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Biochimica et Biophysica Acta 1237 (1995) 70–76

BBA Biochimica et Biophysica Acta

Dipeptide transport and hydrolysis in rat small intestine, in vitro

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Received 2 January 1995; accepted 16 March 1995

Abstract

A range of natural and mixed D-/L-stereoisomer phenylalanine dipeptides was used to investigate peptide uptake and hydrolysis by isolated rings of rat jejunum. Characterisation of dipeptide hydrolysis by the brush border fraction revealed apparent K_m values in the 0.1–1.0 mM range which, except for the charged dipeptides, were significantly higher than those for hydrolysis by the cytosolic fraction. Uptake of L-/L- dipeptides into jejunal rings, which was followed by HPLC, was unaffected by the presence of peptidase inhibitors in the incubation medium although the absorbed peptides were completely hydrolysed in the cytosol; comparison of the effects of excess leucine on dipeptide uptake and on the uptake of the two constituent amino acids were also consistent with absorption of intact dipeptide followed by cytosolic hydrolysis. The uptake of hydrolysis-resistant mixed D-/L- dipeptides was also studied and confirmed that peptide uptake preceded hydrolysis; D-alanyl-L-phenylalanine accumulated within the rings to twice the medium concentration.

Keywords: Dipeptide; Membrane transport; Small intestine; Stereospecificity; (Rat)

1. Introduction

Despite convincing evidence that hydrolysis-resistant dipeptides are absorbed intact [1], some recent reports [1,2] have suggested that natural dipeptides may be split in the intestinal lumen prior to absorption. This is a plausible argument because peptidases with a broad specificity are located on the outer face of the apical membrane. However, the cytosolic peptidases also have the capacity to split any of the natural dipeptides that are absorbed intact.

The view that the small intestine has the capacity to absorb intact dipeptides is consistent with observations (e.g. [3]), that 3 h after the administration of a test protein in humans the protein in the intestinal lumen was predominantly in the form of short peptides. Support for the view that peptides can be absorbed prior to hydrolysis [1] has come from the recent report that the intestinal peptide transporter (PepT1) from rabbit small intestine has been cloned, sequenced and expressed in *Xenopus* oocytes [4]. It is also clear that drugs such as the β-lactam antibiotics [5,6] and the angiotensin-converting enzyme inhibitor [7] appear to be absorbed via the peptide transporter indicating that this is a valuable route for the administration of therapeutic compounds. To optimise the use of the peptide transporter for drug delivery it is necessary to understand the interrelationship between the mucosal peptidases and peptide transport. It is also crucially important to determine how the specificity of the transporter compares with that of the peptidases on the brush border and in the cytosol.

The work presented in this paper had three aims. (1) To use a range of phenylalanine dipeptides to characterise the brush border and cytosolic peptidases of rat small intestine. (2) To use rings of rat jejunum and analysis by HPLC to determine whether or not the natural phenylalanine dipeptides, which have not been studied previously, are hydrolysed prior to uptake. (3) To see whether rat jejunal rings could accumulate hyrolysis-resistant mixed D-/L- dipeptides against a concentration gradient. The use of isolated rings of small intestine has enabled us to use HPLC analysis to determine the extent to which the dipeptides were hydrolysed in the incubation medium and within the cytosol during the incubations.

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SSDI 0005-2736(95)00078-X

2. Materials and methods

2.1. Materials

All purchased chemicals were of analytical grade. The reagents used for peptide synthesis were obtained from the Sigma Chemical Co., Dorset, UK. Sodium pentobarbitone was purchased from May and Baker, Dagenham, UK.

2.2. Animals

Male Wistar rats (250 g) were fed ad libitum on rat and mouse diet (Bantin and Kingham, Humberside, UK) until they reached a weight of 280 g. They were anaesthetised by intraperitoneal injection of sodium pentobarbitone (0.1 ml 100 g^{-1} body weight).

