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Transport of peptides in renal brush border membrane vesicles. Suitability of ^{125}I-labelled tyrosyl peptides as substrates

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Three tyrosine-containing peptides (Tyr-Pro, Tyr-Pro-Phe and Tyr-Pro-Phe-Pro) were [^{125}I]iodo-labelled and their uptake characteristics in renal brush border membrane vesicles was investigated to assess the suitability of these peptides as substrates in peptide transport studies. Hydrolysis of these peptides during uptake measurements was avoided by using brush border membrane vesicles prepared from the kidneys of Japan Fisher 344 rats which genetically lack dipeptidylpeptidase IV activity. The uptake of ^{125}I-Tyr-Pro and ^{125}I-Tyr-Pro-Phe in these membrane vesicles was found to be stimulated by an inwardly directed H^+ gradient. In both cases, the time course of uptake exhibited an overshoot, an indication for active transport. The uptake processes were electrogenic since they were stimulated by an inside-negative membrane potential. Free amino acids had no effect on the uptake of Tyr-Pro and Tyr-Pro-Phe whereas many di- and tripeptides effectively blocked the uptake. These results demonstrated that the [^{125}I]iodo-labelled Tyr-Pro and Tyr-Pro-Phe are suitable substrates for peptide transport studies because their uptake characteristics clearly suggest participation of the peptide transporter in their uptake. In contrast, the uptake of ^{125}I-Tyr-Pro-Phe-Pro was not active, and was not stimulated by the H^+ gradient or by the membrane potential. In addition, competition experiments with unlabelled amino acids and peptides indicated that the uptake did not occur via the peptide transporter. However, when allowed to be hydrolyzed to ^{125}I-Tyr-Pro and Phe-Pro by using dipeptidylpeptidase IV-positive renal brush border membrane vesicles prepared from USA Fisher 344 rats, the radiolabel from the [^{125}I]iodo-labelled tetrapeptide was found to be taken up into the vesicles via the peptide transporter. These data provide direct evidence to show that intact tetrapeptides are not substrates for the renal peptide transporter.

Introduction

Transport studies in brush border membrane vesicles isolated from either small intestine or kidney are usually conducted using radiolabelled substrates. In contrast to the transport systems available for sugars and amino acids, the peptide transport system has a huge number of potential substrates with different amino acid composition and sequence (e.g., 400 dipeptides and 8000 tripeptides). The commercially available radiolabelled peptides are very few. Moreover, many of these peptides are susceptible to hydrolysis by isolated brush border membrane vesicles and thus become unsuitable for investigations involving the transport of intact peptides. Even though the difficulty with the potential hydrolysis of the test peptides has been overcome in the past to some extent by using membrane vesicles whose peptidase activities were either removed by limited proteinase digestion [1–5] or inhibited by specific treatment [6,7], the results in most cases have been less than ideal. Radiolabelled peptides of a desirable amino acid composition and sequence can be custom synthesized, but the cost involved is very high. These conditions put severe limitations on peptide transport studies.

The purpose of the present investigation was to determine the suitability of ^{125}I-labelled peptides in

Abbreviations: DPP IV, dipeptidylpeptidase IV; EGTA, [ethylene bis(oxyethylenenitrilo)]tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; Mes, 4-morpholineethanesulfonic acid; TFA, trifluoroacetic acid.

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peptide transport studies. Three tyrosyl peptides (a dipeptide, a tripeptide and a tetrapeptide) were radiolabelled with ^{125}I and the transport characteristics of these peptides were investigated in isolated renal brush border membrane vesicles. The results of the study clearly indicate that the [^{125}I]iodo-labelled peptides are perfectly suitable for peptide transport studies.

Materials and Methods

Materials. Tyr-Pro-Phe and Tyr-Pro-Phe-Pro were purchased from Bachem Bioscience, Inc., Philadelphia, PA, U.S.A. Tyr-Pro was a gift from Prof. Alfred Barth, Department of Biochemistry, Martin Luther University, Halle/Saale, Germany. All other unlabelled peptides were obtained from Sigma Chemical Co., St. Louis, MO, USA. $Na^{125}I$ (radioactivity, 100 mCi/ml) was obtained from DuPont-New England Nuclear, Boston, MA, U.S.A. Enzymobead radioiodination kit was purchased from Bio-Rad, Richmond, CA, U.S.A.

