Transport of L-alanine into Intestinal and Renal Brush Border Vesicles from Rabbit

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Transport of labeled N-glycol-L-proline has been shown to occur with highly purified brush border membrane vesicles from the epithelial cells of rabbit small intestine and renal cortex. With 1-min incubation, transport occurs mainly as the intact dipeptide since less than 10% of the dipeptide in the medium is hydrolyzed within the period. The properties of the dipeptide transport system are similar in both small intestinal and renal brush border membrane vesicles. The steady state transport varies inversely with medium osmolarity. Extrapolation to infinite medium osmolarity indicates that transport occurs predominantly into an osmotically reactive intravesicular space rather than binding to the membranes. The affinity constants (Kd) for glycyl-L-proline transport in small intestinal and renal brush border membrane vesicles are comparable (0.9 mM in intestine and 1.1 mM in kidney).

Under conditions in which presence of a Na+ gradient between external and intravesicular media stimulated L-alanine transport, glycyl-L-proline transport remains unaffected. Other dipeptides strongly inhibit the transport of glycyl-L-proline but amino acids have no effect. The selective inhibition of glycyl-L-proline transport by other dipeptides is observed in the presence as well as in the absence of a Na+ gradient. Harmaline inhibits Na+-stimulated L-alanine transport but it has no effect on glycyl-L-proline transport even in the presence of Na+. In these respects, dipeptide transport seems to differ from amino acid transport. It is proposed that the Na+ gradient hypothesis of sugar and amino acid transport is not applicable for dipeptide transport. These data provide additional evidence for the distinct nature of amino acid and dipeptide transport systems.

Experimental Procedures

Preparation of Brush Border Membrane Vesicles—The brush border membrane vesicles were prepared from rabbit small intestine and kidney cortex by a combined procedure of Malmot et al. (22) and Kessler et al. (23). After the rabbits were killed by a lethal dose of Nembutal, the entire small intestine from the pyloric end to the ileocecal junction was taken out. The intestine was washed with ice-cold KCl (0.154 M) and cut open longitudinally. The mucosa was scraped off and homogenized in 30 volumes (v/w) of 8-mM Tris-HCl buffer, pH 7.0, for 2 min using a Waring Blender. Then a 1-M CaCl2 solution was added to the homogenate to a final concentration of 10 mM and the mixture was stirred in an ice bath for 10 min. It was then allowed to stand for 15 min before it was centrifuged at 3,000 × g for 15 min in a refrigerated centrifuge. The supernatant was carefully decanted and centrifuged again at 43,000 × g for 20 min. The pelleted material representing brush border membranes was resuspended in an equal volume of the same buffer using a 1-ml syringe and a 25-gauge needle and centrifuged again at 43,000 × g for 20 min. The pellet was then suspended through a 25-g needle in a medium containing 300 mM mannitol, buffered with 1 mM
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Tris-Hepes to pH 7.5. The membrane vesicles were now ready for transport studies.

For the preparation of renal brush border membrane vesicles, the cortices from fresh rabbit kidneys were dissected from the medulla and were subjected to the same procedure described above for intestine except that the homogenization was done for 5 min using a Waring Blender. The quality of the vesicles was regularly evaluated by measuring alkaline phosphatase, a marker enzyme for brush border membrane.

Uptake Experiments—Uptake of glycyl-L-proline and L-alanine was measured by Millipore filtration technique as described by Kippen et al. (24). The uptake buffer was 1 mM Hepes adjusted to pH 7.5 with Tris base. The buffer contained 100 mM mannitol, 100 mM NaCl, 100 mM mannitol plus 100 mM NaCl, or 100 mM mannitol plus 100 mM KCl and, in addition, glycyl-L-proline or L-alanine of required concentrations (including tracer amounts of respective labeled compounds). When the effect of various compounds on the uptake of the dipeptide was studied, the osmolarity of the buffers was adjusted to 300 mosm by appropriately adjusting the concentration of mannitol and pH was adjusted to 7.5 with Tris or HCl. Osmolarity of the solutions used in this study was measured using a Fiske Osmometer (Fiske Associates, Uxbridge, MA).

