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Transport of the Advanced Glycation End Products Alanylpyrraline and Pyrralylalanine by the Human Proton-Coupled Peptide Transporter hPEPT1

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The glycation compound pyrraline, which originates from the advanced Maillard reaction, appears in urine after consumption of pyrraline-containing food. We hypothesized that the absorption of pyrraline occurs in the form of dipeptides rather than the free amino acid. The human intestinal peptide transporter hPEPT1 was transiently expressed in HeLa cells. In hPEPT1-transfected cells but not in cells transfected with empty vector, the uptake of [^{14}C]glycylsarcosine was strongly inhibited by alanylpyrraline (Ala-Pyrr) and pyrralylalanine (Pyrr-Ala). Free pyrraline did not inhibit peptide uptake. In *Xenopus laevis* oocytes expressing human PEPT1, both Ala-Pyrr and Pyrr-Ala generated significant inward directed currents. In a third approach, uptake of the dipeptides into hPEPT1-transfected HeLa cells was analyzed by HPLC. Ala-Pyrr and Pyrr-Ala were taken up by hPEPT1-expressing cells at a 4- to 7-fold higher rate than by HeLa cells transfected with the empty vector. We conclude that pyrraline containing dipeptides are transported by hPEPT1 in an electrogenic manner into intestinal cells.

KEYWORDS: AGE; Maillard reaction; intestine; membrane transport; PEPT1; pyrraline; glycation

INTRODUCTION

Advanced glycation end products (AGEs) originate by the so-called Maillard reaction during thermal processing of food and during food storage (*1*). This reaction ("glycation") occurs between reducing carbohydrates or their degradation products and the ε-amino group of lysine, or the N-termini of proteins or the guanidino group of arginine. Whether dietary AGEs represent a risk to human health is currently under intense discussion (*2*−*5*). The daily intake of AGEs has been estimated to 25−75 mg (*6*). So far, however, no evidence has been brought forward showing that specific diseases result from AGE effects in a causative manner (*7*).

One of the quantitatively most dominating glycation compounds in food is the AGE pyrraline (6-(2-formyl-5-hydroxymethyl-1-pyrrolyl)-L-norleucin) (*8*, *9*). Free or peptide-bound pyrraline is generated during the reaction of 3-deoxy-D-*erythro*-hexos-2-ulose (3-deoxyglucosulose, 3-DG) with the ε-amino group of free lysine or lysine residues, respectively (*10*, *11*). Pyrraline is found predominantly on sites of high thermal impact and low moisture content, like in bread crust, rusk, or crackers, in which the pyrraline content may reach 3.7 g per kg of protein. In milk powder and whey powder (*12*) and pasta products (*13*) pyrraline concentrations vary between 90 and 150 mg per kg of protein.

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After intake of a pyrraline containing diet, significant amounts of free pyrraline appear in urine (*14*, *15*) raising the question how pyrraline can cross the intestinal epithelial barrier protecting the *milieu interieur*. In general, free or peptide/protein-bound AGEs from diet could cross the intestinal epithelium by simple diffusion (paracellularly or transcellularly), by endocytotic processes or mediated by transport proteins. Studying this question at intestinal Caco-2 cells cultured as monolayers on permeable filters, we observed recently that, in contrast to free pyrraline, the peptide derivatives alanylpyrraline (Ala-Pyrr) and pyrralylalanine (Pyrr-Ala) are taken up into the cells and then hydrolyzed intracellularly to alanine and free pyrraline (*16*). Only pyrraline entered the basolateral compartments of the Transwell chambers.

We hypothesized that peptide transporters located at the apical membrane of enterocytes might be responsible for the uptake of the dipeptide derivatives into the cells. Indeed, the uptake of the prototype reference dipeptide [^{14}C]glycylsarcosine ([^{14}C]Gly-Sar) into Caco-2 cells could be inhibited by excess amounts of Ala-Pyrr and Pyrr-Ala but not by free pyrraline. This result suggests competition between the pyrraline-containing dipeptides and Gly-Sar during specific transmembrane transport into the epithelial cells. Actual transport of pyrraline in any form could not be shown. The membrane transporter mainly responsible for uptake of small peptides originating from intestinal protein digestion is the H^+-coupled peptide transporter 1 (PEPT1) (*17*−*19*). This transporter protein accepts most physiologically occurring di- and

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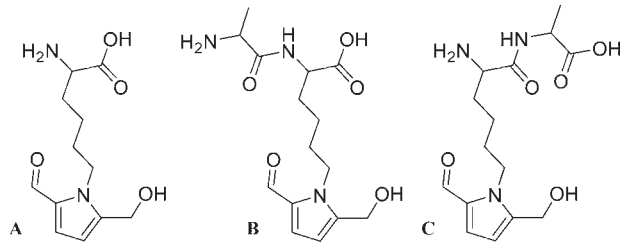

Figure 1. Chemical structures of pyrraline (**A**), Ala-Pyrr (**B**) and Pyrr-Ala (**C**).