Animals. A breeding pair of DPP IV-negative Fisher 344 rats were obtained from Charles River, Inc., Kanagawa, Japan. A colony of these rats is currently being maintained at the Medical College of Georgia. DPP IV-positive control Fisher 344 rats were obtained from Charles River, Inc., Raleigh, NC, U.S.A.

Preparation of brush border membrane vesicles. Isolation of brush border membrane vesicles from kidneys collected from DPP IV-negative and DPP IV-positive rats was done by Mg^{2+}-aggregation in the presence of EGTA [8,9]. The composition of the preloading buffer varied depending upon the experiment, but in most cases it was 50 mM Hepes/75 mM Tris, 100 mM K_2SO_4 (pH 8.3). The protein concentration of the final membrane suspension was adjusted to 6 mg/ml and it was stored in small aliquots in liquid N_2 until use.

Uptake measurements. A rapid filtration technique described earlier [10] was used to determine the radiolabel uptake from peptide substrates. Measurements were made at room temperature (22 °C). Millipore membrane filters (DAWP type, 0.65 μm pore size) were used in uptake measurements.

Uptake measurements were routinely made in duplicate or triplicate and the variation among the replicate values was always less than 10% of the mean value. Each experiment was repeated with two or three different membrane preparations. The results are presented as mean ± S.E.

Quantification of the hydrolysis of unlabelled peptides. The extent of hydrolysis of Tyr-Pro-Phe-Pro by renal brush border membrane vesicles was determined by separating the hydrolytic products from the parent peptide by HPLC [11].

Iodination of peptides. Radiolabelling of tyrosyl peptides with ^{125}I was done using the radioiodination kit (Enzymobeads) supplied by Bio-Rad. The procedure involves solid phase radioiodination and is based on enzymatic iodination rather than oxidative substitution. The exact protocol is described in the Bio-Rad Laboratories Technical Bulletin 1071E. Briefly, 150 nmol of the tyrosyl peptide were incubated for 30 min at 22°C with 1 mCi of $Na^{125}I$ in the presence of the Enzymobead reagent. After the reaction, the Enzymobeads were separated by centrifuging the mixture in a microfuge for 2 min. The supernatant was collected and acidified with 1% TFA. The iodinated peptide was separated from the free iodine by using the PrepSep-C_{18} extraction column. The labelled peptide was eluted with 6 ml of acetonitrile/0.1 M triethyl ammonium formate (pH 4.0)/water (2:1:2, v/v). The eluate was lyophilized to dryness and then dissolved in 1 ml of distilled water. The purity of the labelled peptide was checked by HPLC. Three tyrosyl peptides, Tyr-Pro, Tyr-Pro-Phe and Tyr-Pro-Phe-Pro, were radioiodinated by this procedure, and in each case the radioactivity was found to be eluted as a single peak. The purity of each radiolabelled peptide was greater than 97%. The recovery of each peptide from the PrepSep-C_{18} extraction column was always 100% and therefore the specific radioactivity could be calculated for each radiolabelled peptide. The specific radioactivities on the day of iodination were as follows: ^{125}I-Tyr-Pro, 5 Ci/mmol; ^{125}I-Tyr-Pro-Phe, 3.08 Ci/mmol; ^{125}I-Tyr-Pro-Phe-Pro, 2.38 Ci/mmol.

Results and Discussion

Effects of an inwardly directed H^+ gradient and an inside-negative membrane potential on the uptakes of ^{125}I-Tyr-Pro and ^{125}I-Tyr-Pro-Phe

Hydrolysis of the test peptides by brush border membrane-associated peptidases has been a problem in most peptide transport studies. In the present investigation, we circumvented this problem by judiciously choosing peptides of particular amino acid sequences so that they meet the criteria of resistance to hydrolysis and iodinatability. This was accomplished with the selection of Tyr-Pro as the representative dipeptide and Tyr-Pro-Phe as the representative tripeptide. X-Pro dipeptides are very resistant to hydrolysis by purified renal brush border membrane vesicles [10,12,13], because of the absence of prolidase activity in these membranes [14]. On the other hand, X-Pro-Y tripeptides are easily hydrolyzable by renal brush border membranes. However, since the hydrolysis of these tripeptides is exclusively catalyzed by DPP IV, a peptidase associated with the brush border membrane, the problem can be solved by using brush border membranes isolated from the kidneys of Japanese Fisher 344 rats. These rats exhibit a genetic deficiency of DPP IV [15,16], whereas the same strain of rats obtained within the U.S.A. have normal activity of this enzyme.

