Evidence for Tripeptide-Proton Symport in Renal Brush Border Membrane Vesicles

STUDIES IN A NOVEL RAT STRAIN WITH A GENETIC ABSENCE OF DIPEPTIDYL PEPTIDASE IV*

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We have investigated the transport characteristics of L-phenylalanyl-L-prolyl-L-alanine in renal brush-border membrane vesicles isolated from Japan F344 rats. This particular rat strain genetically lacks dipeptidyl peptidase IV. Owing to the absence of this enzyme, the tripeptide was found to be completely resistant to hydrolysis by the renal brush-border membrane vesicles. Uptake of the tripeptide into these membrane vesicles in the presence of an inwardly directed Na⁺ gradient was slightly greater than in the presence of a K⁺ gradient, but there was no evidence for active transport. On the contrary, uptake was very rapid in the presence of an inside-alkaline transmembrane pH gradient, and accumulation of the tripeptide inside the vesicles against a concentration gradient could be demonstrated under these conditions. The uptake was drastically reduced by dissipation of the pH gradient. The uptake was stimulated by an inside-negative membrane potential and inhibited by an inside-positive membrane potential. Moreover, the uptake was greater in voltage-clamped membrane vesicles than in control vesicles. Many di- and tripeptides inhibited this pH gradient-stimulated uptake of Phe-Pro-Ala. The apparent dissociation constant for the tripeptide was 48 µM. High performance liquid chromatography analysis of the intravesicular content at the peak of the overshoot revealed that the tripeptide was transported across the membrane almost entirely in the intact form. These data provide the first direct evidence for the presence of an electrogenic tripeptide-proton symport in renal brush-border membranes.

However, most of the tripeptides used in these experiments are susceptible to hydrolysis by brush-border peptidases, and it has never been clear from these studies whether the observed inhibition was due to the intact tripeptides or due to the dipeptides which could have arisen as a result of hydrolysis of the tripeptides. Undoubtedly, the potential hydrolysis of tripeptides by brush-border-associated peptidases has been a major hindrance in investigations involving tripeptide transport. There is, however, a single study, reported 15 years ago, in which the intestinal transport of an unhydrolyzable tripeptide, Gly-Sar-Sar, was demonstrated in everted rings of hamster jejunum (19). Transport of this tripeptide was active, was inhibited by metabolic poisons, and was Na⁺-dependent. No definite conclusion can be drawn from this study regarding the driving force for tripeptide transport. Dependence of a transport system on Na⁺ in an intact tissue does not necessarily mean that the system is energized by a Na⁺ gradient. This is particularly obvious from the recent discovery that a H⁺ gradient, not a Na⁺ gradient, is the energy source for active transport of dipeptides in small intestine and kidney, even though dipeptide transport is Na⁺-dependent in intact tissues. Isolated brush-border membrane vesicles have been a valuable tool in identifying the driving force for dipeptide transport. It is therefore clear that investigations of transport of an intact tripeptide with brush-border membrance vesicle preparations would greatly enhance our understanding of the energetics of the transport process.

The purpose of the present investigation was to use a novel experimental animal model to successfully study the characteristics of the transport of an intact tripeptide in renal brush-border membrane vesicles without interference by hydrolysis. Watanabe et al. (20) have recently reported that F344 rats are genetically deficient in dipeptidyl peptidase IV, one of the major renal brush-border peptidases. Our recent studies show that this genetic deficiency is unique to F344 rats obtained from Japan because F344 rats available in the United States have normal dipeptidyl peptidase IV activity.¹ Dipeptidyl peptidase IV releases X-Pro or X-Ala type dipeptides from the amino-terminal end of larger peptides. Since tripeptides of the X-Pro-Y sequence are almost exclusively hydrolyzed by dipeptidyl peptidase IV, it was envisioned that the enzyme-deficient rats (Japan F344) can be used to investigate the renal transport of an intact tripeptide comprising this particular amino acid sequence. We have selected Phe-Pro-Ala as a model tripeptide for this purpose in the present study. The results indicate that (a) Phe-Pro-Ala is not hydrolyzed by renal brush-border membrane vesicles isolated from Japan F344 rats, (b) transport of the tripeptide in the intact form into the vesicles is demonstrable, and (c) transport of the...
Energetics of Renal Tripeptide Transport