2.3. Peptide synthesis

Peptide synthesis was by standard techniques [8]. Each of the dipeptides used in this study was synthesised by condensation of *N*-tertiary-butyl-oxy-carbonyl-derivative of the N-terminal amino acid with the carboxy-benzyl-L-derivative of the C-terminal amino acid. The products were subjected to a series of acid and alkali washes to eliminate starting materials and byproducts before removal of the protecting groups. Finally, the samples were freeze-dried and the purity, which was checked by mass spectrophotometry, NMR and HPLC, was more than 95%.

2.4. Preparation of brush border and cytosolic fractions

The apical and cytosolic peptidase fractions were prepared according to the method of Kessler et al. [9]. Briefly, 1–2 g of mucosa was scraped from an isolated jejunum and placed in 25 ml of buffer (50 mM mannitol, 20 mM Hepes, pH 7.4) per g mucosa. The tissue was dispersed by 20 slow strokes at 10000 rpm with a tight fitting teflon pestle. After adding liquid $CaCl_2$ to a final concentration of 10 mM and stirring the solution for 20 min, the mixture was centrifuged for 15 min at $3000 \times g$ in a Sorvall SS 34 rotor. The resulting supernatant was centrifuged for 30 min at $27000 \times g$ to produce the cytosolic peptidase fraction (supernatant) and the brush border peptidase fraction (pellet). After resuspending the resultant pellet through a 27 gauge needle in 300 mM mannitol, 20 mM Hepes, 0.1 mM $MgSO_4$, pH 7.4, the solution was spun at $27000 \times g$ for 30 min. The pellet was then resuspended as described above.

2.5. Peptidase assay

The protocol for the assay peptidase activity was similar to that described above [9]. A 10 μl aliquot of enzyme fraction was added to 120 μl of reaction medium (pH 7.4) containing 10 mM NaCl, 80 mM NaH_2PO_4, 200 mM mannitol plus the substrate at the desired concentration. The incubation time used (15–60 s) was chosen to ensure that less than 20% of the added peptide was hydrolysed at each substrate concentration. The reaction was terminated by adding an equal aliquot of 6% perchloric acid and frozen in liquid nitrogen. After thawing, the sample was neutralised with 0.6 M KOH and assayed for the concentration of free phenylalanine (Phe) by HPLC.

2.6. Preparation and incubation of tissue rings from rat jejunum

Approximately 20 tissue rings were prepared as previously described [10] and incubated in 10 ml bicarbonate Krebs-Ringer solution (120 mM NaCl, 4.5 mM KCl, 1 mM $MgSO_4$, 1.8 mM Na_2PO_4, 1.25 mM $CaCl_2$ and 25 mM $NaHCO_3$) which was previously gassed at 37.5° C with 95% O_2/5% CO_2 to give a pH of 7.42. The medium continued to be gassed vigorously throughout the experiment. Except where noted, the transport substrate was present in the incubation medium at a final concentration of 0.5 mM. At 4 min intervals two rings were removed from the incubation medium and placed immediately on a Buchner funnel, and washed four times with 5 ml of ice-cold bicarbonate Krebs-Ringer. After blotting on filter paper, the rings were rapidly frozen in liquid nitrogen for subsequent analysis. An estimate of substrate carry-over was made by placing two rings in the incubation medium and then immediately putting them through the washing and freezing procedure mentioned above.

2.7. Preparation of tissue rings for the measurement of solute uptake by HPLC

Frozen rings were weighed and placed in 0.5 ml 6% perchloric acid. The samples were homogenised, deproteinised and desalted as previously described [10]. The samples were analysed by HPLC as described below. An estimate of the wet/dry ratio was obtained by placing four weighed rings in an oven at 110° C for 24 h and reweighing.

2.8. HPLC analysis

The deproteinised, neutralised samples of the perfusate, the serosal secretions and the mucosal tissue samples were analysed for dipeptide and free Phe by isocratic HPLC at 210 nm on a 5 μm ODS C18 column. The mobile phase was 20% methanol/80% 21 mM KH_2PO_4 (pH 5).