These two groups of rats, one DPP IV-positive (U.S.A. Fisher 344) and the other DPP IV-negative (Japan Fisher 344), were very useful in answering certain key questions in the field of peptide transport [11,17]. Owing to the complete absence of DPP IV in the brush border membranes isolated from Japan Fisher 344 rats, Tyr-Pro-Phe is totally resistant to hydrolysis by these membranes. Therefore, Tyr-Pro-Phe is a suitable substrate for tripeptide transport studies in these membrane vesicles. Both Tyr-Pro and Tyr-Pro-Phe are iodinatable due to the presence of the tyrosine residue and hence these peptides can be radiolabelled with ^{125}I. With this logic, we investigated the suitability of ^{125}I-labelled tyrosyl peptides as substrates in peptide transport studies by studying the characteristics of the uptakes of ^{125}I-Tyr-Pro and ^{125}I-Tyr-Pro-Phe in renal brush border membrane vesicles isolated from Japan Fisher 344 rats.

The unique properties of the mammalian peptide transport system include energization by a proton motive force, inhibition by peptides and absence of interaction with free amino acids [18–23]. Therefore, we first investigated the influence of an inwardly directed H^+ gradient and an inside-negative membrane potential on the uptake of ^{125}I-Tyr-Pro and ^{125}I-Tyr-Pro-Phe. Fig. 1 describes the results with the dipeptide and Fig. 2 describes the results with the tripeptide. In both cases, uptake in the absence of a transmembrane H^+ gradient ($pH_o = pH_i = 6.7$) was very slow, but increased with time and reached the equilibrium value at 60 min. On the other hand, the initial uptake rates were many-fold greater in the presence of an inwardly directed H^+ gradient ($pH_i = 8.3$; $pH_o = 6.7$) than in its absence. Under these conditions, the time course of uptake revealed that the dipeptide as well as the tripeptide were accumulated transiently against a concentration gradient. These results show that an inwardly directed H^+ gradient provides the energy source for active transport of ^{125}I-Tyr-Pro and ^{125}I-Tyr-Pro-Phe across the renal brush border membrane. Peptide transport in the small intestine and kidney occurs via the peptide-H^+ symport mechanism and this renders the transport of a zwitterionic peptide electrogenic. This means that an inside-negative membrane potential should accelerate the H^+ gradient-dependent peptide transport. Therefore, we studied the influence of an inside-negative K^+-diffusion potential on the uptake of ^{125}I-Tyr-Pro and ^{125}I-Tyr-Pro-Phe. The potential was generated across the membrane with valinomycin in the presence of an outwardly directed K^+ gradient. It is evident from Figs. 1 and 2 that the uptake of the dipeptide as well as the uptake of the tripeptide were significantly stimulated by this maneuver, indicating that the uptake of the peptides in renal brush border membrane vesicles is electrogenic.

