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EVIDENCE FOR A DIPEPTIDE TRANSPORT SYSTEM IN RENAL BRUSH BORDER MEMBRANES FROM RABBIT

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Summary

Papain treatment of renal brush border vesicles was carried out as a successful first step towards the purification of the membrane components involved in dipeptide transport. The treated vesicles exhibited increased specific transport activity of glycyl-L-proline. In contrast, the specific transport activity of L-alanine in the treated vesicles was less than that in the control vesicles. Papain treatment resulted in the solubilization of 38% of protein, 55% of alkaline phosphatase, 90% of γ-glutamyltransferase and 95% of leucine aminopeptidase. There was no change in the intravesicular volume nor was there any increase in vesicular permeability. Glycyl-L-proline transport was Na^+-independent in the control and papain-treated vesicles. Diamide reduced the Na^+-dependent L-alanine transport while glycyl-L-proline transport remained unaffected in the presence of Na^+. Many dipeptides inhibited glycyl-L-proline transport both in the presence and absence of Na^+. The inhibition by dipeptides was greater than the inhibition by equivalent concentrations of free amino acids. These data demonstrate that renal brush border vesicles can efficiently handle dipeptides by a mechanism completely different from that of amino acid transport.

Introduction

The brush border membrane of renal cortex proximal tubular cells possesses Na^+-dependent transport mechanisms for sugars and amino acids [1—8]. Nu-

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

merous studies also indicate that a variety of small molecular weight proteins and polypeptides filtered at the glomerulus are absorbed by the proximal tubular cells by luminal endocytosis and are hydrolyzed by lysosomal enzymes [9—12]. However, very few studies have been carried out to determine the ability of the kidney to reabsorb small peptides. Renal membranes contain a variety of peptidases [13,14] and studies with small linear peptides have shown [15—19] that these peptidases may play an important role in the hydrolysis of the peptides in the glomerular filtrate with the hydrolyzed products being efficiently reabsorbed. Adibi and co-workers [20,21] have shown that intravenously administered dipeptides are rapidly cleared from the blood and that the kidney has a high capacity for dipeptide uptake compared to other tissues.

Dipeptides have been identified in systemic or portal plasma in a variety of experimental and physiological situations [22—25]. These substrates are freely filtered at the glomerulus and enter the proximal tubule. A highly active particulate renal dipeptidase has been purified and characterized [26,27]. A recent study [28] has established the localization of this dipeptidase in the brush border microvilli. Using L-alanyl-glycine, the most susceptible dipeptide to hydrolysis by this enzyme, the study has shown that the renal brush border dipeptidase may act at the luminal surface of the proximal tubular cells to hydrolyze dipeptides present in the glomerular filtrate, with the subsequent reabsorption of the resultant free amino acids by the Na^+-dependent mechanisms.

However, a few studies have indicated that the kidney may possess a dipeptide transport system distinct from the different group-specific amino acid transport systems. Nutzenadel and Scriver [29] have shown that, in kidney cortex slices, carnosine is taken up by an active transport system which is independent of the mechanism serving the entry of free amino acids. Adibi et al. [20] have found that glycyl-glycine, glycyl-L-leucine and glycyl-sarcosine are taken up intact by the kidney followed by their intracellular hydrolysis. Our own studies with glycyl-L-proline [30] have demonstrated that the renal brush border membrane vesicles are able to transport the dipeptide by a Na^+-independent mechanism, whereas free amino acid transport is Na^+-dependent. The present paper deals with further characterization of the dipeptide transport system using renal brush border membrane vesicles from rabbit.

Methods and Materials

Preparation of brush border membrane vesicles

The brush border membrane vesicles were prepared from rabbit kidney according to the method of Malathi et al. [31]. Freshly prepared brush border vesicles were used throughout the study.

Papain digestion

The 43 000 × *g* pellets obtained from the final step of the membrane isolation procedure were suspended in a 50 mM potassium phosphate buffer, pH 6.2. It was diluted with the same buffer so as to give a final protein concentration of 5 mg/ml. The procedure described below is essentially similar to that of Louvard et al. [32].