2.9. Assay for protein and alkaline phosphatase

The concentration of protein was determined using the BCA protein assay kit (Pierce and Warriner, Warrington, UK) with the automated Cobas Mira analyzer. The purity of the brush border peptidase fraction was established using an alkaline phosphatase assay kit (Roche, Welwyn Garden City, UK) and the automated Cobas Mira analyser.

2.10. Expression of results

All results are expressed as mean ± standard error of the mean (S.E.); n = the number of experiments. Statistical comparisons were made by covariance anaylsis or Student's t-test.

3. Results

3.1. Kinetic parameters of brush border and cytosolic peptidases

The method of Kessler et al. [9] which was used to prepare the cytosolic and brush border fractions resulted in a 10-fold enrichment of the alkaline phosphatase activity in the brush border fraction. Table 1 shows the kinetic parameters for six naturally occurring dipeptides as determined over a concentration range of 0.015 to 4.0 mM. The apparent K_m and V_{max} values were obtained from the Michaelis-Menten plots using a Marquardt least squares curve fitting programme. From the data in Table 1, a number of points are clear. First, with the exception of L-glutamyl-L-phenylalanine (L-Glu-L-Phe) all the brush border peptidases had significantly higher apparent K_m values than those in the cytosolic fraction ($P < 0.05$). Secondly, except for L-Glu-L-Phe, the cytosolic peptidases had significantly higher V_{max} values ($P < 0.05$), suggesting that the bulk of the intestinal peptidase activity was in the cytosol. This was tested for L-methionyl-L-phenylalanine (L-Met-L-Phe) in recovery experiments which showed that more than 80% of the total peptidase activity of the homogenate was in the cytosolic fraction and less than 10% in the brush border fraction. Finally, a comparison of the hydrolysis of L-Met-L-Phe with that of L-phenylalanyl-L-methionine (L-Phe-L-Met), and of L-leucyl-L-phenylalanine (L-Leu-L-Phe) with that of L-phenylalanyl-L-leucine (L-Phe-L-Leu) revealed that for both pairs of dipeptides there were significant differences in the K_m values in the two fractions.

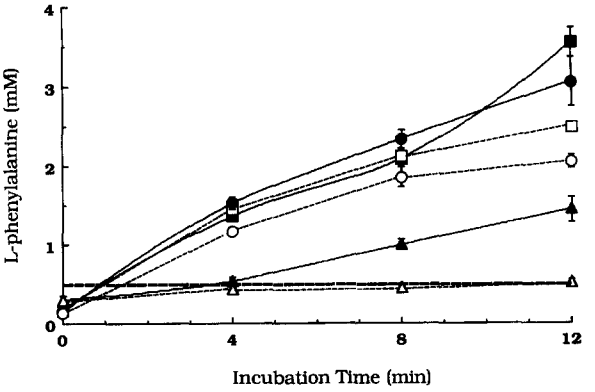

Fig. 1. Time course of L-phenylalanine accumulation in rings of rat jejunum incubated with dipeptides. L-Phe concentration in rings incubated with 0.5 mM L-Leu-L-Phe: ●, control; ○, +10 mM L-Leu; rings incubated with 0.5 mM L-Lys-L-Phe: ■, control; □, +10 mM L-Leu; rings incubated with 0.5 mM L-Glu-L-Phe: ▲, control; △, +10 mM L-Leu. Dashed line shows the medium dipeptide concentration. Values are means ± S.E. ($n = 4$).

