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CHARACTERISTICS OF DIPEPTIDE TRANSPORT IN NORMAL AND PAPAIN-TREATED BRUSH BORDER MEMBRANE VESICLES FROM MOUSE INTESTINE

I. UPTAKE OF GLYCYL-L-PHENYLALANINE

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Papain treatment of isolated brush border membrane vesicles was carried out to study peptide transport in the absence of hydrolytic events associated with the brush border membrane. Such a treatment allowed a 70% decrease in the activity of membrane-associated oligopeptidases and the study of peptide transport in the complete absence of free amino acids up to 1 min of incubation. A comparison between the time course curves of glycyl-L-phenylalanine uptake by normal and papain-treated vesicles showed that the overshoots seen in the presence of Na^+ and K^+ gradients (extravesicular > intravesicular) when using normal vesicles were no longer evident after papain treatment. This result, together with the demonstration of uptake into an osmotically reactive intravesicular space and the analysis of uptake of free phenylalanine, allowed the conclusion that peptide transport was the result of two complementary mechanisms, uptake of free amino acids following hydrolysis by the membrane-bound oligopeptidases, and intact peptide transport down a concentration gradient by a non-Na^+ (and non-K^+)-dependent process. These results also showed the non-involvement of γ-glutamyltransferase and the γ-glutamyl cycle in peptide absorption. A linear relationship has been established between initial dipeptide uptake and glycyl-L-phenylalanine concentration for the intact peptide transport process. However, this process can be inhibited to various extents by other di- and tripeptides but the inhibition never exceeded 43%. These results are consistent with both passive and facilitated diffusion mechanisms of intact peptide transport, the latter occurring by either a low affinity-high capacity or a high affinity-low capacity system.

Introduction

Transmembrane transport of small peptides is a subject that has been extensively reviewed [1,2] and it appears that the process of absorption of di- and tripeptides involves two mechanisms: entry of peptides into the absorptive cells with intracellular hydrolysis, and hydrolysis followed by uptake of free amino acids. Although several of the major features of peptide absorption seem to be well established, several questions remain to be answered: What is the relative importance of the two mechanisms involved in peptide transport? How many systems are there for mucosal peptide uptake? What is the precise relationship between intestinal peptide transport and transport of Na^+? Does intramembrane hydrolysis linked to amino acid transport occur in a way similar to the mechanisms involved in the absorption of disaccharides [3]? Are γ-glutamyltransferase and the γ-glutamyl cycle involved in peptide transport [4,5]?

Abbreviation. Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

A clear-cut answer to the above questions has always been impaired by the experimental methods used for the study of peptide absorption: in vivo studies by perfusion techniques [6] or by measurements of absorption from the lumen of tied loops [7,8], and in vitro studies with rings or everted sacs of small intestine [9,10] The above methods have been unable to clearly differentiate between intact peptide absorption and absorption of free amino acids resulting from luminal or intracellular hydrolysis Recently, the use of purified brush border membrane vesicles [11–14] has overcome some of the problems associated with the preceding techniques but hydrolysis by membrane peptidases is still a factor that complicates the interpretation of results It can be noted, for example, that Ganapathy et al. [14], have not been able to detect intact glycyl-L-proline at any time inside rabbit intestinal brush border membrane vesicles. We therefore reinvestigated the problem of dipeptide absorption using a completely new approach. We have recently introduced the use of papain-treated vesicles for transport studies [15] It has been shown that controlled digestion of vesicles with gel-complexed papain did not modify the diffusional components for uptake of solutes and ions, and did not alter either the intravesicular volume or the carrier-mediated transport for glucose and leucine It was also observed during those studies that the vesicles showed a drastic reduction in oligopeptidase activities while γ-glutamyltransferase was almost completely solubilized by papain treatment. It thus appeared that this new model should be very useful to study the transport of intact peptides without interference from that of free amino acids resulting from hydrolysis and to correlate enzyme activities with transport functions. In this paper, we report for the first time in animal tissues the characteristics of intestinal absorption of a dipeptide per se, glycyl-L-phenylalanine. Our results show that this dipeptide is taken up intact down a concentration gradient by a non-sodium-dependent process.

Materials and Methods

1. Preparation of brush border membrane vesicles. Control mice (strain C57-BL/Ks J-db/m) were killed by decapitation. The whole intestine was then removed, rinsed with cold NaCl (0.9%), and the mucosa was scraped with a spatula. Brush border membranes were purified by the calcium chloride precipitation method of Schmitz et al. [16] and brush border membrane vesicles were obtained by Hopfer's method [17] as already described [15].