The commercial papain (Sigma 3125, 24 BAEE units/mg protein; 1 unit hy-

drolyzes 1.0 μmol of α-*N*-benzoyl-L-arginine ethyl ester per min at pH 6.2, 25°C) was activated before use by incubating papain in a 50 mM potassium phosphate buffer, pH 6.2, for 15 min at 0°C in the presence of 5 mM cysteine and 0.03 mM dithiothreitol. Brush border membrane vesicle suspensions (5 mg protein/ml) in phosphate buffer, pH 6.2, were incubated at 37°C for 30 min with 0.5 mg/ml activated papain. The concentration of papain was varied in one experiment where the effect of different concentrations of papain was studied. At the end of digestion, the suspension was chilled at 0°C and centrifuged at 105 000 × *g* for 45 min. The pellet was suspended in a medium containing 300 mM mannitol, buffered with 1 mM Hepes/Tris to pH 7.5. These membrane vesicles were used for enzyme and transport assays. No inactivation of enzymes by papain was observed in the course of these assays.

Transport assay

Transport of L-alanine and glycyl-L-proline was measured as previously described [30]. When the effects of other dipeptides and amino acids on glycyl-L-proline transport were studied, the osmolarity of the transport buffer [30] was adjusted to 300 mosM by appropriately changing the concentration of mannitol, and the pH was adjusted to 7.5 with Tris base or HCl. The experiments were regularly done in duplicate and sometimes in triplicate and the variations between the individual values and the mean value was always less than ±10%.

Enzyme assays

Leucine aminopeptidase was measured by spectrophotometry using L-leucine *p*-nitroanilide as the substrate [33]. γ-Glutamyl transferase was determined spectrophotometrically, using γ-glutamyl *p*-nitroanilide as the substrate and glycyl-glycine as the acceptor for glutamyl moieties [34].

Protein was determined according to the method of Lowry et al. [35], using crystalline bovine serum albumin as the standard.

Determination of extravesicular space

The control and papain-treated vesicles were incubated in small centrifuge tubes with [*methoxy*-^{14}C]inulin at 25°C for 1 h. After incubation, the samples were centrifuged at 42 000 × *g* for 20 min. A known volume of the supernatant and a known weight of the pellet were transferred to a counting vial with the scintillation cocktail and the radioactivity was counted. The extravesicular space was expressed as the percent of wet weight of the pellet.

Purity of brush border membrane vesicles

Membrane purity was determined routinely by assay of alkaline phosphatase [36], a marker enzyme for brush border membrane. ($Na^+ + K^+$)-ATPase, a marker enzyme for basal-lateral membrane was also routinely determined [37] to monitor the contamination of this membrane. Alkaline phosphatase was enriched 9—12-fold (mean, 10-fold) and the average specific activity in the brush border membrane vesicles was 0.94 ± 0.23 μmol/min per mg protein. The enrichment of alkaline phosphatase in the purified membrane vesicles reported

in the original method [31] was 6.8-fold. ($Na^+ + K^+$)-ATPase was undetectable in most preparations though trace activity was occasionally present. For transport studies, only those membrane preparations completely free of ($Na^+ + K^+$)-ATPase were used. The vesicularity of the membrane preparations was checked by electron microscopy. The purified membranes appeared homogeneous and vesicular.

Materials

Unlabelled dipeptides, Triton X-100 and papain were obtained from Sigma Chemical Company. Unlabelled amino acids were from California Corporation for Biochemical Research. Toluene used in the scintillation cocktail was obtained from Fisher Scientific Company. 2,5-Diphenyloxazole was purchased from Mallinckrodt and *p*-bis(2-(5-phenyloxazolyl)) benzene from Packard Instrument Company. Diamide(diazenedicarboxylic acid bis(*N,N*-dimethylamide) was obtained from Nutritional Biochemicals. All other chemicals were of analytical grade.

