and K_i against phenylalanine was 18·1 ± 0·9 mM ($n = 5$). For methionine, K_i against phenylalanine was 1·1 ± 0·2 mM ($n = 3$) and against alanine was 0·8 ± 0·2 mM ($n = 3$)

5. These data demonstrate that one, and only one, lysine-resistant, sodium-dependent carrier is involved in transport of phenylalanine across the brush-border membrane of rabbit small intestine.

In a study of amino acid uptake by rabbit jejunal brush-border membrane vesicles a contrast between total inhibition of transport of phenylalanine by methionine and apparently only partial inhibition by alanine led Stevens, Ross & Wright (1982) to propose that the rabbit small intestine is equipped with a sodium-dependent transport system for phenylalanine different from the sodium-dependent transporter of neutral amino acids in general, whereby phenylalanine is also transported. In addition, phenylalanine was transported by a saturable, sodium-independent mechanism. The proposal of a separate sodium-dependent carrier of phenylalanine was not further substantiated, and the data on which it was based does not exclude the alternative interpretation that the apparently only partial inhibition by alanine might be caused by a much lower affinity of alanine for the carrier of neutral amino acids. Nevertheless, the proposal has generally been accepted (Barker & Ellory, 1990) and is thought to apply to both the guinea-pig (Del Castillo & Muñiz, 1991), the pig (Maenz & Patience, 1992) and the human small intestine (Malo, 1991).

In the present study we examine the interactions between lysine and phenylalanine; and drawing upon previously reported data on the kinetics of leucine and alanine transport (Munck, 1985*a*) we apply the A–B–C test (Scriver & Wilson, 1964) on the unidirectional, sodium-dependent, lysine-resistant influx of phenylalanine (A) across the brush border of the distal rabbit ileum using leucine (B) and alanine (C) as inhibitors. Previous studies (Munck, 1985*b*; Munck & Munck, 1992*a*, *b*, *c*) indicate that differences in amino acid transport along the total rabbit small intestine exclusively reflect differences in transport capacity while affinities and specificities are constant. However, direct evidence does not exist that this is the case for the sodium-dependent, lysine-resistant carrier of α-amino-monocarboxylic acids. Therefore, the A–B–C test will also be used to examine jejunal transport of phenylalanine (A) and alanine (B) using methionine (C) as the inhibitor of both.

METHODS

Tissue preparation

Female albino rabbits with a body weight of 2500–3000 g were kept with free access to food and water. The rabbits were killed by intravenous injection of pentobarbitone sodium, the abdomen was opened and the most distal 30 cm of ileum or the distal jejunum (70–100 cm from the ileocaecal junction) isolated and excised. The intestinal segments were opened along the mesenteric attachment, rinsed in ice-cold buffer, and cut into halves, which were then cut lengthwise into halves. Hereby tissues were obtained for sixteen measurements from each rabbit.

Chemicals

All solutions were made from a phosphate buffer with a pH of 7·4 and a composition of (mM): Na^+, 140; K^+, 8; Ca^{2+}, 2·6; Mg^{2+}, 1; Cl^-, 140; phosphate, 8; SO_4^{2-}, 1; D-glucose, 5. D-mannitol (80–240 mM) was added to the solutions and replaced by equivalent concentrations of amino acids while maintaining osmolality. Sodium-free solutions were prepared by substituting *N*-methyl-D-glucamine HCl for NaCl. Universally ^{14}C-labelled L-alanine (5·55 GBq mmol^{-1}), L-leucine (11·1 GBq mmol^{-1}), L-lysine (11·1 GBq mmol^{-1}), L-phenylalanine (18·4 GBq mmol^{-1}); and [1,2-^{3}H]-labelled polyethyleneglycol (^{3}[H]PEG) at 37 MBq g^{-1}, mol. wt 4000, were purchased from Du Pont, NEN Research Product, Boston, MA, USA).

Unidirectional influx across the brush-border membrane

Influx across the brush-border membrane (J_{mc}) was measured essentially as described by Schultz, Curran, Chez & Fuisz (1967). Each of the four pieces of intestine was mounted on a lucite plate with the serosal surface resting on moist filter paper, and the mucosal surface facing upwards. A lucite block was clamped on top of the plate exposing four mucosal areas of 0·62 cm^2 in the bottom of wells, where the solutions were oxygenated and stirred by high rates of oxygen flow.