Uptake into brush border membrane vesicles was initiated by addition of 50 μl of the membrane suspension containing 0.6 to 0.8 mg of membrane protein to 100 μl of uptake buffer. The mixture was shaken gently at 25°C in a gyratory water bath shaker (model G76, New Brunswick Scientific, Edison, NJ). At the end of the incubation period, uptake was stopped by adding 2 ml of ice-cold 154 mM NaCl in 50 mM Tris-Hepes buffer, pH 7.5 (4). The suspension was then rapidly filtered through a Millipore filter (HAWP 0.45 μm) and washed twice with 4 ml of the same buffer. The filter was then placed in a counting vial and 10 ml of scintillation mixture were added. The scintillation mixture consisted of toluene-Triton X-100 in a ratio 2:1 (v/v) containing 4 g of 2,5-diphenyloxazole (PPO) and 0.1 g of p- bis[2-(5-phenyloxazolyl)] benzene (POPPOP)/liter of toluene. The radioactive activity was counted in a liquid scintillation spectrometer (Beckman, model LS-230 or LS-3135T). Correction for nonspecific binding to the membranes and filters was done by subtracting from all data the value of a blank prepared routinely by adding membranes to a tube to which stop buffer had already been added. The experiments were regularly done in duplicate and the variation between duplicate experiments was always less than ±10% of the mean value.

Radioactive Isotope—[1-14C]Glycyl-L-proline was first checked for radioactivity by paper chromatography with n-butyl alcohol/acetic acid/water (4:1:1, by volume) as the solvent system. The chromatogram was scanned using a Packard radiochromatogram scanner. It was found that the sample contained a slight contamination (14%) of radioactive impurities. However, it did not contain any ninhydrin-positive material other than glycyl-L-proline. No attempt was made to identify the ninhydrin-negative impurities.

Purity of Brush Border Membrane Vesicles—Membrane purity was determined routinely by assay of alkaline phosphatase (25), a marker enzyme for brush border membrane of both small intestinal and renal epithelial cells. Occasionally (Na+-K+) ATPase, a marker enzyme for basal-lateral membrane was also determined (26) to monitor the contamination of this membrane in the brush border membrane preparations. Alkaline phosphatase was enriched 8- to 17-fold (mean, 14-fold) in the intestinal brush border vesicles and 8- to 12-fold (mean, 10-fold) in renal brush border membrane vesicles. In general, enrichment of alkaline phosphatase in intestinal brush border vesicles was more when compared to that in renal brush border vesicles. The specific activities of alkaline phosphatase in intestinal (1.02 ± 0.41 μmol/min/mg of protein) and renal (0.94 ± 0.33 μmol/min/mg of protein) brush border vesicles were comparable to those reported by other methods (Na+-K+) ATPase activity was undetectable in most preparations although trace activity was occasionally present. The maximum specific activity of (Na+-K+) ATPase observed in these preparations (0.02 μmol/min/mg of protein) was significantly less than that in the homogenate (0.08 ± 0.02 μmol/min/mg of protein). Thus, the brush border membrane preparations employed in these studies were almost completely free from basal-lateral membranes. The vesicularity of the membrane preparations was occasionally checked by electron microscopy. The purified membranes appeared homogeneous and vesicular. The effect of medium osmolarity on the equilibrium uptake of radioactivity into these vesicles (see “Results”) showed that these vesicles contained a tightly closed intravesicular space.

Protein was determined by the method of Lowry et al. (27), with crystalline bovine serum albumin as the standard.

Paper Chromatography—Paper chromatography was used to detect and to quantify the amount of free glycine in the medium after incubating the membrane preparations with labeled glycyl-L-proline for various time intervals. Descending chromatograms were developed with Whatman No. 1 paper using n-butyl alcohol/acetic acid/water (4:1:1, by volume) as the solvent system. An 0.35 ml mixture of glycine, acetone/water (19:1, v/v) was used to detect amino acids and dipeptides on the chromatograms.