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Tritiated tripeptide is active, is driven by an inwardly directed $H^+$ gradient, is electrogenic, and occurs via the tripeptide-proton symporter mechanism. This paper constitutes the first evidence for the $H^+$ gradient-coupled uphill transport of an intact tripeptide across the renal brush-border membrane.

EXPERIMENTAL PROCEDURES

Materials—[Irin-4-3H]Phenylalanyl-prolyl-alanine (specific radioactivity, 22 Ci/mmol) was purchased from the Radiochemical Centre, Amersham, England. Unlabeled peptides were obtained from Bachem Bioscience Inc., Philadelphia, PA (Phe-Pro-Ala) and from Sigma (others). The ionophores, FCF and valinomycin, were also obtained from Sigma. Gly-Pro-p-nitroanilide was a gift from Prof. Alfred Barth, Dept. of Biochemistry, Martin Luther University, Halle, Saale, GDR. All other chemicals used were of analytical grade.

Animals—A breeding pair of dipeptidyl peptidase IV-negative Fischer 344 rats was purchased from Charles River Inc., Kanagawa, Japan. A colony of these rats (Japan F344) is currently being maintained at the Medical College of Georgia. Control F344 rats (dipeptidyl peptidase IV-positive) were obtained from Charles River Inc., Raleigh, NC.

Preparation of Brush-Border Membrane Vesicles—Brush-border membrane vesicles were prepared from rat kidneys by Mg$^2+$-precipitation in the presence of EGTA (21). Briefly, the renal tissue comprising cortical and outer medullary regions are homogenized with 10 volumes of homogenizing buffer (120 mM NaCl, 5 mM EGTA, 300 mM mannitol, pH 7.5) for 90 s using an Ultra-Turrax, with the speed set at 50. To this homogenate, an equal volume of ice-cold distilled water was added. 1 M MgCl$_2$ was added to the above suspension to give a final concentration of 10 mM MgCl$_2$, and the mixture was stirred for 1 min and allowed to stand for 15 min. Then it was centrifuged at 3,000 x g for 10 min. The pellet was discarded. The supernatant was centrifuged at 38,000 x g for 30 min. The pellets containing the brush-border membranes were washed twice with a suitable preloading buffer by suspending the pellets in the buffer with 50 mM Hepes, 75 mM Tris buffer, pH 8.3, containing 100 mM K$_2$SO$_4$. The protein concentration of the membrane suspension was adjusted to 8 mg/ml, distributed in small aliquots in plastic tubes, and stored in liquid nitrogen until use.

Uptake Measurements—Uptake was measured at room temperature (21–22°C) as described earlier (6) using a rapid filtration technique. Millipore membrane filters (DAPW type, 0.65-μm pore size) were employed. Uptake was initiated by rapidly mixing 40 μl of the membrane suspension with 160 μl of uptake buffer containing radio-labeled tripeptide. The composition of the uptake buffer varied depending on the experiment. Uptake was terminated by the addition of 3 μl of ice-cold stop buffer (2 mM Hepes/Tris, 210 mM KCl, pH 7.5) followed by filtration. The filter was washed with the same buffer (3 x 5 ml) and then transferred to a counting vial. The radioactivity associated with the filter was counted by liquid scintillation spectrometry.

Assay of Dipeptidyl Peptidase IV Activity—Dipeptidyl peptidase IV activity was measured in purified brush-border membrane vesicles using Gly-Pro-p-nitroanilide as substrate (22). Brush-border membrane (25 μg of protein) was incubated with 2.5 mM Gly-Pro-p-nitroanilide in 125 mM Tris-HCl, pH 8.0, and the p-nitroanilide formed was monitored continuously over a period of 30 min at 22°C using a Shimadzu recording spectrophotometer (Model UV 160 U).