[1-^{14}C]Glycyl-L-proline (specific radioactivity, 7 mCi/mmol) was purchased from the Radiochemical Center, Amersham, England. L-[U-^{14}C]Alanine (specific radioactivity, 160 mCi/mmol and [*methoxy*-^{14}C]inulin were from New England Nuclear Corporation, Boston, MA.

Results

L-Alanine transport

Transport of L-alanine (20 μM) into the vesicles before and after papain digestion was studied over a period of 10 min, using both NaCl and KCl media. As can be seen from Fig. 1A, the transport of L-alanine into the untreated vesicles, in the presence of a Na^+ gradient, exhibited an overshoot phenomenon with the maximum transport occurring at 1 min incubation. In the absence of a

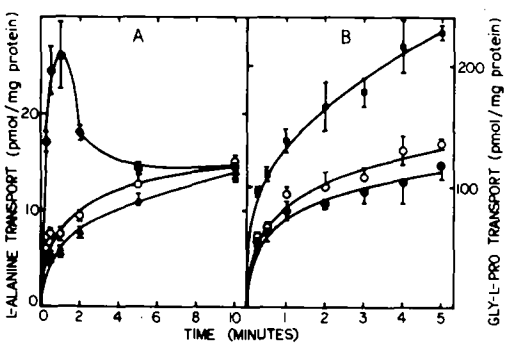


Fig. 1. A. Transport of L-alanine (20 μM) into control and papain-treated vesicles over a period of 10 min. ●, NaCl medium, control vesicles; ○, NaCl medium, papain-treated vesicles; ▲, KCl medium, control vesicles; △, KCl medium, papain-treated vesicles. The results are expressed as mean ± S.D. The data shown are from four different experiments. B. Transport of glycyl-L-proline (GLY-L-PRO) (160 μM) into control and papain-treated vesicles over a period of 5 min in NaCl medium. ●, control vesicles; ■, ○, papain-treated vesicles: ○, transport calculated using the amount of protein present before papain digestion; ■, transport calculated using the amount of protein present after papain digestion. The results are expressed as mean ± S.D. The data shown are from four different experiments.

Na^+ gradient, there was no overshoot, and the transport gradually increased with time to an equilibrium value approximately equal to that obtained in the presence of a Na^+ gradient. At the peak of the overshoot, the presence of a Na^+ gradient stimulated L-alanine transport by 4.3-fold. Papain treatment resulted in a significant reduction of the transport and abolished the Na^+-dependent overshoot. However, the Na^+-independent transport remained unaffected. The treatment of vesicles with papain resulted in 90% reduction in the Na^+-dependent, carrier-mediated L-alanine transport at the overshoot. However, the equilibrium transport of the amino acid in the control and papain-treated vesicles in both NaCl and KCl media remained approximately the same, indicating that papain treatment did not change the intravesicular volume or the ultimate amount of amino acid transported.

Glycyl-L-proline transport

Transport of glycyl-L-proline (160 μM) into the vesicles before and after papain digestion was studied over a period of 5 min using NaCl medium. Since our earlier study [30] had shown that the presence or absence of a Na^+ gradient does not influence glycyl-L-proline transport, only NaCl medium was used in this experiment. Transport studied over a 5 min period represented predominantly the transport of the intact dipeptide because only about 10% of the dipeptide in the medium was hydrolyzed within this time [30].

Fig. 1B shows that glycyl-L-proline transport was slightly stimulated by papain treatment. This stimulation was small but highly reproducible. Since papain treatment released about 40% of the membrane protein, this resulted in a 1.7-fold enrichment of the specific transport activity of glycyl-L-proline.