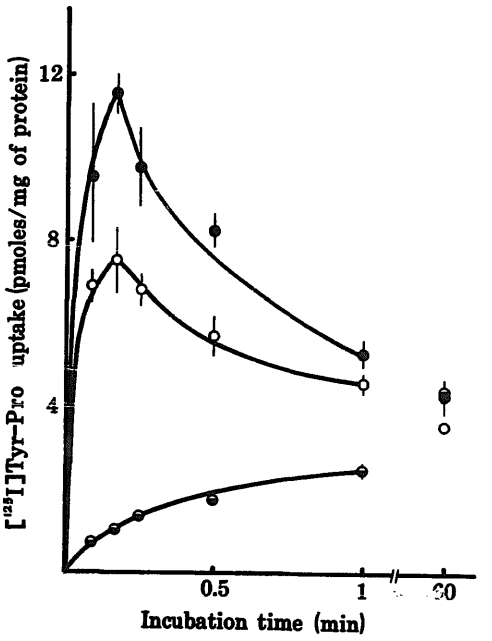

Fig. 1. Effects of an inwardly directed H^+ gradient and an inside-negative K^+-diffusion potential on ^{125}I-Tyr-Pro uptake in DPP IV-negative rat renal brush border membrane vesicles. Brush border membrane vesicles were preloaded with either 50 mM Hepes/75 mM Tris buffer (pH 8.3) containing 100 mM K_2SO_4 (●, ○) or 40 mM Mes/45 mM Hepes/38.5 mM Tris buffer (pH 6.7) containing 100 mM K_2SO_4 (⊖). Uptake of ^{125}I-Tyr-Pro (0.75 μM) was measured in either 50 mM Mes/50 mM Hepes/25 mM Tris buffer (pH 6.0) containing 300 mM mannitol (●, ○) or 40 mM Mes/40 mM Hepes/45 mM Tris buffer (pH 6.7) containing 300 mM mannitol (⊖). An inside-negative K^+-diffusion potential was generated by the addition of 10 μM valinomycin to the uptake buffer (●). The results are given as means ± S.E. ($n = 4$, two membrane preparations). When not shown, the error lies within the symbol.

Influence of free amino acids and peptides on the uptake of ^{125}I-Tyr-Pro and ^{125}I-Tyr-Pro-Phe

We then studied the effects of unlabelled amino acids and peptides on the uptake of radiolabel from ^{125}I-Tyr-Pro and ^{125}I-Tyr-Pro-Phe (Table I). Neither the uptake of Tyr-Pro nor the uptake of Tyr-Pro-Phe was affected by unlabelled amino acids. On the contrary, the uptake of these peptides was markedly reduced in the presence of a number of unlabelled di- and tripeptides. All of the peptides tested in this study were resistant to hydrolysis by renal brush border membrane vesicles prepared from Japan Fisher 344 rats. It is evident from the inhibitory potency of these peptides that the uptake of Tyr-Pro, a dipeptide, was equally susceptible to inhibition by dipeptides and tripeptides. Similarly, the uptake of Tyr-Pro-Phe, a tripeptide, was also inhibited almost to the same extent by di- and tripeptides. These results therefore indicate that a common transport system is responsible for the transport of the dipeptides as well as tripeptides.

Taken collectively, the data presented here demonstrate that the uptake of ^{125}I-labelled tyrosine-containing di- and tripeptides exhibits the following character-

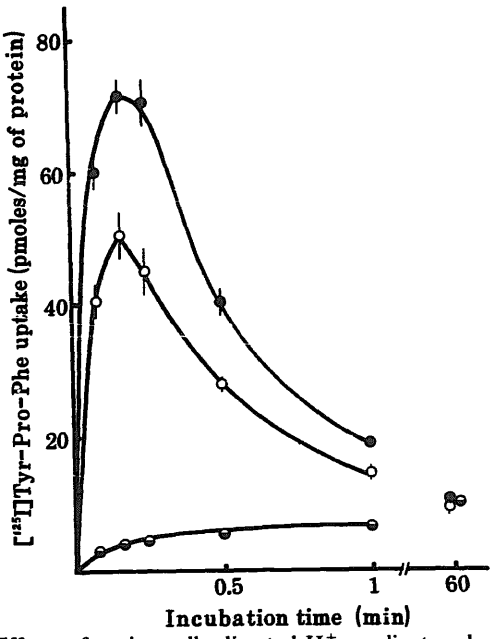

Fig. 2. Effects of an inwardly directed H^+ gradient and an inside-negative K^+-diffusion potential on ^{125}I-Tyr-Pro-Phe uptake in DPP IV-negative rat renal brush border membrane vesicles. Experimental conditions were as those described in the legend to Fig. 1 except that 0.75 μM ^{125}I-Tyr-Pro-Phe was employed. The results are given as means ± S.E. (n = 6, three membrane preparations). When not shown, the error lies within the symbol.