Materials—Unlabeled glycyl-L-proline, glycylglycine, and harmine were obtained from Sigma. Carnosine, L-leucylglycine, and L-amino acids (A grade) were from Calbiochem. Toluene used in the scintillation mixture was obtained from Fisher. Triton X-100 was from Sigma. 2,5-Diphenyloxazole was purchased from Mallinckrodt and p-bis[2-(5-phenyloxazolyl)] benzene from Packard Instrument Co.

Results

Time Course—Fig. 1 and 2 describe the uptake of radioactive activity from 1.2 μM [1-14C]glycyl-L-proline during incubation for different lengths of time into intestinal and renal brush border membrane vesicles, respectively. Three different media were used, 200 mM mannitol, 100 mM mannitol plus 100 mM NaCl, and 100 mM mannitol plus 100 mM KCl. Uptake of radioactive activity from the mannitol medium into intestinal brush border vesicles was greater than that from the NaCl medium at all time periods. However, a slight stimulation of initial uptake by the presence of a Na+ gradient was observed with renal brush border vesicles. Since a complete mannitol medium is unphysiological, it might be more appropriate to compare uptake in a NaCl medium with that in a KCl medium, because K+ is the major biologically counteracting cation. As can be seen from the figures, replacement of Na+ by K+ did not decrease uptake of radioactivity into intestinal or renal brush border vesicles. In fact, uptake was found to be greater than that in the NaCl medium.
in KCl medium than that in NaCl medium after longer periods of incubation. With intestinal brush border vesicles, uptake values in NaCl medium decreased after 10 min (Fig. 1) which was probably due to efflux of radioactivity from the vesicles after initial uptake. This phenomenon was, however, not observed with renal brush border vesicles. When the vesicles were incubated for a short time (5 to 10 min), the qualitative rate of uptake was similar in both NaCl and KCl mediums, although the absolute levels of uptake were almost an order of magnitude higher in intestinal brush border vesicles relative to those of kidney (Figs. 1 and 2). Since the transport observed at longer periods of incubation represents a combination of peptide and free amino acid transport, the results in Figs. 1 and 2 are given as Gly-L-Pro equivalent.

Extent of Hydrolysis of Glycyl-L-proline in the Medium—When intestinal and renal brush border membranes were assayed for the presence of peptidases, it was found that these preparations contained significant glycyl-L-proline hydrolyzing activity. Since hydrolysis of the dipeptide into free amino acids in the medium would make it difficult to interpret the results, it was necessary to quantify the extent of hydrolysis during incubation for different lengths of time. Table I shows that there was more glycyl-L-proline hydrolyzing activity in intestinal brush border membranes compared to renal brush border membranes. With 1-min incubation, 16% of glycyl-L-proline in the medium was hydrolyzed per mg of intestinal brush border protein while there was only 9% hydrolysis in the case of renal brush border preparations. The extent of hydrolysis in the medium increased with longer periods of incubation in both preparations. After 30-min incubation, 75% of the dipeptide was hydrolyzed to free amino acids with intestinal brush border preparations, while only 30% hydrolysis occurred with renal brush border preparations. Since the concentration of amino acids in the medium was very low after 1-min incubation, the uptake of radioactivity would be predominantly in the form of intact dipeptide rather than free amino acids. Hence, subsequent experiments were carried out employing this short incubation period, i.e. 1 min.

Glycyl-L-proline hydrolyzing activity of intestinal and renal brush border membranes could not be removed by repeated washings of the membranes with 50 mM mannitol/2 mM Tris-HCl buffer, pH 7.0, p-Hydroxymercuribenzoate (0.1 mM), a selective inhibitor of cytosol peptidases (28), did not inhibit these activities either in the presence or absence of 0.05% deoxycholate in the assay mixture. The detergent served to break down the vesicular compartmentation and release contaminating cytosol peptidases, if any, trapped within the vesicles. The detergent had no effect on glycyl-L-proline hydrolyzing activity of the membrane preparations. It, therefore, appears that the glycyl-L-proline hydrolyzing activity was, in fact, an integral part of the brush border membranes rather than contamination by cytosol peptidases.