Measurement of Unlabeled Phe-Pro-Ala Hydrolysis by HPLC—Hydrolysis of unlabeled Phe-Pro-Ala by brush-border membrane vesicles was quantitated using HPLC. Brush-border membrane (25 μg of protein) was incubated with 5 mM Phe-Pro-Ala in 15 mM Tris-HCl, pH 8.0, at 37°C for 30 min. After the incubation, an equal volume of 10% TFA was added, and then the mixture was centrifuged to remove precipitated proteins. An aliquot of the clear supernatant was centrifuged at 38,000 x g for 10 min. The supernatant was then adjusted to 8 mg/ml, distributed in small aliquots in plastic tubes, and stored in liquid nitrogen until use.

Results—Each experiment was repeated with two to four different membrane preparations. Uptake measurements were routinely made in duplicate or triplicate, and the variation among the replicate values was always within 10% of the mean value. The data are expressed as the mean ± SD.

RESULTS

Evidence for the Absence of Dipeptidyl Peptidase IV in Renal Brush-Border Membranes Prepared from Japan F344 Rats—Dipeptidyl peptidase IV activity was measured in renal brush-border membrane vesicles prepared from Japan F344 and USA F344 rats using Gly-Pro-p-nitroanilide as the chromogenic substrate. The generation of p-nitroaniline was continuously monitored using these two membranes under strictly identical conditions, and the results are given in Fig. 1. Gly-Pro-p-nitroanilide was hydrolyzed by membranes from USA F344 rats, indicating the presence of dipeptidyl peptidase IV in these membranes, but there was no measurable formation of p-nitroaniline when membranes from Japan F344 rats were employed. It is therefore evident that these membranes possess no or negligible dipeptidyl peptidase IV activity.

Hydrolysis of Phe-Pro-Ala—In order to determine the feasibility of using radiolabeled Phe-Pro-Ala as a model substrate in experiments involving intact tripeptide uptake, preliminary studies were performed to see whether unlabeled Phe-Pro-Ala
was resistant to hydrolysis by renal brush-border membranes isolated from dipeptidyl peptidase IV-negative rats (Japan F344). Renal brush-border membranes from dipeptidyl peptidase IV-positive rats (USA F344) were used as controls. The HPLC elution patterns of the parent tripeptide (Phe-Pro-Ala), and the dipeptidyl peptidase IV-catalyzed hydrolytic product (Phe-Pro) are given in Fig. 2. The membranes from the dipeptidyl peptidase IV-positive rats were found to efficiently hydrolyze the tripeptide. Approximately 40% of the tripeptide was hydrolyzed in 30 min at 37 °C by 25 μg of the membrane protein (concentration of the tripeptide in the reaction medium was 5 mM). Under identical experimental conditions, there was no detectable hydrolysis of the tripeptide when the renal brush-border membranes from dipeptidyl peptidase IV-negative rats were used. These results demonstrate that Phe-Pro-Ala can be conveniently employed to investigate the uptake of an intact tripeptide in these dipeptidyl peptidase IV-negative renal brush-border membrane vesicles.

**Uptake Characteristics of [3H]Phe-Pro-Ala in Renal Brush-Border Membrane Vesicles from Dipeptidyl Peptidase IV-negative Rats**

Effect of a Na+ Gradient and an H+ Gradient on the Initial Rates of Uptake—We have investigated the influence of an inwardly directed Na+ gradient on the initial rate of the radiolabel uptake from [3H]Phe-Pro-Ala by comparing the uptake rates measured in the presence of a Na+ gradient versus a K+ gradient. Table I shows that the uptake rates under these conditions were small. We have also compared the rates of radiolabel uptake measured in the presence and absence of an inwardly directed H+ gradient. The uptake rate in the absence of a H+ gradient (pH = pH0 = 6.7 or 8.3) was small and comparable to the rate measured in the presence of a K+ or Na+ gradient. However, the presence of a H+ gradient (pH = 8.3; pH0 = 6.7) markedly stimulated the uptake rate. Comparison of the rates reveals that the rate was 9 times higher in the presence of a H+ gradient than in its absence. Therefore, uptake of the tripeptide in these vesicles is not dependent upon a Na+ gradient but rather on a H+ gradient.