2. Transport studies. The purified brush border membrane vesicles were resuspended to a final protein concentration of 5–15 mg/ml with 10 mM Tris-Hepes buffer (pH 7.5), 300 mM mannitol and 0.1 mM $MgSO_4$ Incubation media contained, in a 250 μl final volume 10 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 300 mM mannitol or 100 mM mannitol plus 100 mM NaSCN or KSCN, 0.38 mM or 1 mM glycyl-L-phenylalanine and 1.22 μCi glycyl-L-[U-^{14}C]-phenylalanine (Amersham, spec. act. 12.8 mCi/mmol) For transport studies of L-phenylalanine, concentrations of 0.1 and 1 mM with 1 16 μCi L-[U-^{14}C]-phenylalanine (Amersham, spec act. 513 mCi/mmol) were used When the substrate concentration was varied or when the effect of various compounds on the uptake of glycyl-L-phenylalanine was studied, the osmolarity of the buffer was adjusted to 300 mM by appropriately adjusting the concentration of mannitol Transport studies were initiated by the addition of 250–750 μg of brush border membrane vesicles and were conducted at room temperature. At time intervals, 50 μl of the reaction mixture (50–150 μg protein) were mixed with 1 ml of cold stop solution containing 10 mM Tris-Hepes buffer (pH 7.5), 115 mM mannitol, 0.1 mM $MgSO_4$, 100 mM NaCl and 0.9 μCi D-[1-(n)^{3}H]mannitol (New England Nuclear, spec. act. 22.4 Ci/mmol). This step allows the correction for nonspecific adsorption. The resulting mixtures were filtered through 0.45 μm Sartorius filters and were washed with 4 ml nonradioactive stop solution. Filters were then processed for counting as already described [15].

The effect of medium osmolarity on the uptake of glycyl-L-phenylalanine was studied using cellobiose as the impermeant solute [17]. Vesicles were resuspended to a final protein concentration of 20–50 mg/ml with 10 mM Tris-Hepes bufer (pH 7.5), 100 mM cellobiose and 0.1 mM $MgSO_4$. 200–500 μg of brush border membrane vesicles were preincubated for 30 min in a medium containing variable concentrations of cellobiose, 10 mM Tris-Hepes buffer (pH 7.5), 100 mM NaSCN and 0.1 mM $MgSO_4$. Up-

take studies were initiated by adding 0.38 mM radioactive glycyl-L-phenylalanine (with concentrations of all reagents adjusted to the above values). Aliquots were removed after 5 min of incubation and treated as described for transport studies.

Linear regressions were performed using the desk calculator HP-97 and the curve-fitting program (standard pack). Coefficients of determination, r^2, are shown in the legends of the figures when appropriate.

3. Digestion of brush border membrane vesicles with papain. Gel-complexed papain was prepared and digestion was performed as described previously [15] using the determined optimal conditions: 10 min at room temperature with 0.9 U papain/mg protein.

4. Assays. Brush border membrane-catalyzed hydrolysis of L-phenylalanylglycine was studied in the same incubation system employed for analysis of uptake. Hydrolysis was stopped at time intervals by introducing 0.45 ml boiling water into the reaction mixture and transferring the test tube immediately into a boiling water bath and keeping it for 3 min. Free phenylalanine was assayed using L-amino acid oxidase reagent [18].

Oligopeptidase activities were measured according to Fugita et al. [18] using L-leucylglycylglycine and L-phenylalanylglycylglycine as substrates. γ-Glutamyltransferase activity was assayed by the method of Naftalin et al. [19].

Papain was assayed by a titrimetric determination of the acid produced during the hydrolysis of benzoyl-arginyl ethyl ester as described previously [15]. Protein was assayed by the method of Lowry et al. [20] using bovine serum albumin as standard.

Results

1. Uptake of glycyl-L-[U-^{14}C]phenylalanine by normal vesicles

Uptake of glycyl-L-[U-^{14}C]phenylalanine by normal vesicles is shown in Fig. 1A and B for substrate concentrations of 0.38 and 1 mM, respectively. At the lower substrate concentration (Fig. 1A), equilibrium was reached within 1 min in absence of salts (mannitol medium). However, in the presence of NaSCN or KSCN gradients (extravesicular > intravesicular), transient accumulations of substrate over this equilibrium value were clearly seen and higher