3.2. Time course of phenylalanine appearance in rings incubated with dipeptides

In view of the variations in apparent K_m of the brush border peptidases for the neutral, positively charged and negatively charged phenylalanine dipeptides we studied the dipeptide uptake of 0.5 mM L-Leu-L-Phe, L-lysyl-L-phenylalanine (L-Lys-L-Phe) and L-Glu-L-Phe into isolated rings. The HPLC traces showed that the dipeptides were completely hydrolysed by the cytosolic peptidases since no intact dipeptide could be detected in the rings. However, the time course of Phe accumulation in Fig. 1 gives a measure of the dipeptide uptake and shows that there is no significant difference between Phe uptake from L-Leu-L-Phe and L-Lys-L-Phe, but that Phe uptake was significantly less from L-Glu-L-Phe ($P < 0.005$). The Phe accumulation from the three dipeptides appears to be consistent with the differences in the kinetic parameters of the cytosolic peptidases, since L-Glu-L-Phe, which has a lower Phe accumula-

Table 1
Kinetic characteristics of the hydrolysis of a group of phenylalanine dipeptides by brush border and cytosolic fractions from the mucosal layer of rat jejunum

Dipeptide	Brush border fraction		Cytosolic fraction	
	Apparent K_m (mM)	V_{max} (μmol/min per mg protein)	Apparent K_m (mM)	V_{max} (μmol/min per mg protein)
L-Met-L-Phe	0.103 ± 0.010	0.328 ± 0.073	0.031 ± 0.006	1.85 ±0.02
L-Phe-L-Met	0.291 ± 0.028	0.553 ± 0.175	0.046 ± 0.002	1.36 ±0.26
L-Leu-L-Phe	0.279 ± 0.032	0.256 ± 0.036	0.048 ± 0.009	1.33 ±0.06
L-Phe-L-Leu	0.095 ± 0.013	0.285 ± 0.026	0.063 ± 0.003	2.82 ±0.23
L-Glu-L-Phe	0.66 ±0.021	0.58 ±0.032	0.79 ±0.19	0.057 ± 0.001
L-Lys-L-Phe	0.71 ±0.098	0.36 ±0.014	0.312 ± 0.030	7.59 ±0.19

The data were obtained from measurements of the rate of Phe appearance in incubations of the two fractions with each of the dipeptides over a concentration range from 0.015 to 4 mM. The apparent K_m and V_{max} values were obtained from Michaelis-Menten plots using a Marquardt least squares curve fitting program. Values are means ± S.E. for for four determinations.

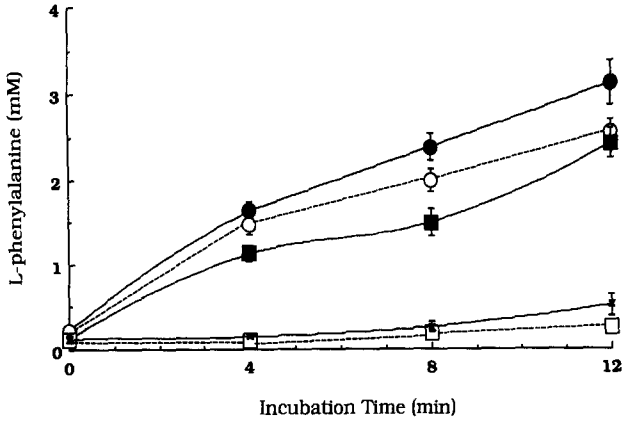

Fig. 2. Accumulation of L-phenylalanine in rings of rat jejunum incubated with L-methionyl-L-phenylalanine or a mixture of L-methionine plus L-phenylalanine. L-Phe concentration in rings incubated with 0.5 mM L-Met-L-Phe: ●, control; ○, +10 mM L-Leu. L-Phe concentration in rings incubated with 0.5 mM L-Met +0.5 mM L-Phe: ■, control; □, +10 mM L-Leu. L-Phe concentration in rings incubated with 0.15 mM Phe, X. Values are means ± S.E. ($n = 4$).

tion, has a peptidase with a higher apparent K_m and a lower V_{max}. The apparent K_m for L-Lys-L-Phe was also significantly higher than that for L-Leu-L-Phe, but this appears to have been compensated for by the high level of peptidase activity in this case. We have also found that the time course of Phe accumulation from L-Phe-L-Leu (data not shown) was identical with that of L-Leu-L-Phe, despite significant differences in the apparent K_m values in the brush border fraction.