The tissues were preincubated for 20 min with an amino acid-free solution of the same ionic composition as the test solution. This solution was withdrawn and adherent solution gently sucked up with soft paper before injection of the test solution. The incubation period of 0·5 min at 37 °C was terminated by aspiration of the test solution and flushing of the well with an ice-cold 300 mM mannitol solution. The exposed tissues were then punched out, briefly rinsed in ice-cold mannitol solution, blotted on hard filter paper, and extracted for 18 h in 0·1 M HNO_3. The extract and the incubation fluid were analysed in a liquid scintillation counter (TRI-CARB 2200 CA, Packard, Downers Grove, IL, USA). The content of ^{3}[H]PEG in the tissue extract was used to correct for extracellular contamination, and thus corrected the content of ^{14}C-labelled substrate was used to calculate the rate of amino acid influx across the brush-border membrane.

For each of the amino acids studied it was assumed that J_{mc} could be described as:

$$J_{mc} = \frac{J_{max}[A]_m}{K_{½} + [A]_m + [I]\,K_{½}/K_i} + P\,[A]_m. \quad (1)$$

where P is the diffusive permeability of A in centimetres per hour, J_{mc} is given in micromoles per square centimetre serosal area per hour, J_{max} is the maximal influx, K_i is the inhibitor constant and $K_{½}$ is the concentration required for half-maximal transport.

RESULTS

Distal ileum

Apparent affinity constants for leucine and alanine

Taking the lysine-resistant, sodium-independent transport as a measure of the diffusive contribution to transport, previously reported data correspond to $K_{½}$ values of

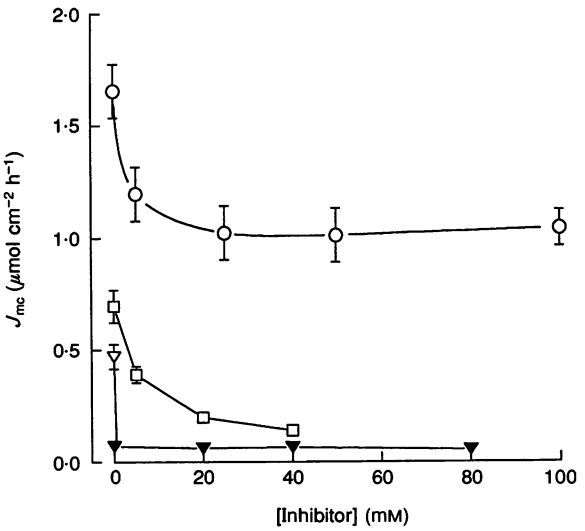

Figure 1. Interaction between lysine and phenylalanine in rabbit ileum
The unidirectional influx (J_{mc}^{Phe}) of phenylalanine (1 mM) was measured (i) at 140 mM sodium with 0, 5, 25, 50 or 100 mM lysine (○), (ii) at 0 mM sodium with 0 mM lysine (▽), and (iii) at 0 mM sodium with 100 mM lysine plus 0, 20, 40 or 80 mM phenylalanine (▼). J_{mc}^{Lys} was measured at 1 mM lysine and 140 mM sodium with 0, 5, 20 or 40 mM phenylalanine (□). Results are means ± S.E.M. of 7–8 observations. The kinetic estimates are given in Table 1.

1·1 ± 0·1 mM ($n = 4$) for leucine and 12·6 ± 1·1 mM ($n = 3$) for alanine for their sodium-dependent, lysine-resistant transport (Munck, 1985*a*). These values are shown in Table 1 together with the estimates for $K_{½}$ and K_i obtained in the present experiments.

Interaction between lysine and phenylalanine

We have previously demonstrated that lysine can exclude neutral amino acids from transport by the β-alanine carrier and from a sodium-dependent as well as a sodium-independent carrier of lysine (Munck, 1985*a*, *b*). For the analysis of phenylalanine transport it is, therefore, necessary to know whether a fraction of it can similarly be inhibited by lysine.