Effect of Harmaline on Glycyl-L-proline and L-Alanine Uptake—Table II shows the effects of Na⁺ gradient and harmaline on the uptake of glycyl-L-proline and L-alanine into intestinal and renal brush border vesicles. The presence of a Na⁺ gradient stimulated L-alanine uptake 4.5-fold in intestine and 2.1-fold in kidney. But, uptake of glycyl-L-proline was not influenced by Na⁺ in either preparation. Harmaline, an inhibitor of Na⁺-dependent transport processes (15, 29) reduced Na⁺-stimulated L-alanine uptake significantly but did not effect glycyl-L-proline uptake.

Effects of Amino Acids and Other Dipeptides on Glycyl-L-proline Uptake in Presence and Absence of a Na⁺ Gradient—Table III shows the effects of certain amino acids and dipeptides on glycyl-L-proline uptake in the presence and absence of a Na⁺ gradient. Glycyl-L-proline uptake into intestinal and renal brush border membrane vesicles was markedly inhibited by dipeptides like glycyl-L-proline, L-histidyl-L-proline, carnosine, and L-leucylglycine. This inhibition was observed both in the presence and absence of a Na⁺ gradient. However, glycylglycine was without any effect. Amino acids like glycine, L-alanine, and L-leucine did not inhibit the uptake. Small but highly reproducible inhibition of uptake of glycyl-L-proline into intestinal brush border vesicles was observed with L-proline in presence of a Na⁺ gradient. In con-

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>Glycyl-L-proline uptake</th>
<th>L-Alanine uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannitol (100 mM) + KCl (100 mM)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mannitol (100 mM) + NaCl (100 mM)</td>
<td>87.6 ± 6.8</td>
<td>450.2 ± 20.7</td>
</tr>
<tr>
<td>Mannitol (98 mM) + NaCl (100 mM) + harmaline (2 mM) (no preincubation)</td>
<td>109.6 ± 4.4</td>
<td>388.8 ± 12.3</td>
</tr>
<tr>
<td>Mannitol (98 mM) + NaCl (100 mM) + harmaline (2 mM) (preincubated with 2 mM harmaline for 10 min)</td>
<td>95.8 ± 5.4</td>
<td>338.5 ± 13.5</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannitol (100 mM) + KCl (100 mM)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mannitol (100 mM) + NaCl (100 mM)</td>
<td>107.9 ± 3.6</td>
<td>208.7 ± 5.0</td>
</tr>
<tr>
<td>Mannitol (98 mM) + NaCl (100 mM) + harmaline (2 mM) (no preincubation)</td>
<td>107.7 ± 4.9</td>
<td>182.2 ± 2.9</td>
</tr>
<tr>
<td>Mannitol (98 mM) + NaCl (100 mM) + harmaline (2 mM) (preincubated with 2 mM harmaline for 10 min)</td>
<td>105.0 ± 2.8</td>
<td>165.4 ± 3.7</td>
</tr>
</tbody>
</table>

**Table II**

**Effects of a Na⁺ gradient and harmaline on glycyl-L-proline and L-alanine uptake**

Brush border membrane vesicles were incubated with 80 µM glycyl-L-proline or 20 µM L-alanine for 1 min at 25°C. The rate of uptake of the dipeptide in KCl medium was 126.6 ± 11.2 pmol × min⁻¹ × mg⁻¹ protein in the intestine and 87.3 ± 10.5 pmol × min⁻¹ × mg⁻¹ protein in the kidney. The rate of L-alanine uptake in KCl medium was 27.1 ± 3.4 pmol × min⁻¹ × mg⁻¹ protein in the intestine and 23.1 ± 2.2 pmol × min⁻¹ × mg⁻¹ protein in the kidney. Results are represented as the average uptake relative to the control uptake ± S.E.
trast, in the absence of a Na\(^+\) gradient, L-proline was not inhibitory. However, L-proline did not affect the uptake into renal brush border vesicles both in the presence and absence of Na\(^+\).