In order to assess the importance of hydrolysis prior to peptide absorption, the incubations shown in Fig. 1 were repeated in the presence of 10 mM leucine (Leu). The addition of a high concentration of another neutral amino acid effectively prevents the uptake of Phe at concentrations of 0.5 mM and below (as shown in Fig. 2). The addition of 10 mM Leu did cause a small (27%) but significant ($P < 0.005$) reduction in Phe accumulation from L-Leu-L-Phe, but it completely blocked Phe accumulation form L-Glu-L-Phe, suggesting that the Phe in this case may have been absorbed after hydrolysis. Although the reduction of Phe accumulation in the rings incubated with L-Leu-L-Phe may indicate that even with this peptide some Phe may be absorbed following hydrolysis, it is worth noting that leucine has been shown to inhibit cytosolic peptidases [11], and we have confirmed this effect.

3.3. Comparison of the time course of phenylalanine appearance from Met-Phe with that from methionine plus phenylalanine

Another approach to the relationship between peptide hydrolysis and uptake is provided by the results reported in Fig. 2. The accumulation of Phe in rings incubated with 0.5 mM L-Met-L-Phe is compared with that from a mixture of 0.5 mM L-Met plus 0.5 mM L-Phe. The accumulation of Phe increased steadily in incubations with the mixture of two free amino acids, and the values obtained were significantly less ($P < 0.005$) than those obtained in the rings incubated with L-Met-L-Phe. However radically different results were found in the presence of 10 mM Leu; uptake of the pair of amino acids was inhibited by more than 90% whereas that from the dipeptide was only 17% ($P < 0.005$). The Phe concentration of the incubation medium after incubation with 0.5 mM L-Met-L-Phe for 12 min was found to be 0.074 ± 0.003 [7], and Fig. 2 also shows the time course of Phe accumulation by rings incubated with L-Phe at twice this level (0.15 mM) was very slow. As pointed out above for the dipeptides of Leu + Phe, incubation of rings with L-Phe-L-Met produced values of Phe accumulation which were not significantly different from those with L-Met-L-Phe (data not shown).

3.4. The effects of brush border peptidase inhibitors on phenylalanine appearance in rings incubated with dipeptides

Amastatin and bestatin have been shown to be potent peptidase inhibitors [12], and when we added them at final concentrations of 30 μM and 60 μM respectively to our brush border fraction the rate of hydrolysis of L-Met-L-Phe was reduced to only 3% of control levels. Fig. 3 shows the effects of the same concentrations of these two inhibitors on Phe accumulation by rings incubated for 8 min with the four dipeptides from Figs. 1 and 2 at a concentration of 0.5 mM. The brush border peptidase inhibitors did not significantly reduce Phe accumulation from any of the four dipeptides.

3.5. Accumulation of Phe and dipeptide in rings incubated with D-Ala-L-Phe

Mixed D-/L- dipeptides have been shown to be relatively resistant to hydrolysis [12,13] and we have synthe-

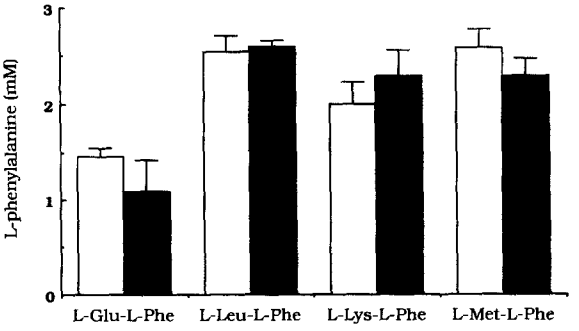

Fig. 3. The influence of brush border peptidase inhibitors on the accumulation of L-phenylalanine in rings of rat jejunum incubated with 0.5 mM dipeptides. L-Phe concentration after incubation for 8 min: □, control; ■, +30 μM amastatin and 60 μM bestatin. Values are means ± S.E. ($n = 4$).