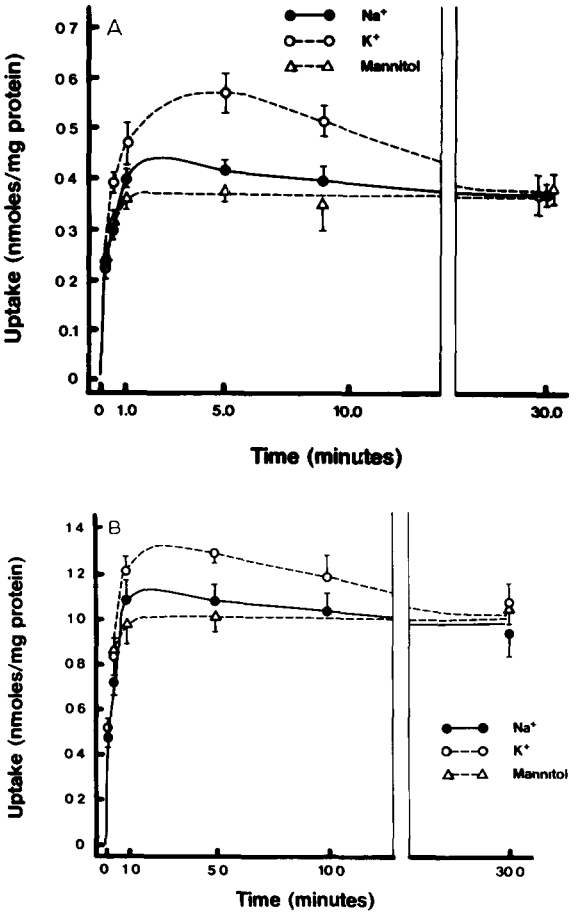

Fig. 1. Time course study of glycyl-L-[U-^{14}C]phenylalanine uptake by normal mouse intestinal brush border membrane vesicles at peptide concentration of 0.38 mM (A) and 1 mM (B), respectively. Uptake studies were performed as discussed under Materials and Methods Three different media prepared in 10 mM Tris-Hepes buffer (pH 7.5) containing 0.1 mM $MgSO_4$ were used. △———△, 300 mM mannitol; •———•, 100 mM mannitol + 100 mM NaSCN; ○———○, 100 mM mannitol + 100 mM KSCN. Values represent the mean ±1 S.E for at least six different preparations of vesicles, and duplicate assays at 0.15 and 0.45 min

overshoot values were obtained in the presence of K^+ as compared to Na^+. Also, after equilibration of the vesicles in NaSCN medium, uptake values followed the mannitol curve (results not shown). At the higher substrate concentration (Fig. 1B), equilibrium was reached within 1 min in the mannitol and NaSCN media but a small overshoot was still visible in the presence of K^+.

2 Uptake of glycyl-L[U-^{14}C]phenylalanine by papain-treated vesicles

Uptake of glycyl-L-[U-^{14}C]phenylalanine by papain-treated vesicles is shown in Fig. 2A and B for substrate concentrations of 0.38 and 1 mM, respectively Similar uptake curves were obtained in these conditions whatever the composition of the media. Equilibrium values were reached within 5 and 1 min for the respective concentrations of 0 38 (Fig. 2A) and 1 mM (Fig. 2B) and no overshoot was present.

3 Brush border membrane-catalyzed hydrolysis of glycyl-L-phenylalanine

Fig 3 shows the time course of peptide hydrolysis

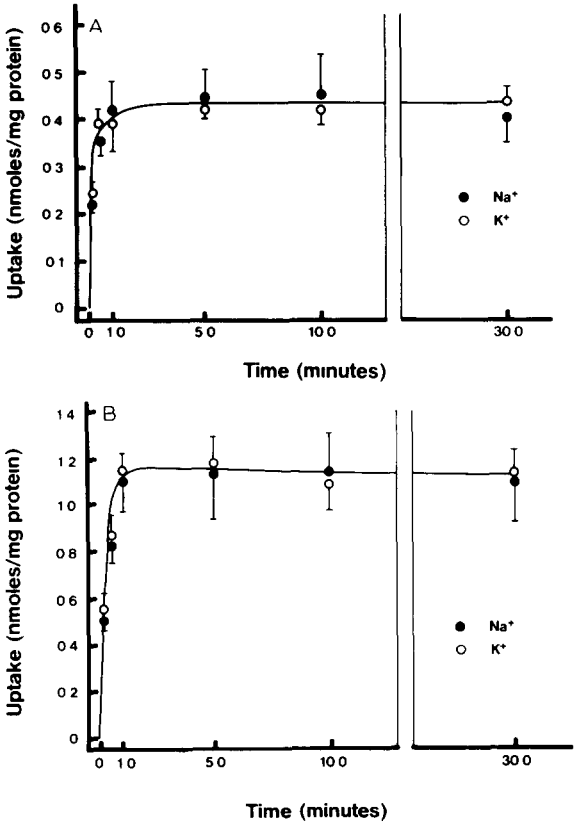

Fig. 2. Time course study of glycyl-L-[U-^{14}C]phenylalanine uptake by papain-treated mouse intestinal brush border membrane vesicles at peptide concentrations of 0.38 mM (A) and 1 mM (B), respectively. Papain treatment and uptake studies were performed as discussed under Materials and Methods. Media and symbols as in Fig. 1 Values represent the mean ±1 S E for 5–6 different preparations of vesicles, and duplicate assays at 0 15 and 0.45 min