**Effect of Medium Glycyl-L-proline Concentration on the Dipeptide Uptake**—The effect of medium concentration of glycyl-L-proline on the uptake into intestinal and renal brush border vesicles was studied over a range from 80 to 1600 \(\mu\text{M}\), using a 1-min incubation period. Since the uptake was not influenced by Na\(^+\), the experiments were done in 300 mM mannitol medium. The uptake over this range of concentration was nonlinear and followed Michaelis-Menten kinetics. The results are shown in Fig. 3 as Lineweaver-Burk plots. The kinetic constants calculated by least squares fit analysis of the data suggested that the apparent \(K_I\) values for glycyl-L-proline uptake into intestinal and renal brush border vesicles were comparable (0.9 mM in intestine and 1.1 mM in kidney), while \(V_{\text{max}}\) values differed significantly. \(V_{\text{max}}\) was 3.3 nmol/min/mg of protein in the case of intestinal brush border vesicles and 0.81 nmol/min/mg of protein in the case of renal brush border vesicles.

Since the concentration of glycyl-L-proline (0.16 mM) employed to check the effect of Na\(^+\) was much lower than the \(K_I\) values observed (kidney, 0.9 mM; intestine, 0.9 mM), it is possible that the effect of Na\(^+\), if any, on glycyl-L-proline transport might have gone unnoticed. Therefore, the effect of Na\(^+\) was studied using 2.5 mM glycyl-L-proline and employing 1-min incubation period. In the kidney, the rate of glycyl-L-proline transport was 606 \(\pm\) 42 pmol/mg of protein in the presence of Na\(^+\) and 624 \(\pm\) 31 pmol/mg of protein in the presence of K\(^+\). In the intestine, it was 1.61 \(\pm\) 0.12 nmol/mg of protein in the presence of Na\(^+\) and 1.86 \(\pm\) 0.17 nmol/mg of protein in the presence of K\(^+\). Thus, the presence of a Na\(^+\) gradient had no effect on glycyl-L-proline transport either in kidney or in intestine even at higher concentration of the dipeptide.

**Table III**

<table>
<thead>
<tr>
<th>Addition</th>
<th>KCl medium</th>
<th>NaCl medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Glycyl-L-proline</td>
<td>30.5 (\pm) 5.2</td>
<td>37.0 (\pm) 3.5</td>
</tr>
<tr>
<td>L-Histidyl-L-proline</td>
<td>55.1 (\pm) 3.4</td>
<td>31.5 (\pm) 4.8</td>
</tr>
<tr>
<td>L-Leucylglycine</td>
<td>80.1 (\pm) 2.5</td>
<td>76.7 (\pm) 2.4</td>
</tr>
<tr>
<td>Carnosine</td>
<td>87.7 (\pm) 4.3</td>
<td>83.6 (\pm) 5.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>93.4 (\pm) 4.8</td>
<td>95.7 (\pm) 7.2</td>
</tr>
<tr>
<td>L-Proline</td>
<td>97.4 (\pm) 3.9</td>
<td>73.5 (\pm) 2.8</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>103.0 (\pm) 7.6</td>
<td>106.2 (\pm) 4.3</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>102.1 (\pm) 6.7</td>
<td>109.1 (\pm) 3.0</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Glycyl-L-proline</td>
<td>56.3 (\pm) 4.0</td>
<td>62.2 (\pm) 3.4</td>
</tr>
<tr>
<td>L-Histidyl-L-proline</td>
<td>52.3 (\pm) 3.2</td>
<td>49.1 (\pm) 5.9</td>
</tr>
<tr>
<td>L-Leucylglycine</td>
<td>65.9 (\pm) 2.6</td>
<td>92.6 (\pm) 1.6</td>
</tr>
<tr>
<td>Carnosine</td>
<td>77.4 (\pm) 6.4</td>
<td>70.5 (\pm) 3.1</td>
</tr>
<tr>
<td>Glycine</td>
<td>93.0 (\pm) 8.4</td>
<td>93.5 (\pm) 3.1</td>
</tr>
<tr>
<td>L-Proline</td>
<td>101.7 (\pm) 7.2</td>
<td>102.1 (\pm) 3.2</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>103.7 (\pm) 2.8</td>
<td>102.5 (\pm) 2.1</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>100.9 (\pm) 3.3</td>
<td>94.5 (\pm) 6.9</td>
</tr>
</tbody>
</table>