tripeptides as substrates. Amino acids and oligopeptides larger than tripeptides are excluded. Interestingly, PEPT1 tolerates a great number of structural modifications of peptide derived substrates without significant alterations in transport capabilities (*17*, *18*). It has been shown that certain amino acid derivatives are recognized and transported by PEPT1. Several studies have demonstrated that dipeptide cores conjugated with different residues in their side chains are transported into intestinal cells. Other peptidomimetics transported by PEPT1 are δ-aminolevulinic acid and alafosfalin (*18*). The system also accepts many peptidomimetic drugs and prodrugs such as β-lactam-antibiotics or valacyclovir as substrates (*18*, *19*). For a PEPT1 substrate with high affinity we presently consider the following structural features as essential: (a) L-amino acids, (b) an acidic or hydrophobic function at the C-terminus, (c) a weakly basic group in α-position at the N-terminus, (d) a ketomethylene or acid amide bond and (e) when present, *trans* conformation of the peptide bond (*18*). Compounds serving as substrate for PEPT1 usually display apparent affinity constants (K_i, K_t) between 50 μM and 10 mM (*18*, *19*).

The purpose of this study was to investigate whether the uptake of Ala-Pyrr and Pyrr-Ala into intestinal cells is catalyzed by the proton-coupled peptide transporter. We employed two techniques that specifically allow measurement of substrate transport by PEPT1, namely, the two-electrode voltage-clamp technique at *Xenopus laevis* oocytes expressing human PEPT1 and the transfection of PEPT1-negative human host cells with hPEPT1-cDNA.

MATERIALS AND METHODS

Materials. The epithelial cervical cancer cell line HeLa was obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cell culture media, supplements and trypsin solution were purchased from Life Technologies, Inc. (Karlsruhe, Germany) or PAA (Pasching, Austria). Fetal bovine serum was from Biochrom (Berlin, Germany). [Glycine-1-^{14}C]glycylsarcosine (Gly-Sar; specific radioactivity 56 mCi/mmol) was custom synthesized by GE Healthcare (Little Chalfont, U.K.). Gly-Sar and Ala-Lys were ordered from Sigma-Aldrich (Deisenhofen, Germany). 3-Deoxyglucosulose (3-DG) was synthesized according to Henle and Bachmann (*10*). For the synthesis of pyrraline, Ala-Pyrr and Pyrr-Ala (for structures see **Figure 1**), we followed our protocol described previously (*16*). Briefly, Boc-Lys-OH or the protected dipeptides Boc-Ala-Lys-OH and Boc-Lys-Ala-OH were incubated in dry state with a 4-fold molar excess of 3-DG for 4 h at 70 °C in a drying oven. After extraction of the crude reaction product, the Boc protecting group was cleaved and the deprotected pyrraline derivatives were finally purified by semipreparative ion exchange chromatography yielding the substances with an overall content of about 90% with residual formate as assessed by nuclear magnetic resonance spectroscopy. The absence of impurities, especially other amino acids or dipeptides, was verified by amino acid analysis and mass spectroscopy (data not shown). Spectroscopic data were in accordance with those published previously (*16*).

Cell Culture. HeLa cells were routinely cultured in 75 cm^2 culture flasks with D-MEM (with Glutamax; 4500 mg/L glucose) supplemented with 10% fetal bovine serum and gentamicin (50 μg/mL) at 37 °C in a humidified atmosphere containing 5% CO_2 (*20*). Subconfluent cultures (80% of confluence) were treated for 5 min with Dulbecco's phosphate-buffered saline and trypsinized with 1% trypsin solution for 2 min. For uptake experiments, cells were seeded in 24-well plates (Greiner Bio-One GmbH, Frickenhausen, Germany) at a density of 0.75×10^6 cells per well (*20*).

Heterologous Expression of hPEPT1 in HeLa Cells and Uptake Measurements. The cDNA of human PEPT1 was cloned into pcDNA3 using the pBluescript constructs as a template for the PCR and *Xho*I and *Bam*HI as restriction sites (*20*). The resulting pcDNA3-hPEPT1 construct was confirmed by sequencing. Human PEPT1 was heterologously expressed in HeLa cells using the pcDNA3-hPEPT1 cDNA construct (1 μg/well) and Turbofect (1.5 μL/well; Fermentas, St. Leon-Rot, Germany) according to manufacturers' protocols. The transfection was done 1 h post seeding in 24-well plates, and 20−24 h post transfection the uptake of [^{14}C]Gly-Sar (20 μM, pH 6.0) in the absence or presence of increasing concentrations of Gly-Sar, pyrraline, Ala-Pyrr or Pyrr-Ala was measured at room temperature. The uptake buffer contained 25 mM Mes/Tris (pH 6.0), 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$ and 5 mM glucose (*20*). After incubation for 10 min the monolayers were quickly washed four times with ice-cold uptake buffer, solubilized and prepared for liquid scintillation spectrometry. The nonsaturable component of [^{14}C]Gly-Sar uptake (diffusion, adherent radioactivity) determined by measuring the uptake of [^{14}C]Gly-Sar in the presence of 50 mM unlabeled Gly-Sar represented only 3.7% of the total uptake. This value was taken into account during nonlinear regression analysis of inhibition constants (*20*).