Effect of papain concentration on glycyl-L-proline and L-alanine transport

The brush border membrane vesicles were treated with different concentrations of papain. Using the control and treated vesicles, the transport of glycyl-L-proline (160 μM) in NaCl medium and L-alanine (20 μM) in NaCl and KCl media was studied with a 1 min incubation period. This incubation period was employed in transport studies because we have earlier shown [30] that with 1 min incubation, less than 10% of the dipeptide in the medium was hydrolyzed. Therefore, the transport in this time period represented dipeptide transport rather than free amino acid transport. In the case of L-alanine, 1 min incubation period represented the overshoot. The concentration of papain was varied from 5 μg/mg membrane protein to 200 μg/mg membrane protein. The results are given in Fig. 2. The enrichment of specific transport activity of glycyl-L-proline was 1.6-fold over that of the control vesicles, and the transport remained almost unaffected at all concentrations of papain.

In the case of L-alanine, the Na^+-dependent, carrier-mediated transport was calculated by subtracting the Na^+-free transport from the transport measured in the presence of Na^+ at each concentration of papain. The lowest papain concentration used led to an enrichment of specific transport activity of L-alanine by about 1.2-fold. However, the enrichment factor steeply decreased as the papain concentration increased. There was only about 20% of the transport activity in the papain-treated vesicles when compared to the control vesicles, when the papain concentration was 200 μg/mg membrane protein.

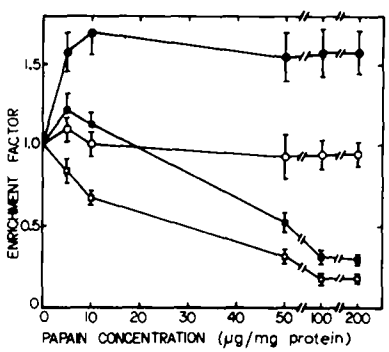
Effect of diamide on glycyl-L-proline and L-alanine transport

The effect of diamide on the transport of glycyl-L-proline (160 μM) in NaCl medium and L-alanine (20 μM) in NaCl and KCl media was also studied. Brush border membrane vesicles were preincubated with 5 mM diamide at 0°C for 10 min before they were used in transport studies. Fig. 3A shows that diamide inhibited the initial transport of L-alanine in the presence of a Na^+ gradient. Diamide had no effect on L-alanine transport in the absence of a Na^+ gradient. The equilibrium transport of L-alanine was also not affected by diamide, indicating that the intravesicular volume was not altered. When the Na^+-dependent, carrier-mediated L-alanine transport was calculated by subtracting the transport in KCl medium from the transport in NaCl medium, diamide caused about 25% inhibition.

Fig. 3B shows the effect of diamide on glycyl-L-proline transport in NaCl medium. Diamide did not affect the dipeptide transport.

Effect of medium osmolarity on the equilibrium transport of glycyl-L-proline into papain-treated vesicles

In a separate experiment, it was found that the transport of radioactive label from [1-^{14}C]glycyl-L-proline (160 μM) in a complete mannitol medium reached a steady state after 1 h incubation. This time period was employed to study the osmotic behavior of the papain treated vesicles. Increasing osmolarity



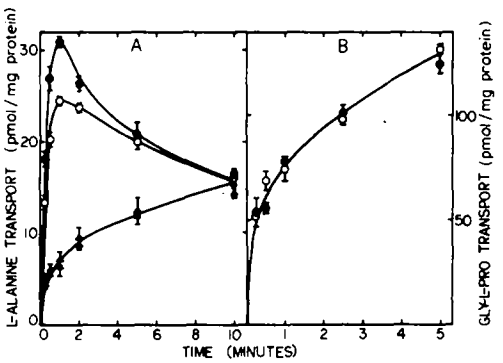


Fig. 2. Effect of papain concentration on the transport of L-alanine (20 μM) and glycyl-L-proline (160 μM). Incubation period was 1 min. In the case of L-alanine, both NaCl and KCl media were used. In the case of glycyl-L-proline, only NaCl medium was used. Na^+-dependent L-alanine transport was calculated by subtracting the transport in KCl medium from that in NaCl medium. Transport observed in the control vesicles is taken as 1 and the results are expressed relative to this value. ●, Glycyl-L-proline transport, calculated with protein after papain solubilization; ○, glycyl-L-proline transport, calculated with protein before papain solubilization; ■, L-alanine transport, calculated with protein after papain solubilization; □, L-alanine transport, calculated with protein before papain solubilization. The results are expressed as mean ± S.D. The data shown are from three different experiments.