**Effect of Medium Osmolarity on the Uptake**—The osmotic behavior of intestinal and renal brush border membrane vesicles was studied by measuring the uptake of radioactivity after 40-min incubation in 300 mM mannitol medium. A 40-min incubation period was chosen because uptake of radioactivity into vesicles reached a saturable stage at this period (Figs. 1 and 2). Increasing concentrations of mannitol to which the vesicles were impermeant were correlated with decreasing accumulation of radioactivity within the intravesicular space (Fig. 4). Extrapolation to infinite medium osmolarity (zero intravesicular space) in the case of intestinal brush border vesicles resulted in no uptake and this indicated that uptake of radioactivity was completely due to transport into intravesicular space rather than binding to the membrane. However, in the case of renal brush border vesicles, there was some residual uptake of radioactivity even at infinite medium osmolarity suggesting that a portion of the uptake may have been due to binding to the membranes. This residual uptake
represented less than 20% of the total uptake under isosmolar conditions (300 mosm).

It was also noticed with intestinal as well as renal brush border membrane vesicles that uptake was inversely related to medium osmolarity only if osmolarity was above 200 mosm (osmolarity \( < 5 \)). With medium osmolarity below 200 mosm (osmolarity \( > 5 \)), the uptake no longer increased with decreasing osmolarity. Under these very low osmolar conditions, the uptake decreased. This was probably due to osmotic swelling of the vesicles under these conditions, leading to increased efflux of radioactivity from leaking or partially torn vesicles.

**DISCUSSION**

Studies on amino acid and peptide transport in the mammalian intestine have established the distinct nature of these two transport systems (for a review see Refs. 30 and 31). One of the main differences was their differential dependence on Na\(^+\). Although most studies have shown that the presence of a Na\(^+\) gradient stimulated transport of amino acids and peptides, the kinetics of stimulation differed. The presence of Na\(^+\) increased the affinity of amino acids to their transport system without altering the \( V_{\text{max}} \), while the effect of Na\(^+\) on peptide transport was to cause an increase in \( V_{\text{max}} \) without changing affinity. It, thus, appears that the effect of Na\(^+\) on amino acid transport is to facilitate binding of amino acids to the carrier. There is growing evidence for the Na\(^+\) gradient hypothesis of sugar and amino acid transport postulated by Crane (1) which suggests that sugars and amino acids are cotransported with Na\(^+\). In the intestinal and renal brush border membrane largely accounts for the transport of these solutes. This hypothesis is strongly supported by the results of recent studies on amino acid and sugar transport into highly purified brush border membrane vesicles from small intestine and renal cortex (2-9). One of the main advantages of this system is that these preparations are completely devoid of the (Na\(^+-\)K\(^+)\)-ATPase whose presence would otherwise make it difficult to interpret the exact role of Na\(^+\).

Peptide transport has so far been studied using a wide variety of biological preparations including intact animals, everted intestinal sacs, and tissue slices. In all of these complex preparations, net transport represents the sum of numerous processes functioning both in series and in parallel to peptide transport. In such systems, the relative contribution of peptide carrier(s), tissue metabolism, and ion gradients across the cell is difficult to determine. Removal of Na\(^+\) from the incubation medium of an intact cellular preparation may alter oxygen consumption, K\(^+\) gradients, and cytoplasmic ATP concentrations independent of any direct effect it has on peptide carrier(s) affinities.