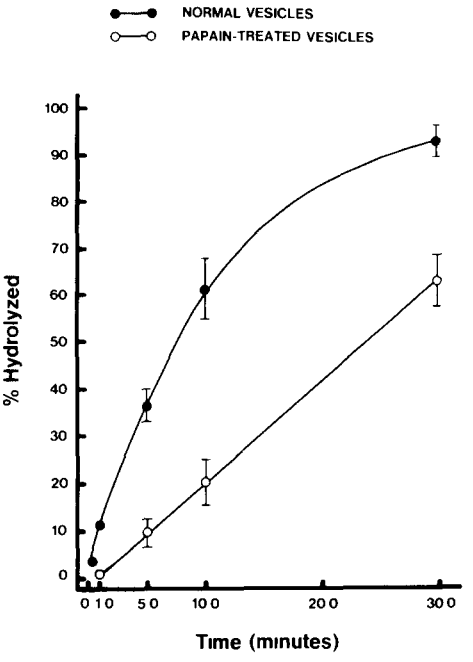

Fig. 3. Time course study of brush border membrane catalyzed hydrolysis of glycyl-L-phenylalanine under conditions of uptake studies at a peptide concentration of 1 mM The vesicle preparations used are the same as those used in Figs. 1 and 2 Values represent the mean ±1 S.E of 5–6 different experiments in either mannitol, NaSCN or KSCN media, as it has been found that ions do not influence rates of dipeptide hydrolysis.

during uptake experiments using the higher substrate concentration (1 mM). With normal vesicles, there was an appreciable hydrolysis of dipeptide, even in the early time points However, after papain treatment, the incubation medium was found to be completely free of free phenylalanine up to 1 min, of incubation Similar results were found when using the lower substrate concentration of 0.38 mM (results not shown). Table I shows that the reduction in hydrolysis after papain treatment was the consequence of the removal of 66–74% of brush border membrane oligopeptidase activities by papain digestion as estimated from total activities recovered in the vesicle fractions. However, two different peptidases might be responsible for the hydrolysis of the two substrates chosen, as phenylalanylglycylglycine hydrolase was inactivated upon solubilization by papain while that for leucylglycylglycine was resistant. It was also observed that hydrolysis of glycyl-

TABLE I

EFFECT OF DIGESTION WITH GEL-COMPLEXED PAPAIN ON SOLUBILIZATION OF OLIGOPEPTIDASE ACTIVITIES OF MOUSE INTESTINAL BRUSH BORDER MEMBRANE VESICLES

The values given represent the mean total amounts ± 1 S E. recovered in the respective fractions and were obtained with the preparations of brush border membrane vesicles used in Figs. 1 and 2 The activities referred to as soluble were determined in the supernatant recovered after 15 min centrifugation at 31 000 × *g* following papain digestion. See details under Materials and Methods. The ratios of activities between papain-treated and normal vesicles × 100 are given within brackets.

	Oligopeptidase activities (nmol amino acid/min)					
	Phe-Gly-Gly			Leu-Gly-Gly		
	Membranes	Soluble	Total	Membranes	Soluble	Total
Normal vesicles	2409 ± 57	315 ± 20	2723 ± 66	25 586 ± 2642	1412 ± 204	26 998 ± 2542
Papain-treated vesicles	639 ± 48 (26.5 ± 1.4)	388 ± 45	1027 ± 60 (37.7 ± 2.4)	8143 ± 628 (33.2 ± 5.4)	21 995 ± 1456	30 137 ± 1675 (116.5 ± 17.1)

L-phenylalanine was independent of the presence of Na^+ or K^+ in the incubation media (results not shown).

As previously [15], we also found that γ-glutamyltransferase activity has been solubilized 90–95% with quantitative recovery (results not shown).