Fig. 3. Effect of 5 mM diamide on L-alanine (20 μM) transport in both NaCl and KCl media and glycyl-L-proline (160 μM) transport in NaCl medium. Brush border membrane vesicles were preincubated with 5 mM diamide at 0°C for 10 min before transport assay. A. Time course of L-alanine transport: ●, transport in NaCl medium in the absence of diamide; ○, transport in NaCl medium in the presence of diamide; ▲, transport in KCl medium in the absence of diamide; △, transport in KCl medium in the presence of diamide. B. Time course of glycyl-L-proline transport in NaCl medium: ●, in the absence of diamide; ○, in the presence of diamide. The results are expressed as mean ± S.D. The data shown are from four different experiments.

of the medium was correlated with decreasing accumulation of radioactive label within the vesicles. Extrapolation to infinite medium osmolarity (zero intravesicular space) showed some residual transport, suggesting that a portion of the transport may have been due to nonspecific binding to the membranes. This residual transport represented less than 15% of the total transport under isosmolar conditions (300 mosM). This shows that the bulk of the transport was due to the accumulation of the radioactive label in an osmotically responsive intravesicular space. Because of the long period of incubation, the transport observed in this experiment represented transport of radioactive label in the form of [1-^{14}C]glycyl-L-proline as well as [1-^{14}C]glycine resulting from hydrolysis.

Effects of amino acids and dipeptides on glycyl-L-proline transport into control and papain-treated vesicles

The effects of five dipeptides and equivalent concentrations of the constituent amino acids on glycyl-L-proline transport into control and papain-treated vesicles were studied using both NaCl and KCl media. The results are given in Table I. All five dipeptides were potent inhibitors of glycyl-L-proline transport in the control as well as in the papain-treated vesicles. The inhibition was seen both in the presence and absence of a Na^+ gradient. In all cases, the inhibition by dipeptides was significantly greater than the inhibition by an equivalent concentration of the constituent amino acids. Glycyl-L-proline transport was completely Na^+-independent in control and papain-treated vesicles. In the case of control vesicles, the transport measured was 120.6 ± 1.0 pmol/mg protein in NaCl medium and 118.8 ± 4.6 pmol/mg protein in KCl medium. In the case of

TABLE I

EFFECTS OF DIPEPTIDES AND AMINO ACIDS ON GLYCYL-L-PROLINE TRANSPORT

Untreated and papain-treated vesicles were incubated with 160 μM [1-^{14}C]glycyl-L-proline for 1 min at 25°C either in NaCl or KCl medium. In untreated vesicles, the transport measured was 120.6 ± 1.5 pmol/mg protein in NaCl medium and 118.8 ± 4.6 pmol/mg protein in KCl medium. In papain-treated vesicles, the transport measured was 176.9 ± 18.8 pmol/mg protein in NaCl medium and 168.7 ± 10.9 pmol/mg protein in KCl medium. These transport rates are assigned a relative value of 100. Results are expressed as the average transport relative to the control transport ± S.D. The data are from two experiments done in duplicate.

Addition	Relative transport			
	Untreated vesicles		Papain-treated vesicles	
	NaCl	KCl	NaCl	KCl
Control	100 ± 2	100 ± 4	100 ± 11	100 ± 6
L-Alanyl-L-proline (8 mM)	33 ± 1	36 ± 2	38 ± 4	36 ± 3
L-Alanine (8 mM) + L-proline (8 mM)	96 ± 6	100 ± 6	90 ± 9	93 ± 6
L-Prolyl-L-leucine (8 mM)	46 ± 2	54 ± 3	44 ± 4	43 ± 2
L-Proline (8 mM) + L-leucine (8 mM)	90 ± 7	91 ± 3	82 ± 8	76 ± 5
Glycyl-glycine (8 mM)	94 ± 3	81 ± 3	39 ± 4	39 ± 2
Glycine (16 mM)	89 ± 2	98 ± 5	71 ± 7	82 ± 5
Glycyl-L-leucine (8 mM)	36 ± 1	42 ± 3	33 ± 4	36 ± 2
Glycine (8 mM) + L-leucine (8 mM)	80 ± 1	85 ± 5	72 ± 8	80 ± 5
Carnosine (β-alanyl-L-histidine) (8 mM)	46 ± 2	54 ± 4	39 ± 4	51 ± 3
β-Alanine (8 mM) + L-histidine (8 mM)	80 ± 1	96 ± 7	74 ± 8	74 ± 5