The recent finding that (Na\(^+-\)K\(^+)\)-ATPase containing basolateral membrane vesicles are also capable of transporting solutes via carrier-mediated processes (32, 33) makes it imperative that this system as well as the brush border membrane vesicle system be carefully purified free from one another, and extensively characterized to define the role of Na\(^+\) in the primary peptide translocation step. The vesicles used in the current investigation were almost free of (Na\(^+-\)K\(^+)\)-ATPase activity, and hence presumably free of basolateral membrane contamination. Since they were also free of cytoplasmic components, they represent an ideal preparation for studying peptide transport. The only complicating factor was that as the membranes were purified, a number of peptidases in the preparation were also enriched. The presence of peptidases in the brush border membrane of the enterocyte has always made it difficult to interpret the results of peptide transport. This led investigators of peptide transport to use peptides which are rather unphysiological but highly resistant to hydrolysis (11-14). We selected glycyl-L-proline as a model dipeptide to study peptide transport because it has been found that there was no glycyl-L-proline hydrolyzing activity in the brush border membrane of monkey (15) and rabbit (34) small intestines. Even in rabbit kidney brush border preparations, no prolidase or prolinase activity was demonstrable (35).

However, in the present study (Table I), we found that there was significant glycyl-L-proline hydrolyzing activity in intestinal and renal brush border membranes from rabbit. The discrepancy in these studies may be due to the fact that previous investigators used crude pellet preparations of the intestinal homogenate to check the activity and in the present study, we used highly purified brush border membranes for this purpose. We also found glycyl-L-proline hydrolyzing activity in the purified renal brush border membranes from dog and pig.

There is an active prolidase in the soluble fraction of the epithelial cells of small intestine (36) and renal tubules (37). It is possible that glycyl-L-proline hydrolyzing activity detected in the brush border membranes may be the soluble enzyme adsorbed onto the membranes. However, the facts that repeated washings failed to remove the activity and p-hydroxymercuribenzoate (0.1 mM) did not inhibit it suggest that glycyl-L-proline hydrolyzing activity was an integral part of these membrane preparations.

Studies on the uptake of radioactivity from different media (mannitol, NaCl, and KCl) showed that the initial uptake was similar in NaCl and KCl media. The replacement of Na\(^+\) by K\(^+\) did not decrease the uptake either in intestinal or in renal brush border membrane vesicles. The decrease in the uptake values after 10-min incubation in the case of intestinal brush border vesicles in NaCl medium was probably due to the efflux of radioactive glycine from the intravesicular space. Since the concentrations of free amino acids in the medium after prolonged incubation of the dipeptide with the intestinal brush border preparation were very high (Table I), the uptake of radioactivity under these conditions partly represented free amino acid uptake. The efflux of free glycine would be facilitated in NaCl medium, and not in KCl medium, because of the Na\(^+\)-dependent mechanism of glycyl-L-proline transport. In the case of renal brush border membrane vesicles, there was no decrease in the uptake after long incubation even in NaCl medium. This was probably because the concentration of free glycine in the medium was very low even after 30-min incubation of the dipeptide with renal brush border membrane preparation, due to the much lower level of peptidase activity in the preparation.

The presence of Na\(^+\) gradient across the brush border membrane stimulated L-alanine transport significantly, 4.5-fold in small intestine and 2.1-fold in kidney. Under similar conditions, glycy-L-proline transport was not stimulated by the Na\(^+\) gradient. Harmaline, a well known inhibitor of Na\(^+\)-dependent transport processes inhibited Na\(^+\)-stimulated L-alanine transport in both intestine and kidney but was without any effect on glycyl-L-proline transport.