4. Uptake of L-[U-^{14}C]phenylalanine by normal vesicles

Uptake of L-[U-^{14}C]phenylalanine by normal vesicles is shown in Fig. 4A and B for substrate concentrations of 0.1 and 1 mM, respectively. For both concentrations, an overshoot phenomenon in the presence of a Na^+ gradient (extravesicular > intravesicular) was clearly visible, indicating an active transport process by a sodium-dependent carrier-mediated mechanism. However, an unexpected finding is that phenylalanine at low concentrations can also respond to a K^+ gradient (extravesicular > intravesicular). When incubated with choline chloride,

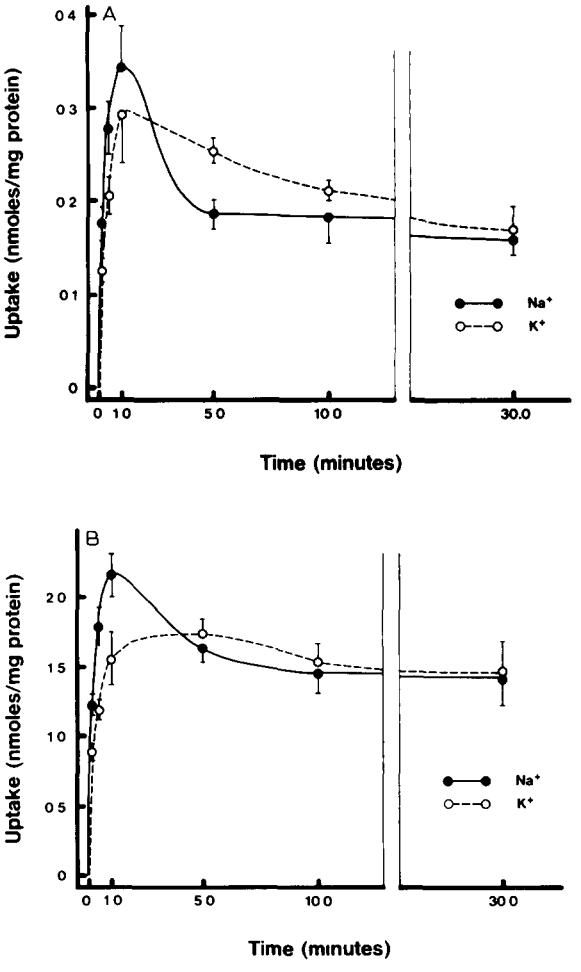

Fig. 4. Time course study of L-[U-^{14}C]phenylalanine uptake by normal mouse intestinal brush border membrane vesicles at amino acid concentrations of 0.1 mM (A) and 1 mM (B), respectively. Uptake studies were performed as discussed under Materials and Methods. Media contained 10 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$ and either 100 mM mannitol + 100 mM NaSCN (●———●) or 100 mM mannitol + 100 mM KSCN (○———○). Values represent the mean ±1 S.E. for five different preparations of vesicles and duplicate assays at 0.15 and 0.45 min.

uptake reached equilibrium values within 5 min and no overshoot was present (results not shown). It can also be seen that uptake of phenylalanine was faster than uptake of glycyl-L-phenylalanine for a concentration of 1 mM, the ratios of initial uptake over equilibrium uptake being 0.85 and 0 52, respectively.

5. Uptake as a function of osmolarity

Uptake of radioactive label from glycyl-L-[U-^{14}C]-phenylalanine at equilibrium (5 min incubation) as a function of medium osmolarity is demonstrated in Fig. 5. This uptake was quite sensitive to changes in medium osmolarity, sharply decreasing with increases in osmolarity When extrapolated to infinite medium osmolarity, uptake was negligible. The above indicates uptake into an osmotically reactive intravesicular space without appreciable binding to the surface of the vesicles

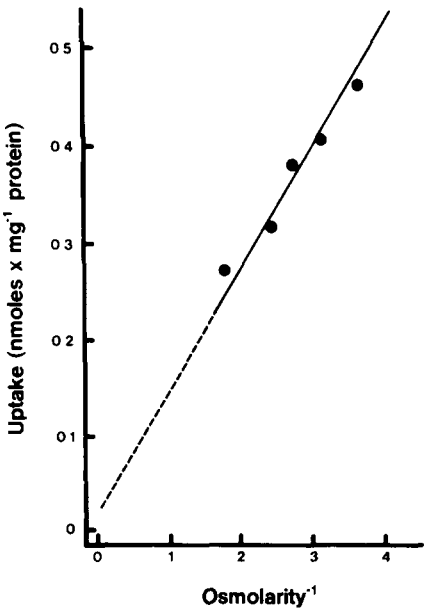

Fig 5. Uptake of glycyl-L-[U-^{14}C]phenylalanine by mouse intestinal brush border membrane vesicles as a function of incubation medium osmolarity. Uptake studies were performed as discussed under Materials and Methods in media containing 10 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 100 mM NaSCN, 0.38 mM glycyl-L-[U-^{14}C]phenylalanine and cellobiose added to give the desired medium osmolarity. Values represent the mean of duplicate assays at 5 min incubation. A linear regression program has been used to fit the data and a coefficient of determination $r^2 = 0.987$ has been found.