TABLE I

Effects of amino acids and peptides on the uptake of ^{125}I-Tyr-Pro and ^{125}I-Tyr-Pro-Phe in DPP IV-negative renal brush border membrane vesicles in the presence of an inwardly directed H^+ gradient

Uptake was measured in the presence of an inwardly directed H^+ gradient (pH_i = 8.3; pH_o = 6.7). Incubation time was 10 s. Concentration of the radiolabelled peptides was 0.75 μM and that of unlabelled amino acids and peptides was 250 μM. The values are means ± S.E. (n = 6; three membrane preparations)

Unlabelled amino acid or peptide	Uptake of ^{125}I-Tyr-Pro		Uptake of ^{125}I-Tyr-Pro-Phe	
	pmol/mg per 10 s	%	pmol/mg per 10 s	%
None	6.58 ± 0.12	100	41.92 ± 1.45	100
Alanine	6.79 ± 0.51	103	42.14 ± 2.90	101
Glycine	6.61 ± 0.25	100	43.00 ± 2.67	103
Proline	6.79 ± 0.33	103	44.49 ± 3.21	106
Phenylalanine	6.78 ± 0.24	103	41.29 ± 2.89	98
Ala-Pro	1.92 ± 0.08	29	12.99 ± 0.19	31
Gly-Pro	2.81 ± 0.18	43	18.81 ± 0.51	45
Phe-Pro	1.76 ± 0.17	27	11.06 ± 0.28	26
Val-Pro	0.63 ± 0.12	10	6.15 ± 0.24	15
Gly-Sar	2.81 ± 0.11	43	20.34 ± 0.84	49
Leu-Pro	1.14 ± 0.14	17	6.68 ± 0.10	16
Tyr-Pro	2.11 ± 0.05	32	13.41 ± 0.24	32
Ala-Pro-Gly	1.96 ± 0.13	30	16.41 ± 1.10	39
Phe-Pro-Ala	2.27 ± 0.16	34	11.03 ± 0.27	26
Phe-Pro-Gly	2.75 ± 0.24	42	11.44 ± 0.11	27

istics: energization by an inwardly directed H^+ gradient, electrogenicity and inhibition by peptides. These characteristics are similar to those described for other di and tripeptides. Therefore, the ^{125}I-labelled tyrosine-containing peptides are very much suitable for peptide transport studies.

Characteristics of ^{125}I-Tyr-Pro-Phe-Pro uptake

Most of the studies in peptide transport have so far been carried out using either dipeptides or tripeptides as substrates. To date, there is no direct demonstration of whether or not an intact tetrapeptide is a substrate for the peptide transport system. The major reason for the lack of studies on tetrapeptide transport is the unavailability of radiolabelled tetrapeptides which would remain intact during incubation with purified brush border membrane vesicles. Since the experiments described thus far in this paper demonstrate the suitability of ^{125}I-labelled tyrosine peptides in peptide transport studies, we investigated the possibility of an intact tetrapeptide being a substrate for the renal peptide transport system by studying the uptake characteristics of ^{125}I-Tyr-Pro-Phe-Pro in renal brush border membrane vesicles. This peptide is primarily hydrolyzed by DPP IV and therefore the use of renal brush border membrane vesicles prepared from Japan Fisher 344 rats should eliminate this hydrolysis to a large extent. That this indeed is the case became apparent when the hydrolysis of Tyr-Pro-Phe-Pro was compared upon incubation with the renal brush border membranes isolated from DPP IV-positive (USA Fisher 344) and DPP IV-negative (Japan Fisher 344) rats (Fig. 3). Incubation of 2.5 mM of Tyr-Pro-Phe-Pro with DPP IV-positive membranes (100 μg of protein) for 1 h at 22 °C resulted in almost total hydrolysis of the tetrapeptide (Fig. 3A). There were only two hydrolytic products, Tyr-Pro and Phe-Pro, indicating that DPP IV was most likely responsible for the hydrolysis. This conclusion is supported by the observation that the hydrolysis was almost completely abolished when the peptide was incubated with the DPP IV-negative membranes (Fig. 3B). However, there was a small but significant hydrolysis of the tetrapeptide still detectable with these membranes. Even though DPP IV is completely absent, there is another enzyme in these membranes which is capable of generating the same hydrolytic products from the tetrapeptide. Angiotensin converting enzyme (peptidyldipeptidase, ACE) is one of the peptidases associated with the renal brush border membrane [24] and this enzyme splits dipeptides from the carboxy terminus of larger peptides in a sequential manner [25]. To test whether this enzyme was responsible for the small hydrolysis of Tyr-Pro-Phe-Pro observed with the DPP IV-negative renal brush border membranes, we studied the influence of captopril on the hydrolytic activity of these membranes. Captopril is