**Uphill Transport of Phe-Pro-Ala in the Presence of a H+ Gradient**—Since an inwardly directed H+ gradient stimulated the uptake of radiolabel from [3H]Phe-Pro-Ala, we further investigated the characteristics of this H+ gradient-dependent tripeptide uptake. Fig. 3 describes the time course of Phe-Pro-Ala uptake in the presence and absence of a H+ gradient. In the absence of a H+ gradient (pH = pH0 = 6.7), the uptake of radiolabel was very slow, increased with time, and finally reached the equilibrium value. On the other hand, the uptake of radiolabel was manifold greater in the presence of a H+ gradient (pH = 8.3; pH0 = 6.7). The time course of radiolabel uptake under these conditions exhibited the "overshoot" phenomenon which demonstrates the transient uphill transport of the label inside the vesicles against a concentration gradient. The intravesicular concentration of the radiolabel at the peak of the overshoot (10 s) was 13 times greater than the equilibrium value (60 min). The vesicular volume and/or integrity was not affected by the H+ gradient because the equilibrium value remained the same in the presence and absence of the H+ gradient.

**Influence of a Membrane Potential on [3H]Phe-Pro-Ala Uptake**—Fig. 3 also describes the influence of a valinomycin-induced, inside-negative, K+-diffusion potential on the uptake of radiolabel from [3H]Phe-Pro-Ala in the presence of an inwardly directed H+ gradient. The initial uptake rates were almost doubled in the presence of the inside-negative mem-

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**Table I**

| Incubation condition | Phe-Pro-Ala uptake (pmol/mg protein/10 s) |
|----------------------|------------------------------------------|
| H+ gradient          |                                          |
| pH = 8.3; pH0 = 6.7   | 2.21 ± 0.27                              |
| pH = pH0 = 6.7        | 0.24 ± 0.03                              |
| pH = pH0 = 8.3        | 0.13 ± 0.01                              |
| Na+ gradient          |                                          |
| [Na+] = 120 mM; [Na+] = 0 | 0.24 ± 0.01 |
| [K+] = 120 mM; [K+] = 0 | 0.21 ± 0.03 |

**Fig. 2.** HPLC analysis of unlabeled Phe-Pro-Ala hydrolysis by renal brush-border membrane vesicles from dipeptidyl peptidase IV-positive (USA F344) and dipeptidyl peptidase IV-negative (Japan F344) rat strains.
brane potential, and the concentration of the radiolabel inside the vesicles at the peak of the overshoot was equally increased. But, the equilibrium value remained unaltered.

The influence of a membrane potential was analyzed in more detail as described in Table II. Initial rates of radiolabel uptake from [3H]Phe-Pro-Ala were measured in the presence of a H⁺ gradient. K⁺ was present in equal concentrations (200 mM) both inside and outside of the membrane vesicles. Generation of an FCCP-induced, inside-positive, H⁺-diffusion potential greatly reduced the uptake of radiolabel under these conditions, whereas voltage-clamping the membrane by the addition of valinomycin significantly stimulated the radiolabel uptake. These data collectively provide strong evidence for the electrogenic nature of the H⁺ gradient-dependent Phe-Pro-Ala uptake. They also suggest that the uptake of the tripeptide was accomplished by a net transfer of positive charge across the membrane.