6. Concentration dependence of glycyl-L-phenylalanine transport

The concentration dependence of glycyl-L-phenylalanine uptake is shown in Fig. 6. Initial velocities have been estimated from uptake values at 9 s for dipeptide concentrations varying from 0.38 up to 72 mM. In these conditions, there was a linear relationship of initial uptake to substrate concentrations whether using normal ($r^2 = 0.992$) or papain-treated ($r^2 = 0.996$) vesicles. Absence of saturation in the range of 0.38–5 mM dipeptide concentrations is also shown in the inset in Fig. 6 It also has to be noted that a ratio of 1.51 has been found between

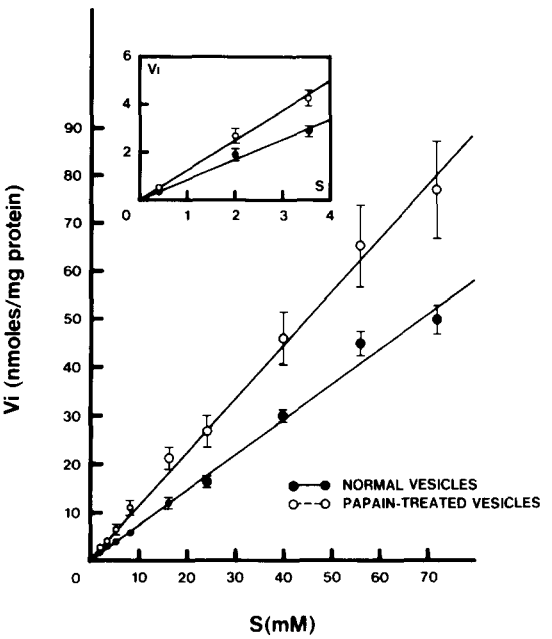

Fig 6. Concentration dependence of glycyl-L-phenylalanine initial uptake by normal (●———●) and papain-treated (○———○) vesicles. Uptake studies were performed as discussed under Materials and Methods in media containing 10 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 100 mM NaSCN, and variable concentrations of substrate and mannitol as to keep a 100 mM total concentration Initial uptake has been measured at 0.15 min. Values represent the mean ±1 S.E. for duplicate assays on two different preparations A linear regression program has been used to fit the data and coefficients of determination $r^2 = 0.992$ and 0.996 have been found for normal and papain-treated vesicles, respectively. Inset shows the absence of saturation in the low range of concentrations. $r^2 = 0\,989$ and 0.995 have been found by linear regression analysis for normal and papain-treated vesicles, respectively.

the slopes obtained for papain-treated and normal vesicles, a value similar to the 1.6-fold increase in specific activity of leucine transport after papain treatment reported earlier [15].

7. Effects of peptides and free amino acids on glycyl-L-phenylalanine uptake

A variety of peptides and free amino acids have been tested for their ability to inhibit glycyl-L-phenylalanine uptake and the results are described in Table II. For these experiments, only normal vesicles have been used as the initial velocity at 9 s selected for this study is independent of papain treatment. In these conditions, free amino acids did not inhibit significantly uptake of the dipeptide and only the dipeptides glycylglycine, glycyl-L-proline, glycyl-L-leucine, carnosine, triglycine, glycylglycylsarcosine and L-leucylglycylglycine were able to inhibit glycyl-L-phenylalanine uptake with statistical significance ($P < 0.05$). However, this inhibition never exceeded 43% of control values.

TABLE II

EFFECTS OF PEPTIDES AND FREE AMINO ACIDS ON GLYCYL-L-PHENYLALANINE UPTAKE BY MOUSE INTESTINAL BRUSH BORDER MEMBRANE VESICLES

Uptake measurements at 0 15 min were performed as discussed under Materials and Methods in a medium containing 10 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 100 mM NaSCN, 0.38 mM of radioactive substrate, 40 mM mannitol and 60 mM of the different compounds tested. The given values represent the mean ± 1 S.E. for duplicate assays on two different preparations. Values of uptake relative to control uptake are given within brackets