papain-treated vesicles, the transport measured was 176 ± 18.8 pmol/mg protein in NaCl medium and 168.7 ± 10.9 pmol/mg protein in KCl medium.

Solubilization of enzymes with papain

Papain treatment (100 μg/mg) for 30 min at 37°C solubilized 55 ± 4% of alkaline phosphatase, 90 ± 7% of γ-glutamyltransferase 95 ± 3% of leucine aminopeptidase and 38 ± 4% of protein. The hydrolysis of glycyl-L-proline was similar in control and papain-treated vesicles, indicating that glycyl-L-proline hydrolase activity was not affected by papain treatment.

Extravesicular space in control and papain-treated vesicles

In order to look for possible increased permeability of the vesicles by papain treatment, the extravesicular space was determined using [*methoxy*-^{14}C]inulin. The extravesicular space in control vesicles was 40 ± 3% of the wet weight of the vesicles, whereas the extravesicular space in papain-treated vesicles was 31 ± 3% of the wet weight of the vesicles.

Discussion

There are different mechanisms present in the tubular cells to reabsorb the solutes filtered through the glomerulus. Large polypeptides and proteins are reabsorbed by endocytosis and are hydrolyzed by lysosomal enzymes. Glucose and amino acids are reabsorbed by specific Na^+-dependent, carrier-mediated transport mechanisms. Recent studies [15—19] indicate that small linear peptides are handled in the proximal tubule by a mechanism, different from that of large polypeptides and proteins. Small linear peptides appear to be hydrolyzed at the luminal membrane and the liberated amino acids are probably taken up by the amino acid transport systems.

The information on the handling of di- and tripeptides by renal tubular cells is scanty. Welch and Campbell [28] recently studied the transport of L-alanylglycine into renal brush border vesicles. They found that the hydrolysis of this dipeptide in the medium was instantaneous (5 s or less) and the characteristics of the transport of radiolabel from L-alanyl-[^{3}H]glycine and [^{14}C]glycine were similar. They concluded that the transport of dipeptide amino acids by renal tubular cells occur by hydrolysis of the dipeptides at the external surface of the membrane, followed by transport of the released amino acids by the same Na^+-dependent transport systems known to exist for free amino acids.

Treatment of intestinal brush border vesicles with papain has been shown to solubilize a number of membrane hydrolases effectively [32]. Recently, Berteloot et al. [38] have shown that the use of high concentrations of papain led to the solubilization of amino acid and glucose carriers from the intestinal brush border vesicles. We used papain in the present study to see whether the membrane components involved in dipeptide transport are equally susceptible to papain treatment. Papain reduced the Na^+-dependent L-alanine transport measured at the overshoot by 90% whereas transport of glycyl-L-proline was slightly stimulated. The data on L-alanine transport can be interpreted in two ways. First, papain either partially solubilized or inactivated the amino acid carrier(s), or that part of the carrier(s) which determines the Na^+-dependence.

Second, papain treatment may have rendered the vesicles leaky because of the removal of most of the surface components and the increased leakiness accounted for the apparent reduction of amino acid transport.