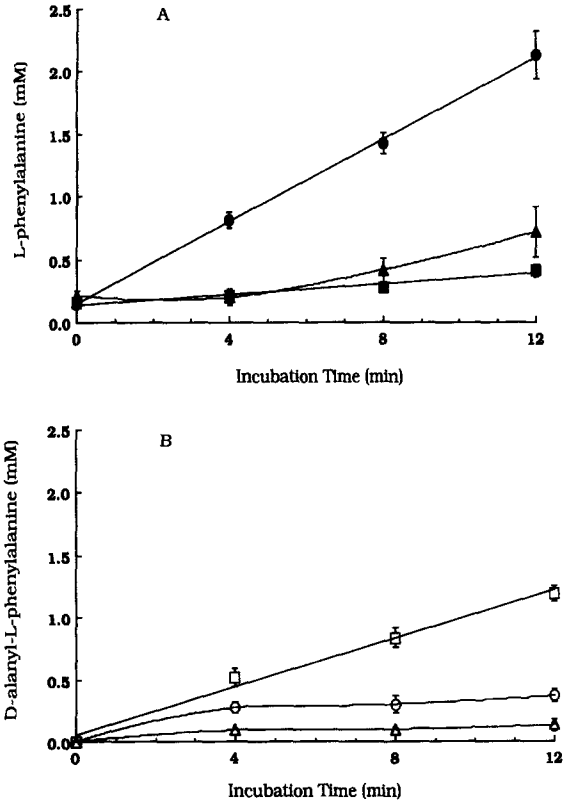

Fig. 4. Transport and hydrolysis of D-alanyl-L-phenylalanine in rings of rat jejunum. Rings incubated with 0.5 mM D-Ala-L-Phe. (A) L-phenylalanine concentration: ●, control; ■, +10 mM L-Leu; ▲, +10 mM Gly-L-Pro. (B) D-Ala-L-Phe concentration: ○, control; □, +10 mM L-Leu; △, +10 mM Gly-L-Pro. Values are means ± S.E. ($n = 4$).

sised D-Ala-L-Phe in order to study its uptake and hydrolysis. Because of the limited solubility of this dipeptide it was difficult to obtain an accurate measure of the apparent K_m and V_{max} for its hydrolysis by the cytosolic fraction; but the apparent K_m was approximately 5 mM and the V_{max} was less than 10% of that shown in Table 1 for the natural dipeptides. Fig. 4 shows the results obtained when 0.5 mM D-Ala-L-Phe was incubated with rings. Although the cytosolic peptidase activity is low, Phe accumulated to about 4-times the medium substrate concentration by the end of the 12 min incubation. Furthermore. we were able to detect significant amounts of the intact peptide in the rings during the incubation (Fig. 4B). Fig. 4 also shows the effects of duplicate sets of incubations in which either 10 mM glycyl-L-proline (Gly-L-Pro) or 10 mM Leu were present in addition to 0.5 mM D-Ala-L-Phe. The Gly-L-Pro inhibited the accumulation of both Phe and the dipeptide, indicating that both were entering the rings via the peptide transporter. The fact that 10 mM Leu inhibited the accumulation of Phe suggested that it was inhibiting hydrolysis of the mixed dipeptide in the cytosol, and this was confirmed by the finding that in the presence of Leu the dipeptide accumulated to more than twice the medium concentration.

3.6. Accumulation of D-Phe and dipeptide in rings incubated with L-Ala-D-Phe

We also studied uptake and hydrolysis of the complementary mixed dipeptide L-Ala-D-Phe, since we have shown in other studies [14] that the rat small intestine does not concentrate D-Phe. Fig. 5 shows the results obtained when rings were incubated with 0.5 mM L-Ala-D-Phe in the absence and presence of 10 mM Gly-L-Pro or 10 mM Leu. In our HPLC analyses we could not distinguish between D-Phe and L-Phe, but because no L-Phe was present in our incubations with L-Ala-D-Phe we have assumed that the increase in Phe that we observed in these experiments was due to D-Phe. The zero time values in Figs. 1, 2 and 4 show that the rings initially contained L-Phe and in Fig. 5A these initial values have been subtracted from the data obtained at 4, 8 and 12 min. The controls in Fig. 5A show that D-Phe was accumulated to more than 2-times the concentration of the dipeptide in the medium, and Fig. 5B shows that some intact dipeptide could also be detected in the rings. Both the accumulation