The Essential Nature of the Inwardly Directed H⁺ Gradient—Table II also describes an experiment in which the effect of dissipation of the H⁺ gradient on [3H]Phe-Pro-Ala uptake was investigated. When FCCP and valinomycin were added together to the uptake system, the uptake of radiolabel was drastically reduced. Under these conditions, there will be no change in the membrane potential across the membrane, but the transmembrane H⁺ gradient will collapse. These results, together with those presented in Fig. 3, suggest that the inwardly directed H⁺ gradient is the driving force for the tripeptide uptake.

The role of the H⁺ gradient was further probed by using nigericin, an ionophore for Na⁺, K⁺, and H⁺. We studied the influence of this ionophore on [3H]Phe-Pro-Ala uptake in the absence of a H⁺ gradient, but in the presence of an inwardly directed Na⁺ or K⁺ gradient. Under these experimental conditions, nigericin would catalyze the influx of Na⁺ or K⁺ coupled to the efflux of H⁺ and thus generate an inwardly directed H⁺ gradient. The results of these experiments, given in Fig. 4, show that, in the absence of nigericin, the uptake rates of the radiolabel were very slow, but the rate was nonetheless significantly higher in the presence of a Na⁺ or K⁺ gradient. Addition of nigericin stimulated the uptake rate manyfold in the presence of both a Na⁺ and K⁺ gradient. The time course of the uptake revealed transient accumulation of the tripeptide in the presence of the ionophore, indicating the H⁺ gradient-driven active transport.

TABLE II

| Incubation conditions | Extravesicular Phe-Pro-Ala uptake |
|-----------------------|----------------------------------|
| pH | K⁺ | pH | K⁺ | Pmol/mg protein/10 s |
| 8.3 | 200 | 6.7 | 200 | 1.25 ± 0.07 |
| 8.3 | 200 | 6.7 | 200 | 0.12 ± 0.02 |
| 8.3 | 200 | 6.7 | 200 | 2.16 ± 0.21 |
| 8.3 | 200 | 6.7 | 200 | 0.25 ± 0.04 |

Effect of Amino Acids and Peptides on Phe-Pro-Ala Uptake

Membrane vesicles were preloaded with 5 mM Hepes, 75 mM Tris buffer, pH 8.3, containing 100 mM K₂SO₄. Uptake of 0.1 μM [3H]Phe-Pro-Ala was measured with a 10-s incubation in 50 mM Mes, 50 mM Hepes, 25 mM Tris buffer, pH 6.0, containing 300 mM mannitol. Concentration of amino acids and peptides were 1 mM during uptake. The data represent the mean ± S.D. (n = 4, two membrane preparations).

| Unlabeled amino acids and peptides | Phe-Pro-Ala uptake |
|-----------------------------------|-------------------|
| None                              | 1.90 ± 0.12       |
| Alanine                           | 1.99 ± 0.06       |
| Proline                           | 1.99 ± 0.08       |
| Phenylalanine                     | 1.84 ± 0.11       |
| Phe-Pro                            | 0.11 ± 0.01       |
| Tyr-Pro                            | 0.15 ± 0.01       |
| Gly-Pro                            | 0.28 ± 0.01       |
| Ala-Pro                            | 0.17 ± 0.02       |
| Val-Pro                            | 0.04 ± 0.02       |
| Gly-Sar                            | 0.35 ± 0.01       |
| Ala-Pro-Gly                        | 0.16 ± 0.01       |
| Gly-Pro-Ala                        | 0.31 ± 0.01       |
| Val-Pro-Leu                        | 0.02 ± 0.02       |
| Phe-Pro-Gly                        | 0.07 ± 0.02       |
| Phe-Pro-Ala                        | 0.04 ± 0.02       |

Effect of Amino Acids and Peptides on Phe-Pro-Ala Uptake—The ability of amino acids and peptides to compete with [3H]Phe-Pro-Ala for the uptake process was investigated in the presence of an inwardly directed H⁺ gradient (Table III). Free amino acids did not interact with the tripeptide uptake system. However, all the dipeptides and tripeptides tested were found to be potent inhibitors of radiolabel uptake from [3H]Phe-Pro-Ala. The tripeptides used in this experiment were specifically selected because of their X-Pro-Y sequence and were expected to remain unhydrolyzed upon incubation with the renal brush-border membranes from the...
dipeptidyl peptidase IV-negative rats. The inhibition of \([^3H]Phe-Pro-Ala\) uptake by dipeptides and tripeptides suggests that the transport system responsible for Phe-Pro-Ala uptake also interacts with various di- and tripeptides.