The unidirectional influx of phenylalanine (J_{mc}^{Phe}) was measured at 1 mM phenylalanine at 140 mM NaCl with 0–100 mM lysine, at 0 mM sodium with 0 or 100 mM lysine, and at 0 mM with 100 mM lysine and 0–80 mM phenylalanine. Unidirectional influx of lysine (J_{mc}^{Lys}) was measured at 1 mM lysine at 140 mM NaCl in the presence of 0–40 mM phenylalanine (Fig. 1). Lysine is a partial inhibitor of phenylalanine transport, as previously observed for lysine inhibition of alanine, glycine and leucine transport (Munck, 1985*a*). The data of Fig. 1 indicate that the maximum effect of lysine is already reached at 25 mM. The degree of inhibition attained by 5 mM lysine corresponds to a K_i of 1·8 ± 0·6 mM ($n = 6$). It is also demonstrated that 0·07 μmol cm^{-2} h^{-1} of the total transport of lysine is resistant to inhibition by phenylalanine; assuming this to be the diffusion contribution, the estimate of K_i^{Phe} against the transport of lysine is the same for all three concentrations, 2·6 ± 0·04 mM ($n = 3$). The data of Fig. 1 demonstrate that a sodium-dependent as well as a sodium-independent fraction of phenylalanine transport is inhibited by lysine and that all saturable sodium-independent transport of phenylalanine is inhibited by lysine, as previously shown for alanine and leucine (Munck, 1985*a*).

These results demonstrate a relationship between the transport of phenylalanine and lysine closely resembling that observed for lysine, alanine and leucine (Munck, 1985*a*). This demonstrates the necessity of examining the transport of phenylalanine under maximum inhibition by lysine. In the following kinetic analyses of the interactions of alanine and leucine with phenylalanine the lysine- and phenylalanine-resistant, sodium-independent transport of phenylalanine will be assumed to represent a diffusive contribution to the transport of this amino acid.

Kinetics of the sodium-dependent, lysine-resistant J_{mc}^{Phe}

The unidirectional influx of phenylalanine (J_{mc}^{Phe}) was measured at 1 mM phenylalanine, 140 mM NaCl and 100 mM lysine with 0–80 mM phenylalanine. Assuming a diffusive contribution of 0·07 μmol cm^{-2} h^{-1}, the results (Fig. 2) correspond to a $K_i = K_{½}$ of 3·1 ± 0·2 mM ($n = 7$). With this $K_{½}$ the influx at 1 mM phenylalanine corresponds to a J_{max}^{Phe} of 3·6 ± 0·3 μmol cm^{-2} h^{-1}.

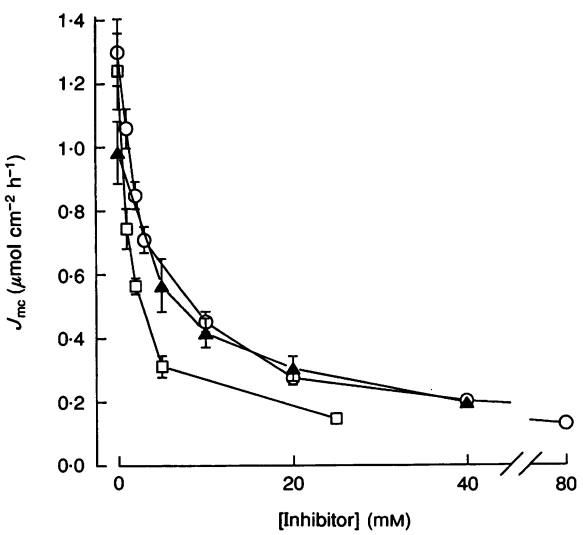

Figure 2. Interaction between lysine-resistant fluxes of leucine and phenylalanine in rabbit ileum

The unidirectional influx of phenylalanine (1 mM) was measured at 140 mM sodium and 100 mM lysine with 0, 1, 2, 10, 20, 40 or 80 mM phenylalanine present (○). Fitting of these data to eqn (1) by non-linear regression (± S.D.) gives $K_{½}^{Phe} = 3·8 \pm 0·7$ mM, $J_{max}^{Phe} = 5·5 \pm 0·6$ μmol cm^{-2} h^{-1} and $P = 0·07 \pm 0·01$ cm h^{-1} ($\chi^2 = 0·670$, $P = 0·98$). In the presence of 140 mM sodium, 100 mM lysine and 1 mM substrate J_{mc}^{Phe} (□) was measured with 0, 1, 2, 5 or 25 mM leucine and J_{mc}^{Leu} was measured with 0, 5, 10, 20 or 40 mM phenylalanine (▲). The results are means ± S.E.M. of 7–8 observations. The kinetic estimates are given in Table 1.