Test compound	Peptide uptake (nmol/mg protein)
None	0.237 ± 0.019 (100)
Glycine	0 218 ± 0.011 (92)
L-Leucine	0.227 ± 0 006 (96)
L-Phenylalanine	0.230 ± 0.002 (97)
Glycylglycine	0.166 ± 0.008 [a] (70)
Glycylsarcosine	0 171 ± 0.017 (72)
Glycyl-L-proline	0.162 ± 0.011 [a] (68)
Glycyl-L-leucine	0.178 ± 0.011 [a] (75)
L-Prolylglycine	0.192 ± 0.017 (81)
Carnosine	0.153 ± 0 016 [a] (65)
β-Alanylglycine	0.201 ± 0 019 (85)
Triglycine	0.163 ± 0.009 [a] (69)
Glycylglycylsarcosine	0 156 ± 0 009 [a] (66)
Glycyl-L-leucyl-L-tyrosine	0.205 ± 0.013 (87)
Glycyl-L-prolyl-L-alanine	0.188 ± 0.013 (79)
L-Leucyl-glycyl-glycine	0.134 ± 0.015 [a] (57)
L-Phenylalanylglycylglycine	0.166 ± 0 012 (70)
Glutathione	0.217 ± 0.024 (92)

[a] Statistical significance relative to control values ($P < 0$ 05) as estimated by Student's *t*-test.

Discussion

Following uptake by the absorptive cells of the small intestine, most small peptides are so rapidly hydrolyzed by the cytosolic peptidases that peptide uptake by small intestine in vitro is represented only by an increase in the constituent amino acids of the peptide taken up [1,2]. The use of slowly hydrolyzable peptides [10,21,22] has not completely overcome the difficulty in assessing Na^+-dependency [21–27] and determining precise kinetic parameters [7,10,12,14,23,25,28–34]. The introduction of purified brush border membrane vesicles for peptide transport studies, therefore, appeared valuable as they would permit the study of membrane phenomena in the absence of cytosolic events. However, the first attempts using such vesicles [11–14] were not very conclusive, as hydrolysis of peptides by brush border membrane hydrolases complicated the interpretation of results. For example, intact peptides have not been detected at any time inside the vesicles [12,14]. Our studies with papain-treated brush border membrane vesicles have been designed to investigate membrane events in almost complete absence of peptide hydrolysis. It has been shown recently [15] that controlled papain digestion of brush border membrane vesicles did not modify the characteristics of sugar and amino acid transport. Also, the treatment with papain allowed the removal of approx. 70% of membrane oligopeptidase activities (Table I), so that free amino acids cannot be detected in the media up to 1 min incubation (Fig. 3), a time at which equilibrium uptake values have been reached. The above results clearly show that peptide transport per se can be investigated using papain-treated vesicles. It also has to be mentioned that glycyl-L-phenylalanine was not extensively hydrolyzed even by normal vesicles as compared to glycyl-L-leucine [11], L-alanylglycine [12], and glycyl-L-proline [14] used in earlier studies.

An examination of the time course of glycyl-L-[U-^{14}C]phenylalanine uptake by normal (Fig. 1) and papain-treated (Fig. 2) vesicles revealed the following. At the lower substrate concentration (0.38 mM), peptide uptake showed active transport of labeled products in the presence of salt gradients (medium > vesicle) as evidenced by the overshoot seen on the time course curves for normal vesicles. However, after papain-treatment, this transient accumulation of substrate over equilibrium values was no longer observed. Also, at the higher substrate concentration (1 mM), the overshoot was seen only in the presence of a KSCN gradient when using normal vesicles and was not evident after papain treatment. These results are best interpreted as follows transport of glycyl-L-phenylalanine is independent of sodium (and/or potassium) and the overshoots seen with normal vesicles are the consequence of uptake of free phenylalanine released from the dipeptide by brush border membrane oligopeptidases. This interpretation is strengthened by considering the characteristics of uptake of free phenylalanine (Fig. 4), with an unusual feature for amino acid transport, namely a dependency on K^+ gradient (outside > inside) Such a K^+-dependent phenylalanine uptake has been described in membrane vesicles isolated from the midgut of *Philosamia cynthia* larvae [35] but, to the best of our knowledge, this is the first time that K^+-dependent amino acid transport is reported in mammalian small intestine (unpublished results). However, Fig. 4B shows that the K^+-dependent uptake of L-phenylalanine was saturated faster than when Na^+ was present. Also, Fig. 4A shows almost the same maximum overshoot values in the presence of Na^+ or K^+, though the overshoot phenomenon lasted a longer time in the presence of K^+ as compared to Na^+. The above results can be interpreted as due to lower permeability of the membranes to K^+ as compared to Na^+, allowing a longer time for the dissipation of the K^+-gradient This lower permeability to K^+ would also explain the higher overshoot values obtained in K^+ media during uptake studies of radioactive label from glycyl-L-phenylalanine by normal vesicles. It would thus appear that peptide transport is the result of two complementary mechanisms: (1) uptake of free amino acids following hydrolysis by the brush border membrane oligopeptidases and (2) intact peptide transport down a concentration gradient by a non-sodium-dependent process. It also appears that phenylalanine is absorbed faster in the free state than in the peptide bound state at the 1 mM concentration used for the comparison.