**Kinetics of Phe-Pro-Ala Uptake**—We have investigated the dependence of Phe-Pro-Ala uptake on the concentration of the peptide over a range of 5–150 \(\mu\)M. Uptake measurements were made in the presence of an inwardly directed \(H^+\) gradient (pH 8.3; pH 6.7). The uptake of the tripeptide was very rapid as shown in Fig. 5 under these conditions. For kinetic analysis, 6-s incubations were employed to determine the uptake rate. Even with this short incubation, the uptake rate represented only the near initial uptake rate and therefore the kinetic constants calculated from this experiment only represent near approximate values. Fig. 5 describes the experimental data given in the form of a Eadie-Hofstee plot (uptake rate/peptide concentration versus uptake rate). The linearity of the plot \((r^2 > 0.96)\) indicates that the uptake system obeyed simple Michaelis-Menten kinetics describing the participation of a single system. The kinetic parameters, \(K_r\) and \(V_{max}\), were determined by linear regression analysis of the data. The \(K_r\) for Phe-Pro-Ala was 48 ± 3 \(\mu\)M, and the \(V_{max}\) was 0.50 ± 0.01 nmol/mg of protein per 6 s.

**Analysis of Intravesicular Contents**—We have demonstrated in Fig. 2 that while the dipeptidyl peptidase IV-positive renal brush-border membranes hydrolyzed Phe-Pro-Ala, the dipeptidyl peptidase IV-negative membranes failed to show any detectable hydrolysis. These experiments were, however, done at a peptide concentration of 5 \(\mu\)M and in the absence of a \(H^+\) gradient. But the characteristics of radiolabel uptake from \([^3H]\)Phe-Pro-Ala were investigated at a peptide concentration of 0.1 \(\mu\)M and in the presence of a \(H^+\) gradient. In order to show that the observed characteristics indeed represented those of the uptake of an intact tripeptide, it was crucial to demonstrate that there was no hydrolysis of the tripeptide in the incubation medium under the uptake conditions and that the tripeptide was detected in the intact form inside the vesicles. A 10-s incubation period was chosen for this experiment because the accumulation of the radiolabel from the tripeptide was maximal at this time. The results are shown in Table IV. Analysis of the radioactive peptides in the total incubation medium revealed that approximately 40% of the tripeptide was hydrolyzed upon incubation with dipeptidyl peptidase IV-positive membranes. But, under the same conditions, only about 3% hydrolysis was observed with dipeptidyl peptidase IV-negative membranes. Analysis of intravesicular contents showed that only 36% of the radioactivity was present as the intact tripeptide in dipeptidyl peptidase IV-positive membrane vesicles, whereas more than 90% of the radioactivity was associated with the intact tripeptide in dipeptidyl peptidase IV-negative membrane vesicles.

**DISCUSSION**

We have used in the present investigation an experimental animal model which is unique and novel to examine the characteristics of renal transport of an intact tripeptide. The experimental animals were F344 rats obtained from Charles River Inc., Kanagawa, Japan. These rats are genetically deficient in dipeptidyl peptidase IV, which is an integral membrane peptidase associated primarily with the brush-border membrane in small intestine and kidney. Owing to the near total absence of the enzyme, purified renal brush-border membranes from these rats are unable to hydrolyze tripeptides of the X-Pro-Y type. This provided us the opportunity to investigate the renal uptake of intact Phe-Pro-Ala, a physiological tripeptide, and seek an answer to a long standing question, i.e. whether or not tripeptides can cross the brush-border membrane intact.