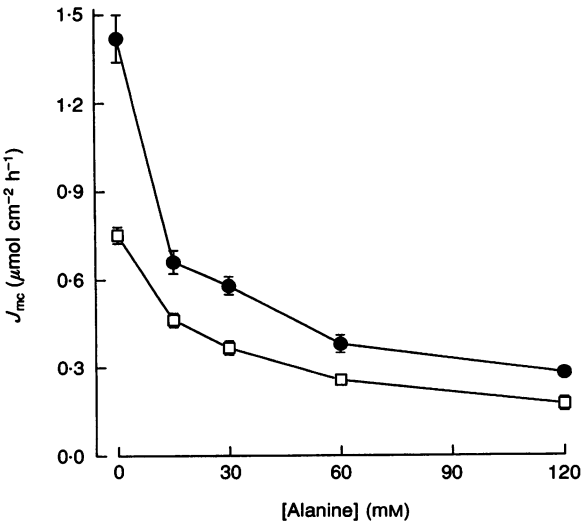

Figure 3. Alanine inhibition of lysine-resistant J_{mc}^{Leu} and J_{mc}^{Phe} in rabbit ileum
The unidirectional influxes of leucine (J_{mc}^{Leu}, □) and phenylalanine (J_{mc}^{Phe}, ●) were measured at 1 mM with 100 mM lysine and 0, 15, 30, 60 or 120 mM alanine present. The results are means ± S.E.M. of 7–8 observations. The kinetic estimates are given in Table 1.

Mutual inhibition between phenylalanine and leucine

The unidirectional influx of phenylalanine was measured at 1 mM phenylalanine and 100 mM lysine with 0–25 mM leucine (Fig. 2). Using the data from Fig. 1 and the estimate of $K_{½}^{Phe}$, these results correspond to a K_{i}^{Leu} against J_{mc}^{Phe} of 1·1 ± 0·1 mM ($n = 4$) (Table 1), a value identical to the previously determined $K_{½}$ for leucine (Munck, 1985*a*).

The unidirectional influx of leucine (J_{mc}^{Leu}) was measured at 1 mM leucine and 100 mM lysine with 0–40 mM

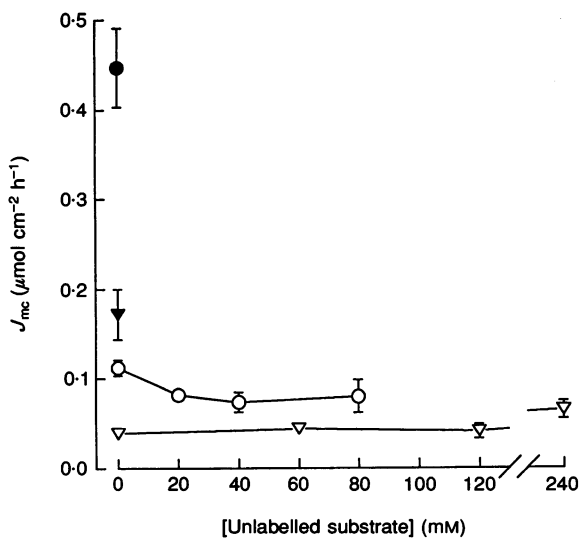

Figure 4. Sodium-independent transport of alanine and phenylalanine in rabbit jejunum
The unidirectional influx was determined in paired experiments at 0 mM sodium after preincubation at 0 mM sodium. J_{mc}^{Phe} at 1 mM phenylalanine was measured in the presence of 100 mM D-mannitol (●) or 100 mM lysine and 0, 20, 40 and 80 mM phenylalanine (○). J_{mc}^{Ala} at 1 mM alanine was measured in the presence of 100 mM D-mannitol (▼) or 100 mM lysine and 0, 60, 120 and 240 mM alanine (▽). Results are means ± S.E.M. of 5 observations. For alanine the diffusive contribution is assumed to be described by the average, 0·05 μmol cm^{-2} h^{-1}, of the data measured at 100 mM lysine, for phenylalanine by the average, 0·09 μmol cm^{-2} h^{-1}, of the data at 100 mM lysine and 20, 40 and 80 mM phenylalanine.