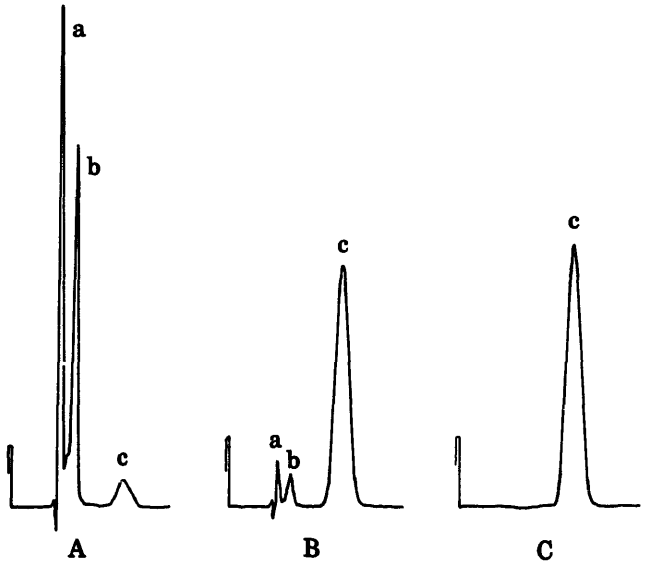

Fig. 3. Hydrolysis of Tyr-Pro-Phe-Pro by renal brush border membrane vesicles isolated from DPP IV-positive and DPP IV-negative rat strains. The experimental conditions for hydrolysis and for separation and detection of the hydrolytic products by HPLC are described in Materials and Methods. The absorbance peaks a, b and c identify the elution of Tyr-Pro, Phe-Pro and Tyr-Pro-Phe-Pro, respectively. A, DPP IV-positive membranes; B, DPP IV-negative membranes; C, DPP IV-negative membranes plus 10 μM captopril.

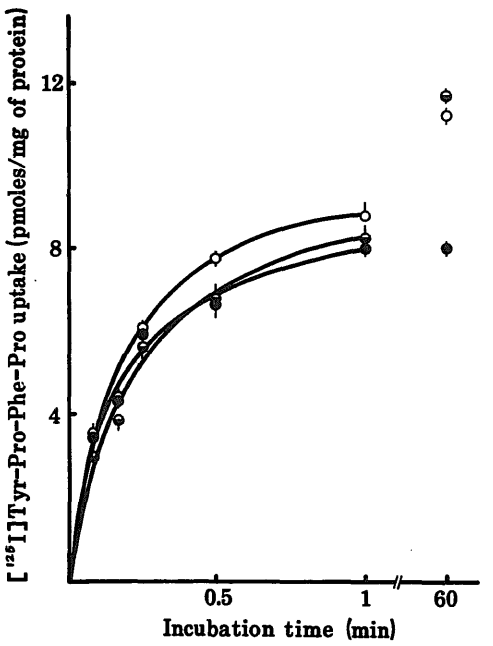

Fig. 4. Effects of an inwardly directed H^+ gradient and an inside-negative K^+-diffusion potential on ^{125}I-Tyr-Pro-Phe-Pro uptake in DPP IV-negative rat renal brush border membrane vesicles. Experimental conditions were similar to those described in the legend to Fig. 1, except that the membrane vesicles were preincubated with 50 μM captopril for 30 min at 22 °C before use in uptake experiments. Uptake of 0.75 μM ^{125}I-Tyr-Pro-Phe-Pro was measured. The results are given as means ± S.E. (n = 6, three membrane preparations).

a specific inhibitor of ACE [26]. The results in Fig. 3C show that preincubation of these membranes with 10 μM captopril completely prevented the tetrapeptide hydrolysis. It is therefore apparent that the handling of an intact tetrapeptide by renal brush border membrane vesicles can be investigated by studying the uptake of ^{125}I-Tyr-Pro-Phe-Pro with DPP IV-negative renal brush border membrane vesicles which had been pretreated with captopril. The presence of captopril during uptake measurements does not interfere with the peptide transport activity of the renal brush border membrane vesicles (data not shown).