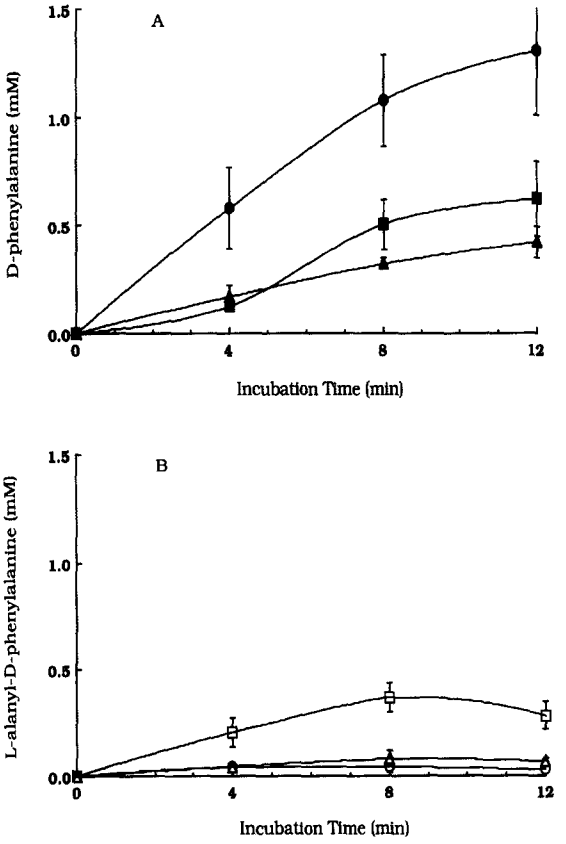

Fig. 5. Transport and hydrolysis of L-alanyl-D-phenylalanine in rings of rat jejunum. Rings incubated with 0.5 mM L-Ala-D-Phe. (A) D-phenylalanine concentration: ●, control; ■, +10 mM L-Leu; ▲, +10 mM Gly-L-Pro. (B) L-Ala-D-Phe concentration: ○, control; □, +10 mM L-Leu; △, +10 mM Gly-L-Pro. Values are means ± S.E. ($n = 4$).

of the D-Phe and the dipeptide were inhibited by Gly-L-Pro, indicating that uptake was by the peptide transporter. In the presence of 10 mM Leu there was a marked increase in the content of intact dipeptide and more or less complete inhibition of D-Phe accumulation, showing again that hydrolysis of the mixed dipeptide was inhibited by excess leucine.

4. Discussion

In this paper we report results of the first study of the hydrolysis and absorption of a range of charged and neutral phenylalanine dipeptides. By using rings of intact intestine and employing HPLC, we were able to determine the extent of dipeptide hydrolysis in the medium and the cytosol during the uptake studies. Although it would have been interesting to complement our work by investigating transport across the apical membrane of the enterocytes using brush-border membrane vesicles, this was not possible because the phenylalanine dipeptides were not available in labelled form. It should also be pointed out that membrane vesicles are not suitable for an investigation of the relationship between peptide transport and hydrolysis.

Our comparison of the characteristics of the peptidase activity in the brush border and cytosolic fractions demonstrates that the apparent K_m of peptidases in the brush border fraction was in the millimolar range and for four of the six natural peptides the apparent K_m was an order of magnitude higher than that in the cytoplasm. As a consequence, low concentrations of peptides will tend to escape hydrolysis in the lumen, but once absorbed they will be split in the cytosol creating a zero-trans configuration for peptide uptake. However the data in Table 1 show that this was not the case for the two charged dipeptides, for which the apparent K_m of the cytosolic peptidase was comparable to that on the brush border. Furthermore, the V_{max} of the cytosolic fraction for the negatively charged L-Glu-L-Phe was an order of magnitude lower than for the other dipeptides tested.