The available evidence indicates that papain does not alter the permeability of the vesicles. Papain is known to remove a number of surface components from membrane vesicles, without affecting the lipid bilayers [32]. Hughey et al. [39] have shown that papain treatment of lecithin vesicles containing Triton-purified γ-glutamyl transferase resulted in the release of 95% of the enzyme activity without releasing the internally trapped [^{3}H]sucrose. If papain treatment results in a nonspecific increased permeability, the effects on amino acid and dipeptide transport should be similar, but they are not. Also, the extravesicular space of the papain-treated vesicles was less than that of the control vesicles. Increased permeability would have resulted in increased extravesicular space. The reduced extravesicular space of the treated vesicles may be due to removal of most of the surface components by papain, thereby allowing closer approximation of the treated vesicles. The equilibrium transport of L-alanine was the same in the control and papain-treated vesicles (Fig. 1A), indicating that the intravesicular volume remained unchanged. The normal osmotic behavior of papain-treated vesicles shows that the vesicles still contain a tightly closed, osmotically responsive intravesicular space, regardless of whether the label was in the form of dipeptide, amino acids or mixtures.

There is a remarkable similarity in the structure, morphology, and enzyme pattern of renal and intestinal brush border membranes. The characteristics of sugar and amino acid transport systems in these membranes are also remarkably similar. Nevertheless, it is widely believed that, while intestine can transport di- and tripeptides by a carrier-mediated mechanism, the proximal tubule does not possess such a transport system [19].

Recently we have become interested in the mechanisms of the handling of dipeptides by tubular cells. We have selected glycyl-L-proline for this purpose because of its very slow hydrolysis by renal brush border vesicles. Our earlier study [30] showed that more than 80% of the dipeptide remained intact in the medium even after 30 min incubation. The transport of glycyl-L-proline was completely Na^+-independent whereas L-alanine transport was stimulated 4.3-fold in the presence of a Na^+ gradient. These results are in obvious disagreement with those of Welch and Campbell [28]. The reason for this disagreement is due to the differences in the rates of hydrolysis of the dipeptides employed in these two studies.

The difference in the effects of papain and diamide on the transport of L-alanine and glycyl-L-proline suggests that the mechanisms of handling of these solutes by the vesicles are quite different. Diamide has been shown to inhibit Na^+-dependent sugar and amino acid uptake by renal cortical slices [37,40,41] as well as by purified renal brush border vesicles [42].

Table I shows that the transport of glycyl-L-proline was inhibited by other dipeptides more effectively than by an equivalent concentration of the constituent amino acids. If the hydrolysis at the external surface of the membrane followed by the transport of released free amino acids is the actual mechanism of glycyl-L-proline transport, the inhibition by free amino acids would be greater than the inhibition by the corresponding dipeptides.

This study also shows that neither leucine aminopeptidase nor γ-glutamyl transferase plays any role in the dipeptide transport because glycyl-L-proline transport remained unaffected even after removal of 90—95% of these enzymes by papain treatment. Since papain treatment leads to significant enrichment of dipeptide transport, this can be successfully employed as an initial step in the studies intended to purify the dipeptide carrier.

The data presented in this paper indicate that, like intestinal brush border vesicles, renal brush border vesicles also can transport dipeptides by a mechanism completely different from that for free amino acids. Elevated plasma levels of dipeptides have been demonstrated after a protein meal [23]. Recent studies [20,21] have shown that various dipeptides, when infused intravenously, are efficiently utilized in the body, and the kidney apparently plays a predominant role in the clearing of these dipeptides from the plasma. A number of peptide hormones are filtered through the glomeruli. The action of several endopeptidases and dipeptidylpeptidase IV of renal brush border membrane on these hormonal peptides would result in the elevated levels of dipeptides in the filtrate. The presence of a specific mechanism in the renal brush border membrane to reabsorb dipeptides would prevent the urinary loss of amino acids in the form of dipeptides, thereby conserving nitrogen, calories and essential amino acids.

Renal tubular cells contain a very active cytosol glycyl-L-proline hydrolase [43]. One of the functions of this enzyme may be to hydrolyze glycyl-L-proline entering the cell through this dipeptide transport system under normal physiological conditions. This would result in a very low cytoplasmic dipeptide concentration which would maintain a favorable peptide concentration gradient for further peptide transport by facilitated (carrier-mediated) diffusion.

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