Fig. 4 describes the characteristics of ^{125}I-Tyr-Pro-Phe-Pro in DPP IV-negative renal brush border membrane vesicles in the presence of captopril. The time course of uptake was similar in the absence as well as in the presence of a transmembrane H^+ gradient. More notably, there was no evidence for an overshoot in the presence of an inwardly directed H^+ gradient alone or in the presence of the H^+ gradient plus an inside-negative K^+-diffusion potential. These characteristics are in contrast with those of the uptake of ^{125}I-Tyr-Pro and ^{125}I-Tyr-Pro-Phe in these membrane vesicles. Further studies on the influence of medium osmolality on the equilibrium uptake of ^{125}I-Tyr-Pro-Phe-Pro indicated that more than 90% of uptake was really due to binding to the membrane vesicles rather than transport into an osmotically-responsive intravesicular space (data not shown).

In order to find out whether the tetrapeptide binds to the renal peptide transporter but is not transported across the membrane, we investigated the effects of

TABLE II

Effects of amino acids and peptides on the uptake of ^{125}I-Tyr-Pro-Phe-Pro in DPP IV-negative renal brush border membrane vesicles in the presence of captopril and an inwardly directed H^+ gradient

Membrane vesicles were preincubated with 50 μM captopril before use in uptake measurements. Uptake was measured in the presence of an inwardly directed H^+ gradient (pH_i = 8.3; pH_o = 6.7) and with a 30 s incubation. Concentration of the radiolabelled peptide was 0.75 μM and that of unlabelled amino acids and peptides was 250 μM. The values are means ± S.E. (n = 6; three membrane preparations)

Unlabelled amino acid or peptide	Uptake of ^{125}I-Tyr-Pro-Phe-Pro	
	pmol/mg per 30 s	%
None	6.25 ± 0.29	100
Tyrosine	3.58 + 0.39	57
Leucine	3.56 ± 0.25	57
Phenylalanine	2.45 ± 0.25	39
Ala-Pro	5.01 ± 0.25	80
Gly-Pro	5.83 ± 0.71	93
Gly-Sar	5.65 ± 0.41	90
Phe-Pro	1.91 ± 0.14	31
Tyr-Pro	2.15 ± 0.42	34
Ala-Pro-Gly	6.90 ± 0.12	110
Tyr-Pro-Phe-Pro	0.25 ± 0.03	4

unlabelled amino acids and peptides on the binding (Table II). Peptides such as Ala-Pro, Gly-Pro, Gly-Sar and Ala-Pro-Gly which were potent inhibitors of ^{125}I-Tyr-Pro uptake and ^{125}I-Tyr-Pro-Phe uptake were without any effect on the binding of the tetrapeptide. On the other hand, phenylalanine, a free amino acid, which had no effect on the uptake of the dipeptide and the tripeptide, markedly inhibited the tetrapeptide binding. Tyrosine and leucine, two other hydrophobic amino acids, were also equally effective in inhibiting the binding. These differences between the effects of free amino acids and peptides on the uptake of Tyr-Pro and Tyr-Pro-Phe and on the binding of Tyr-Pro-Phe-Pro strongly suggest that the peptide transporter was not responsible for the binding of the tetrapeptide observed in these membrane vesicles. However, certain peptides (e.g., Tyr-Pro, Phe-Pro and Tyr-Pro-Phe-Pro) did have a significant inhibitory effect on the binding. But, these inhibitory peptides, unlike the aforementioned peptides which are non-inhibitory, contain amino acids which are highly hydrophobic. It appears therefore that the hydrophobicity of ^{125}I-Tyr-Pro-Phe-Pro is responsible for the interaction of the tetrapeptide with the membrane vesicles and that hydrophobic amino acids and peptides effectively block this interaction.

Characteristics of ^{125}I-Tyr-Pro-Phe-Pro uptake in DPP IV-positive renal brush border membrane vesicles

The experiments with ^{125}I-labelled tetrapeptide show that if hydrolysis of the peptide is prevented, the radiolabel is not taken up into the renal brush border membrane vesicles, strongly indicating that the intact tetrapeptide is not a substrate for the renal peptide transporter. In order to see whether ^{125}I-Tyr-Pro-Phe-Pro is handled differently if hydrolysis of the peptide is allowed to proceed during uptake measurements, we investigated the characteristics of the radiolabel uptake from the tetrapeptide in DPP IV-positive renal brush border membrane vesicles. Under these conditions, ^{125}I-Tyr-Pro-Phe-Pro is expected to be hydrolyzed very rapidly to generate ^{125}I-Tyr-Pro and Phe-Pro. Since ^{125}I-Tyr-Pro is a very good substrate for the renal peptide transporter, the uptake of radiolabel from the medium should then exhibit characteristics which are similar to those of ^{125}I-Tyr-Pro uptake. The results of the experiment given in Fig. 5 indicate that this indeed is the case because the uptake of radiolabel under these conditions was H^+-dependent and was stimulated by an inside-negative membrane potential. Furthermore, the radiolabel uptake was markedly inhibited by many di- and tripeptides but not by amino acids (Table III).