Table 1. A (Phe)–B (Leu)–C (Ala) test of phenylalanine influx in rabbit distal ileum

		K_i ($Y \rightarrow X$) (mM)		
	$X \rightarrow$	Phe	Leu	Ala
Y	Phe	3·1 ± 0·2 (7)	3·1 ± 0·2 (4)	—
↓	Leu	1·1 ± 0·1 (4)	1·1 ± 0·1 (4)*	—
	Ala	13·1 ± 1·8 (4)	11·0 ± 0·4 (4)*	12·6 ± 1·1 (3)*
	Lys	1·8 ± 0·6 (6)	1·8 ± 0·8 (6)*	5·0 ± 3·1 (3)*

The apparent affinity and inhibitory constants for sodium-dependent, lysine-resistant transport of phenylalanine, leucine and alanine, and of lysine in rabbit distal ileum calculated from Figs 1–3 and from Munck (1985*a*) (values marked by asterisks). The values are means ± S.E.M. of the number of inhibitory concentrations tested (n, in parentheses).

phenylalanine (Fig. 2). Assuming a diffusive contribution of 0·07 μmol cm^{-2} h^{-1} and using a $K_{½}^{Leu}$ of 1·1 mM, the results of this experiment correspond to a K_i for phenylalanine against the transport of leucine of 3·1 ± 0·2 mM ($n = 4$), an estimate not significantly different from the estimate of $K_{½}$ for the sodium-dependent, lysine-resistant transport of phenylalanine (Table 1).

Alanine inhibition of J_{mc}^{Leu} and J_{mc}^{Phe}

The unidirectional influxes of leucine and phenylalanine were measured at 1 mM of the amino acids with 100 mM lysine and 0–120 mM alanine (Fig. 3). Using 0·07 μmol cm^{-2} h^{-1} as a measure of the diffusive contribution to these fluxes, 1·1 mM as the $K_{½}^{Leu}$ and 3·1 mM as the $K_{½}^{Phe}$, the data correspond to a K_i^{Ala} of 13·1 ± 1·8 mM ($n = 4$) against J_{mc}^{Phe} and a K_i^{Ala} of 11·1 ± 0·4 mM ($n = 4$) against J_{mc}^{Leu} (Table 1). These estimates do not differ significantly, nor are they significantly different from the previous estimate of 12·6 ± 1·1 mM ($n = 3$) for $K_{½}^{Ala}$.

Together the data of Figs 2 and 3 fulfil the criteria of the A–B–C test (Scriver & Wilson, 1964) demonstrating that one, and only one, carrier is responsible for the sodium-dependent, lysine-resistant transport of the neutral amino acids alanine, leucine and phenylalanine. In addition, the data of Fig. 1 demonstrate that phenylalanine shares a sodium-independent as well as a sodium-dependent carrier of lysine with alanine, glycine, leucine and phenylalanine.

Jejunum

We have previously demonstrated (Munck & Munck, 1992*b*) that in the jejunum, as in the distal ileum (Munck,

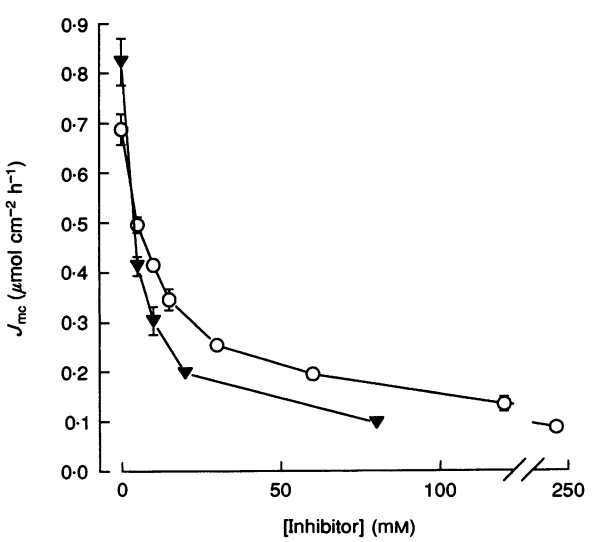

Figure 5. Lysine-resistant alanine transport in rabbit jejunum
The unidirectional influx of alanine (J_{mc}^{Ala}) (1 mM) was measured at 140 mM sodium plus 100 mM lysine and 0–240 mM alanine (▼) or 0–80 mM phenylalanine (○). Fitted to eqn (1) by non-linear regression, the data from alanine self-inhibition (▼) are described by a $K_{½}^{Ala}$ of 21 ± 11 mM, a J_{max}^{Ala} of 11·7 ± 4·1 μmol cm^{-2} h^{-1} and a P value of 0·04 ± 0·02 cm h^{-1} ($\chi^2 = 3·95$, $P = 0·56$). Results are means ± S.E.M. of 4–6 observations. The kinetic estimates are given in Table 2.