Another important difference between amino acid and dipeptide transport into brush border membrane vesicles was their inhibition by either amino acids or dipeptides. Fass et al. (7) have shown that L-alanine transport into renal brush border vesicles was strongly inhibited by other amino acids in the presence of a Na\(^+\) gradient. But, in the absence of a Na\(^+\) gradient, the inhibition was small and statistically insignificant. In the present study, glycyl-L-proline transport was inhibited significantly by glycyl-L-proline, L-histidyl-L-proline,

1 V. Ganapathy and F. H. Leibach, unpublished observations.

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Dipeptide Transport in Intestinal and Renal Brush Border Vesicles

L-leucylglycine, and carnosine and the inhibition was observed in the presence as well as in the absence of a Na" gradient. Carnosine and L-leucylglycine were relatively less potent inhibitors when compared to glycyl-L-proline and L-histidyl-L-proline. Carnosine was not hydrolyzed by the brush border membrane preparations and hence its low inhibitory capacity is probably due to its low affinity for the dipeptide transport system. L-Leucylglycine is significantly hydrolyzed by these preparations even with a short incubation period and this may be one of the reasons for its low inhibitory capacity since free amino acids, except L-proline, did not inhibit glycyl-L-proline transport. Glycylglycine was totally ineffective as an inhibitor in either NaCl or KCl medium. L-Proline inhibited glycyl-L-proline uptake into intestinal brush border vesicles in the absence of a Na" gradient. The inhibition was small but highly reproducible.

This work clearly demonstrates that the dipeptide transport into intestinal and renal brush border vesicles is totally Na," independent. The partial dependence of dipeptide transport on Na" as observed by other investigators (10, 15) may be due to the fact that the systems employed in these earlier studies were more complex and the transport was studied in the presence of normal metabolic activity. The presence of basolateral membranes with their intrinsic (Na"-K")-ATPase in these systems also has made it difficult to pinpoint the site of action of Na". It now appears that Na" does not play any role in these preparations to hydrolyze this dipeptide (39). Amino acids like glycine, L-leucine, and L-alanine were not inhibitory to the peptide transport, because the transport measured at a single concentration of these peptides by the renal brush border membrane as it does in amino acid or sugar transport is probably due to its low affinity for the dipeptide transport system (38) and the presence of an active enzyme in these preparations to hydrolyze this dipeptide (39). Amino acids like glycine, L-leucine, and L-alanine were not inhibitory in either NaCl or KCl medium. L-Proline inhibited glycyl-L-proline uptake into intestinal brush border vesicles in the presence of a Na" gradient but was without effect in the absence of a Na" gradient. The inhibition was small but highly reproducible.

We were not able to detect intact glycyl-L-proline at any time inside the vesicles. Since there was no intravesicular, soluble glycyl-L-proline hydrolase, the hydrolysis of the peptide must have occurred because of the presence of the membrane bound hydrolase. This, however, does not mean that the transport was exclusively the result of free amino acid transport, because the transport measured at 1 min was significantly inhibited by other peptides, but not by free amino acids (Table III). This means that hydrolysis and transport of the peptide are closely integrated and the membrane bound hydrolase may be the site of integration, as suggested by Ugolev et al. (41).

Very little is known about handling of peptides by the renal tubules but it appears that some small peptides, especially those containing L-proline and hydroxy-L-proline, are transported by the tubules (42). Transport of intact carnosine by kidney cortex slices has been demonstrated recently (18). Adibi (19) has shown that the kidney plays an important role in clearing intravenously injected dipeptides. They have also shown that the kidney is capable of transporting intact glycylcarnosine. It has also been demonstrated recently that the major portion of labeled angiotensin II (20) and bradykinin (21) microinfused into proximal convoluted tubules of the rat kidney was reabsorbed and a small portion of the labeled material was excreted in the urine largely in the form of their smaller peptide fragments. These various studies indicate that the epithelial cells of renal tubules are capable of transporting small peptides. The present work shows that renal brush border membrane vesicles can transport glycyl-L-proline rapidly and that the K{sub}i value for glycyl-L-proline transport into kidney is comparable to that in small intestine. These studies suggest that the kidney may play an important role in transporting and subsequently catabolizing small peptides from plasma. It is relevant here to point out that a number of peptides have been identified both in plasma and in urine under physiological conditions (43, 44).

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REFERENCES

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