The conclusions from this investigation are straightforward and can be summarized as follows: 1) Phe-Pro-Ala is totally resistant to hydrolysis upon incubation with renal brush-border membrane vesicles obtained from Japan F344 rats, 2) the tripeptide is taken up into the vesicles in intact form, 3) the uptake is only slightly stimulated by a \(Na^+\) gradient compared to a \(K^+\) gradient, but is markedly stimulated by a
H\(^+\) gradient, 4) the tripeptide transiently accumulates inside the vesicles against a concentration gradient in the presence of a H\(^+\) gradient, 5) the uptake process is electrogenic, accompanied by simultaneous transfer of positive charge across the membrane, 6) tripeptide-H\(^+\) symport is the most likely mechanism of the uptake process, and 7) many di- and tripeptides share the transport system.

In recent years, many transport systems in the renal brush-border membranes have been shown to be energized by an inwardly directed H\(^+\) gradient. The brush-border membrane Na\(^+-\)H\(^+\) exchanger plays an important role in the generation of the transmembrane H\(^+\) gradient in vivo. It is likely that the H\(^+\)-translocating ATPase, recently described in purified renal brush-border membranes (25), may also make a significant contribution to the generation of this driving force. Under in vitro conditions, whether the Na\(^+-\)H\(^+\) exchanger present in purified brush-border membrane vesicles is able to generate a transmembrane H\(^+\) gradient in response to the experimentally imposed Na\(^+\) gradient will largely depend upon the buffer capacity of the intravesicular and extravesicular media. When the buffer capacity is very high, the influence of a Na\(^+\) gradient on tripeptide H\(^+\) co-transport can hardly be seen (Table I). This means that, under these conditions, the Na\(^+-\)H\(^+\) exchanger is not able to generate a H\(^+\) gradient in response to a Na\(^+\) gradient. On the contrary, when the buffer capacity is low, the stimulatory effect of a Na\(^+\) gradient readily becomes apparent (Fig. 4). These data show that, if the experimental conditions are optimal, the involvement of the Na\(^+-\)H\(^+\) exchanger in converting a Na\(^+\) gradient to a H\(^+\) gradient across the brush-border membrane can be demonstrated even in vitro using purified brush-border membrane vesicles.

Since the transport characteristics of dipeptides in intestinal and renal brush-border membrane vesicles are similar, particularly with respect to the energization of the transport process by a H\(^+\) gradient (24), it is very likely that the intestinal transport of intact tripeptides is also driven by a H\(^+\) gradient. The intestinal brush-border membrane possesses an active Na\(^+-\)H\(^+\) exchanger and a transmembrane H\(^+\) gradient is known to exist across this membrane under physiological conditions (25). The Na\(^+\) dependence of Gly-Sar-Sar uptake in intact intestinal tissue described by Addison et al. (19) can be easily explained because of the involvement of the Na\(^+-\)-H\(^+\) exchanger in converting a transmembrane Na\(^+\) gradient to a transmembrane H\(^+\) gradient.

A clear understanding of the energetics involved in the intestinal and renal transport of intact peptides is of importance because this process plays a significant role in physiological as well as in certain clinical situations. The function of the intestinal peptide transport hardly needs to be elaborated here since detailed reviews have been devoted to discussing its nutritional relevance (26-29). The physiological function of the renal transport of intact peptides is not as obvious as that of its intestinal counterpart, but it is likely to participate in the conservation of amino nitrogen by reabsorbing small peptides present in the tubular fluid (30). Peptide absorptive mechanisms in the small intestine and kidney may also become relevant in therapeutic applications. There is increasing evidence that inclusion of small peptides in the place of free amino acids in enteral and parenteral solutions has certain advantages in providing efficient protein nutrition (31-33). If and when the addition of peptides to these solutions becomes a routine in clinical practice, intestinal transport of peptides will play a vital role in the assimilation of the enterally administered peptides. Similarly, the capacity of the kidneys to absorb and hydrolyze circulating peptides will contribute significantly to the efficient utilization of parenterally administered peptides.

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