1985*a*), lysine at a concentration of 100 mM exerts its maximal inhibitory effect on the transport of neutral amino acids. Transport of alanine and phenylalanine was therefore measured in the presence of 100 mM lysine.

Lysine-resistant, sodium-independent transport of alanine and phenylalanine

After preincubation at 0 mM sodium the influx of alanine (1 mM) and phenylalanine (1 mM) was measured at 0 mM sodium, and at 0 mM sodium with 100 mM lysine in the presence of 0–240 mM alanine and 0–80 mM phenylalanine, respectively. The results from these experiments (Fig. 4) demonstrate a sodium-independent transport of both alanine and phenylalanine which is completely inhibited by 100 mM lysine. This lysine-resistant contribution to the transport of the two amino acids is assumed to represent diffusion and is used as such for estimates of $K_{½}$ and K_i for alanine and phenylalanine.

Transport interactions between alanine and phenylalanine

The unidirectional influx of alanine (J_{mc}^{Ala}) (1 mM) was measured at 140 mM sodium, and 100 mM lysine in the presence of 0–240 mM alanine or 0–80 mM phenylalanine (Fig. 5). Influx of phenylalanine (1 mM) was measured at 140 mM sodium and 100 mM lysine in the presence of 0–80 mM phenylalanine or 0–240 mM alanine (Fig. 6). Using 0·05 cm h^{-1} and 0·09 cm h^{-1} (Fig. 4) as the diffusive permeability (P) of alanine and phenylalanine respectively, the data of Figs 5 and 6 correspond to a $K_{½}^{Ala}$ of 15·6 ± 0·8 mM ($n = 7$), a K_i^{Ala} against transport of phenylalanine of 18·1 ± 0·9 mM ($n = 5$), a $K_{½}^{Phe}$ of 4·7 ± 0·2 mM ($n = 7$), and a K_i^{Phe} against transport of alanine of 4·8 ± 0·2 mM ($n = 4$) (Table 2).

Methionine as inhibitor of the transport of alanine and phenylalanine

Influx of alanine (1 mM) and phenylalanine (1 mM) was measured at 140 mM sodium and 100 mM lysine with 0–80 mM methionine (Fig. 7). Since the data correspond to a K_i^{Met} of approximately 1 mM it is assumed that the rates of transport observed in the presence of 80 mM methionine represent the diffusive contribution to influx of alanine and phenylalanine. With this assumption the data correspond to a K_i^{Met} of 0·8 ± 0·2 mM ($n = 3$) against alanine and 1·1 ± 0·2 mM ($n = 3$) against phenylalanine.

The estimates of the kinetics of alanine and phenylalanine transport, their mutual inhibitory interactions, and their sensitivity to inhibition by methionine are summarized in Table 2. As in the distal ileum these data fulfil the criteria of the A–B–C test for the involvement of only one lysine-resistant and sodium-dependent carrier of α-amino-monocarboxylic acids. These studies of the distal ileum and jejunum used the same amino acids as in the original study which led to the proposal of the existence of a special phenylalanine carrier (Stevens *et al.* 1982). In addition, the results support the conclusion that variation of transport along the rabbit small intestine reflects variation in transport capacity only.