Further studies on the analysis of the nature of the intact peptide transport process revealed a linear relationship between initial uptake and substrate concentrations (Fig. 6) using either normal or papain-treated vesicles. These results are at variance with those obtained earlier and showing mediated uptake conforming to Michaelis-Menten kinetics [7,10,12,14,23,25,28–34]. However, it has to be stressed that important simple diffusional components in transport of dipeptides have been reported recently [33,34] and that no evidence for saturation was obtained for β-alanylglycylglycine [22] and propylhydroxyproline [36], peptides which are not hydrolyzed by rings of everted hamster jejunum. Although our results seem compatible with an intact peptide transport process occurring by passive diffusion, it has to be stressed that two other possibilities have to be considered: facilitated diffusion processes by either a high affinity-low capacity system or by a low affinity-high capacity system. The first possibility seems to be unlikely in view of the previously reported K_t values in the mM range [7,10,12,14,23,25,28–34]. The physiological significance of such a high affinity-low capacity system would also be questionable in view of the kinetic parameters for hydrolysis (estimated K_m and V values of 3.96 mM and 389 nmol/min per mg protein, respectively). Finally, the detection of such a high affinity-low capacity system would have been impaired by the low specific activity of the radioactively labelled peptide used for the present study. The second possibility, namely the presence of a low affinity-high capacity system is also difficult to be tested because of the solubility of the dipeptide, and therefore cannot be rejected on the basis of our studies. It also has to be noted that higher uptake values were obtained after papain treatment, the increase closely matching the extent of protein removal during digestion, and showing that papain digestion did not remove any protein essential in peptide transport. It can thus be concluded that neither oligopeptidases nor γ-glutamyltransferase are involved in group translocation of peptides.

In further attempts to characterize the nature of

the mechanism(s) involved in intact peptide transport, inhibition studies by a variety of peptides and free amino acids have been undertaken (table II). Our results showed that glycyl-L-phenylalanine transport was not inhibited by free amino acids, in accordance with the previously established independence of mucosal uptake of peptides from those of free amino acids [1,2]. However, when dipeptides and tripeptides were incubated with glycyl-L-[U-^{14}C]-phenylalanine, some degree of inhibition in glycyl-L-phenylalanine transport can be achieved. Highest inhibitions (greater than 30%) were obtained with the tripeptides L-leucylglycylglycine, glycylglycylsarcosine and triglycine and the dipeptides carnosine and glycyl-L-proline. Weaker inhibitions (between 20 and 30%) were obtained with L-phenylalanylglycylglycine, diglycine, glycylsarcosine and glycyl-L-leucine. Non-significant inhibitions were obtained with the other di- and tripeptides. Such results agree with the possible involvement of carrier-mediated system in intact peptide transport, as discussed previously when considering the high concentrations (60 mM) of tested compounds used in these studies. However, the failure to inhibit glycylphenylalanine transport more than 43% also points out the presence of an important diffusible component in peptide uptake, even at low peptide concentrations (0.38 mM used in this study), or that peptides are transported by several systems with overlapping specificities.

In conclusion, the results presented in this study clearly show that glycyl-L-phenylalanine is taken up intact by mouse intestinal brush border membrane vesicles by a non-Na^+-dependent process but also that this dipeptide undergoes a certain amount of superficial hydrolysis followed by uptake of the free amino acids liberated. However, the relative importance of both processes cannot yet be established. Three possible schemes have been considered by Matthews [1] in order to explain peptide absorption, and one of the models in which di- and tripeptides are taken up at the brush border by one or more peptide-specific active transport mechanisms and hydrolyzed in the cytosol deep to the peptide transport mechanism(s) has been favored. Our results, however, failed to show any Na^+-dependent active transport of intact peptide but are consistent with both passive and facilitated diffusion mechanisms of uptake, the latter occurring by either a low affinity-high capacity or a high affinity-low capacity system. It has to be noted, however, that a model of active peptide transport cannot be definitively rejected as energizing processes other than the sodium gradient might be involved. Finally, our results showing a lack of an effect of the removal of γ-glutamyltransferase and oligopeptidases by papain-treatment on peptide transport are proof against the involvement of these enzymes in group translocation mechanisms [1,4,5].

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