In conclusion, the data presented in this paper clearly demonstrate that ^{125}I-labelled tyrosine-containing peptides are suitable substrates for peptide trans-

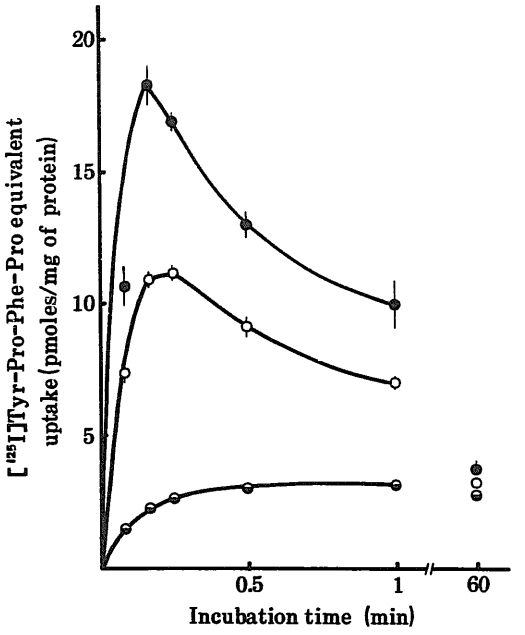

Fig. 5. Effects of an inwardly directed H^+ gradient and an inside-negative K^+-diffusion potential on ^{125}I-Tyr-Pro-Phe-Pro uptake in DPP IV-positive rat renal brush border membrane vesicles. Experimental conditions were similar to those described in the legend to Fig. 1, except that 0.75 μM ^{125}I-Tyr-Pro-Phe-Pro was used in uptake measurements. The results are given as means ± S.E. ($n = 4$, two membrane preparations). When not shown, the error lies within the symbol.

TABLE III

Effects of amino acids and peptides on the uptake of radiolabel from ^{125}I-Tyr-Pro-Phe-Pro in DPP IV-positive renal brush border membrane vesicles in the presence of an inwardly directed H^+ gradient

Uptake was measured in the presence of an inwardly directed H^+ gradient ($pH_i = 8.3$; $pH_o = 6.7$) with a 10 s incubation. Concentration of the radiolabelled peptide was 0.75 μM and that of unlabelled amino acids and peptides was 250 μM. The values are means ± S.E. ($n = 6$; three membrane preparations)

Unlabelled amino acid or peptide	Uptake of ^{125}I-Tyr-Pro-Phe-Pro equivalent	
	pmol/mg per 10 s	%
None	11.93 ± 0.45	100
Alanine	11.70 ± 0.26	98
Glycine	12.12 ± 0.52	102
Proline	11.52 ± 0.14	97
Phenylalanine	10.58 ± 0.36	89
Ala-Pro	3.03 ± 0.08	25
Gly-Pro	4.37 ± 0.34	37
Phe-Pro	1.21 ± 0.28	10
Val-Pro	1.16 ± 0.16	10
Gly-Sar	5.28 ± 0.18	44
Leu-Pro	1.04 ± 0.44	9
Tyr-Pro	2.21 ± 0.35	19
Ala-Pro-Gly	2.58 ± 0.29	22
Phe-Pro-Ala	0.77 ± 0.08	6
Phe-Pro-Gly	1.21 ± 0.45	10

port studies in isolated renal brush border membrane vesicles. This approach has enabled us to provide direct conclusive evidence for the first time that tetrapeptides are not substrates for the renal peptide transporter. The wide spectrum of tyrosine-containing peptides commercially available, the ease with which these peptides can be [^{125}I]iodo-labelled and the cost efficiency of the procedure compared to custom-synthesizing either ^{3}H-labelled or ^{14}C-labelled peptides should help expand peptide transport studies in renal as well as intestinal brush border membrane vesicles.

Acknowledgements

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