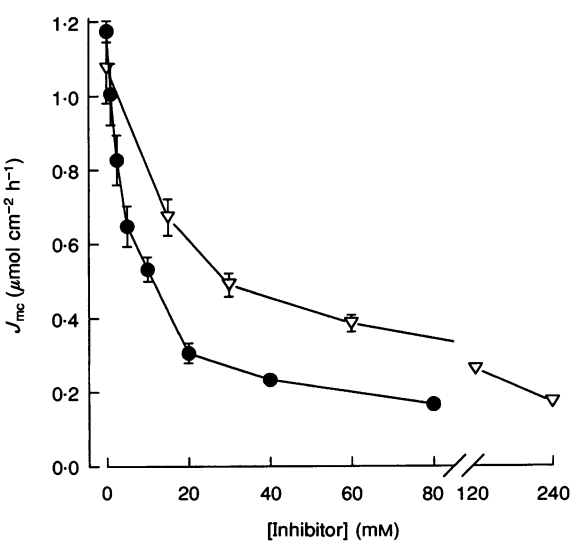

Figure 6. Lysine-resistant phenylalanine transport in rabbit jejunum
The unidirectional influx of phenylalanine (J_{mc}^{Phe}) (1 mM) was measured at 140 mM sodium plus 100 mM lysine with the addition of (i) 0–80 mM phenylalanine (●) (fitting these data to eqn (1) by non-linear regression (± S.D.) gives a K_i^{Phe} of 4·6 ± 0·8 mM, a J_{max}^{Phe} of 6·1 ± 0·7 μmol cm^{-2} h^{-1} and a P value of 0·09 ± 0·01 cm h^{-1} ($\chi^2 = 0·237$, $P = 0·99$)); or (ii) 0–240 mM alanine (▽). Results are means ± S.E.M. of 4–6 observations. The kinetic estimates are given in Table 2.

Table 2. A (Phe)–B (Ala)–C (Met) test of phenylalanine influx in rabbit jejunum

		K_i ($Y \rightarrow X$) (mM)	
	$X \rightarrow$	Phe	Ala
Y	Phe	4·7 ± 0·2 (7)	4·8 ± 0·2 (4)
↓	Ala	18·1 ± 0·9 (5)	15·6 ± 0·8 (7)
	Met	1·1 ± 0·2 (3)	0·8 ± 0·2 (3)

The apparent affinity and inhibitory constants for sodium-dependent, lysine-resistant transport of phenylalanine, alanine and methionine in rabbit jejunum calculated from Figs 5–7. The values are means ± S.E.M. of the number of inhibitory concentrations tested (n, in parentheses).

DISCUSSION

The proposal (Stevens *et al.* 1982) that a separate, sodium-dependent carrier exists for phenylalanine and methionine was based on the observed contrast between a fully competitive inhibition by methionine of sodium-dependent uptake of phenylalanine by rabbit intestinal brush-border membrane vesicles, and on data which suggested only partial competitive inhibition by alanine. However, rather than steadily increasing estimates of K_i with increasing alanine concentration, their data corresponded to a K_i of 5 mM at 5 mM alanine and a K_i of 4 mM at 10 mM alanine; only at 25 and 50 mM alanine did their estimates increase to 13 and 16 mM, respectively. It seems possible that these results could reflect uncertainties introduced with the corrections used for sodium-independent transport of phenylalanine.

The proposal of Stevens *et al.* (1982) is not supported by data from a systematic study of amino acid transport in mouse small intestine (Karasov *et al.* 1986), which demonstrated complete mutual inhibition between leucine and methionine, nor by studies of neutral amino acid transport in rabbit small intestine (Hajjar & Curran, 1970; Preston, Shaeffer & Curran, 1974), which reported values of K_i^{Ala} almost the same against methionine and phenylalanine and not differing much from $K_{\frac{1}{2}}^{Ala}$ itself. In addition, $K_{\frac{1}{2}}$ for phenylalanine did not differ from its K_i against alanine and methionine. It must, however, be noted that these data on transport by intact epithelia were analysed without correction for diffusive contributions and without precautions against the involvement of several transport systems. For guinea-pig, pig and human small intestine the proposal of a separate phenylalanine/methionine carrier has been accepted without further testing (Del Castillo & Muñiz, 1991; Malo, 1991; Maenz & Patience, 1992).