The anomalous kinetic characteristics of the L-Glu-L-Phe peptidases were reflected in the differences between the accumulation of Phe in rings incubated with 0.5 mM L-Glu-L-Phe and the other dipeptides. Fig. 3 shows that Phe accumulation from L-Leu-L-Phe, L-Met-L-Phe, L-Lys-L-Phe and L-Glu-L-Phe was unaffected by the presence of the inhibitors amastatin and bestatin, indicating that hydrolysis by the brush border peptidases was not important for Phe uptake. Although it may be a consequence of the negative charge on the peptide, the lower levels of Phe accumulation from L-Glu-L-Phe is consistent with its low rate of hydrolysis by the cytosolic fraction despite the fact that we were unable to detect intact L-Glu-L-Phe in the rings. Phe accumulation from L-Glu-L-Phe was also much more strongly affected by the addition of 10 mM Leu than was the case with the other dipeptides. In view of the lack of inhibition by amastatin and bestatin, the leucine appears to be acting as an inhibitor of the cytosolic peptidases [12,13], an effect which is much more severe for L-Glu-L-Phe where the peptidase activity is low. It is worth noting that in our peptide uptake studies with rings no pH gradient was imposed, since the medium pH was maintained at 7.4. The fact that our studies with peptidase inhibitors indicate that L-Glu-L-Phe is absorbed prior to hydrolysis despite the high capacity of the brush-border peptidase relative to that in the cytosol is in agreement with data obtained by Minami et al. [15] for the absorption of glutamine dipeptides by human intestine.

Further evidence to support the view that the natural dipeptides are absorbed prior to hydrolysis is provided by Fig. 2, which shows that Phe accumulation from 0.5 mM L-Met-L-Phe was only slightly affected by the presence of 10 mM Leu, whereas that from 0.5 mM Met plus 0.5 mM Phe was virtually abolished.

Hydrolysis-resistant peptides such as Gly-L-Pro have been used to study peptide uptake [16,17] in the past and we attempted to follow the same approach by synthesising mixed D-/L- dipeptides which have been shown to be relatively resistant to hydrolysis [14,18]. The results obtained in Figs. 4 and 5 with D-Ala-L-Phe and L-Ala-D-Phe show clearly that both of these dipeptides are hydrolysed to some extent, although in both cases we were able to detect intact dipeptide in the rings during the 12 min incubations. The inhibitory effects of Gly-L-Pro show that the Ala-Phe mixed dipeptides are using the same transporter, and the increase in dipeptide content of the rings in the presence of 10 mM Leu indicates that the amino acid is inhibiting hydrolysis of the mixed dipeptides by the cytosolic peptidases.

The fact that D-Ala-L-Phe accumulated to more than 4-times the medium concentration in the presence of leucine (Fig. 4B) shows that the intestinal mucosa is actively transporting this mixed dipeptide. The data in Fig. 5A demonstrates that by incorporating D-Phe into a dipeptide the intestine is capable of concentrating this amino acid to more than 2-times the medium level, despite the fact that D-Phe itself is not accumulated against a concentration gradient [14]. This observation has clear implications for the absorption of chemotherapeutic agents.

The data we have presented in this paper show: (1) That for neutral phenylalanine dipeptides the brush border peptidases of rat small intestine have a much higher apparent K_m and lower V_{max} than those in the cytosolic fraction; significant differences in apparent K_m were not observed for the charged dipeptides. (2) The natural phenylalanine dipeptides are not hydrolysed prior to uptake into rings of rat jejunum. (3) Rat jejunal rings can accumulate D-Ala-L-Phe against a concentration gradient. (4) By incorporating D-Phe into L-Ala-D-Phe it is possible to accumulate passively transported D-Phe against a concentration gradient in intestinal rings.

Acknowledgements

We are grateful to The Wellcome Trust for support and to the Medical Research Council for providing a studentship for A.P. Sykes.

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