We have shown that, in the rabbit distal jejunum (Munck & Munck, 1992*b*) as well as distal ileum (Munck, 1985*b*), lysine, alanine, glycine and leucine share pathways of sodium-dependent as well as sodium-independent

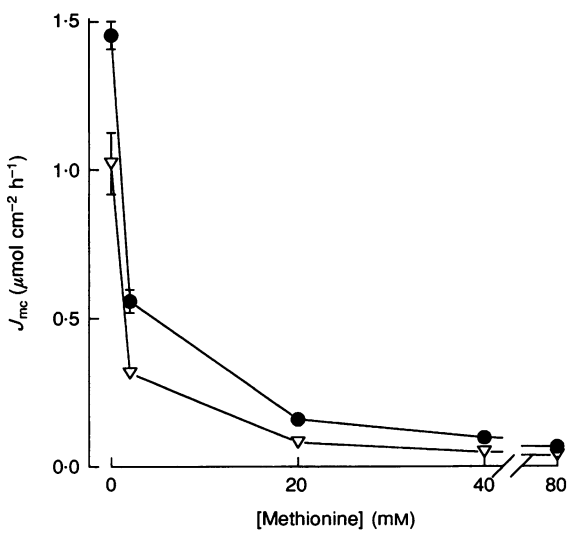

Figure 7. Methionine inhibition of alanine and phenylalanine transport in rabbit jejunum
The unidirectional influxes of 1 mM alanine (▽) and 1 mM phenylalanine (●) were measured at 140 mM sodium and 100 mM lysine with 0–80 mM methionine added. Results are means ± S.E.M. of 4–5 observations. The kinetic estimates are given in Table 2.

transport. In studies based on the use of brush-border membrane vesicles the sodium-dependent carrier of neutral amino acids, the NBB (neutral brush border) system, is defined as lysine resistant. For this reason alone the proposal of a separate carrier of phenylalanine must be examined under conditions of maximum inhibition by lysine. Our results demonstrate that this goal is reached with 100 mM lysine. As shown in Fig. 1 and by previous data on transport of alanine and leucine by the distal ileum (Munck, 1985*a*) and the jejunum (Munck & Munck, 1992*b*), the use of 100 mM lysine has the additional advantage of completely inhibiting the saturable, sodium-independent transport of these amino acids. As a consequence the kinetics derived from the data of Figs 2–3 and 5–7 (which are summarized in Tables 1 and 2) describe transport by the carrier of neutral amino acids, the NBB system. This is the case also for the previously determined kinetics of alanine and leucine transport (Table 1).

In all of the present series of experiments it was seen that the estimates of K_i and $K_{½}$ were invariable over a wide range of inhibitor concentrations and up to 85–94% inhibition. This aspect of the data alone makes it highly improbable that more than one system is involved in the sodium-dependent, lysine-resistant transport of alanine, leucine and phenylalanine. This interpretation is confirmed by the compliance of alanine, leucine and phenylalanine with the criteria of the A–B–C test for the involvement of one and only one common carrier (Tables 1 and 2).

Phenylalanine has been added to the series of α-amino-monocarboxylic acids which share both sodium-dependent and sodium-independent means of transport with lysine. It is not clear whether the lysine inhibited, sodium-dependent carriers of these neutral amino acids are also sodium-dependent carriers of lysine. However, previously reported results (Munck, 1985*b*) strongly indicate that this is the case at least in the distal ileum, where the β-alanine carrier is the best candidate for such a role. By inhibition and cross-inhibition studies the β-alanine carrier has been identified as a high-affinity carrier of alanine, amino-butyric acid, glycine, leucine and lysine (Munck, 1985*b*), and more recently as both a sodium- and chloride-dependent carrier of β-alanine, leucine (Munck & Munck, 1990), lysine and glycine (B. G. Munck & L. K. Munck, unpublished data). In terms of specificity, the β-alanine carrier comes close to the B^{0+} carrier described for mouse blastocytes (Van Winkle, Christensen & Campione, 1985). Since this carrier is reported to be chloride dependent (Van Winkle, 1988), it is an example (so far unique) of close similarity between a non-epithelial amino acid carrier and an amino acid carrier from an intestinal brush-border membrane. However, evidence is accumulating (Stevens *et al.* 1982; Munck, 1984, 1985*a*; Munck & Munck, 1992*b*) for a sodium-independent mutual carrier of α-amino-monocarboxylic acids and cationic amino acids, which indicates that intestinal epithelia possess an equivalent of the b^{0+} carrier described for mouse blastocysts (Van Winkle, Campione & Gorman, 1988). In this situation it may be advantageous to use 'B' as the signature for the sodium-dependent carrier of α-amino-monocarboxylic acids (Stevens, 1992).

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