Transfected HeLa cells were also used for uptake studies with the unlabeled compounds. HeLa cells transfected with the empty vector pcDNA3 (control) or pcDNA3-hPEPT1, respectively, were incubated with unlabeled pyrraline, Ala-Pyrr or Pyrr-Ala (1 mM) for 30 min at pH 6.0 and 37 °C. After incubation, the cells were quickly washed four times with ice-cold uptake buffer, and 500 μL of aqua bidest. was added. After freezing and thawing the cell layers three times, the cell suspension was transferred in 1.5 mL reaction tubes, homogenized with a 25 gauge needle and centrifuged in a table top centrifuge at 13000 rpm for 45 min. The supernatant was centrifuged again at 13000 rpm for 45 min and prepared for HPLC measurements (*20*).

High Pressure Liquid Chromatography (HPLC). All analytical HPLC analyses of pyrraline and pyrraline-containing dipeptides were performed using a high pressure gradient system from Amersham Pharmacia Biotech (Uppsala, Sweden), consisting of a pump P-900 with an online degasser (Knauer, Berlin, Germany), a column oven, and a UV detector UV-900. The mobile phases consisted of 5 mM sodium heptanesulfonate, pH 2.0 (solvent A) and a mixture of 50% solvent A in acetonitrile (solvent B). Separations were performed at a flow rate of 1 mL/min using a polymer-based RP-18-column (PLRP-S, 100 Å, 8 μm, 250 mm × 4.6 mm, Polymer Laboratories, Darmstadt, Germany). The column temperature was set to 30 °C, and UV detection was performed at 297 nm. A linear gradient from 4 to 54% B in 23 min was used for the measurements of pyrraline and Ala-Pyrr containing samples (gradient A). The gradient for Pyrr-Ala samples was from 14 to 37% B in 35 min (gradient B). External calibration was performed with the synthesized standards. 300 μL of the supernatants were added to 300 μL of solvent A. The mixture was centrifuged at 10 000 rpm for 15 min and 50 μL were injected.

***Xenopus laevis* Oocytes Expressing hPEPT1 and Electrophysiology.** The *Xenopus laevis* oocyte expression vector pNKS was kindly provided by Prof. G. Schmalzing (RWTH, Aachen, Germany). This vector contains the 5′ and 3′ UTRs of the *X. laevis* oocyte β-globin gene. To clone the transporter's cDNA into pNKS, *Aat*II and *Xba*I restriction sites were introduced at the 5′ and 3′ end, respectively, by PCR. As template the pBluescript-hPEPT1 vector was used. After restriction enzyme digestion the PCR product was ligated into the digested pNKS vector. The insertion of the correct cDNA was verified by sequencing. The pNKS-hPEPT1 construct served as template for cRNA synthesis. After linearizing the plasmids with *Not*I, cRNAs were synthesized using the mMESSAGE mMACHINE SP6 kit (Ambion, Huntingdon, U.K.). The cRNAs were purified with the MEGAclear kit (Ambion) and the concentration was determined by UV absorbance at 260 nm. The cRNAs were stored at −80 °C (*21*).

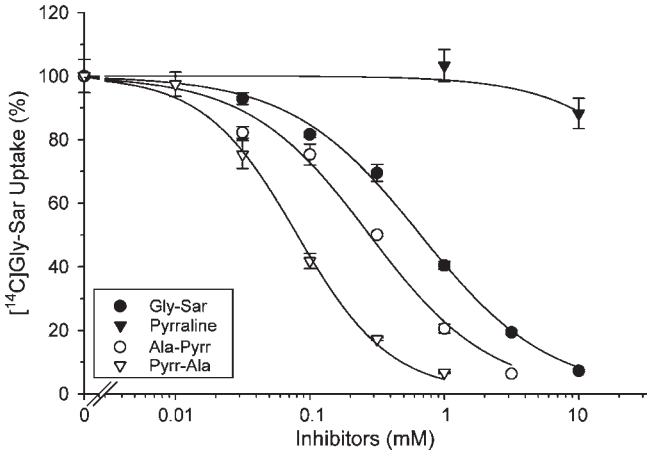

Figure 2. Inhibition of [^{14}C]Gly-Sar uptake into hPEPT1-transfected HeLa cells. Uptake of 20 μM [^{14}C]Gly-Sar was measured for 10 min at pH 6.0 in the presence of increasing concentrations of pyrraline, Ala-Pyrr, Pyrr-Ala and Gly-Sar. $n = 3-4$.

Oocytes were surgically removed from anesthetized *X. laevis* frogs, dissected and defolliculated as described by Riedel and co-workers (*22*). Tricaine methane sulfonate (Sigma) was used for anesthesia. The removed oocytes were separated by collagenase treatment (2 mg/mL) for 2 h. Healthy-looking oocytes (stages V−VI) were manually selected, and 23 nL (1.1 μg/μL) of cRNA solution of hPEPT1 was injected per oocyte. Water injected oocytes were used as control. Injected oocytes were maintained at 19 °C in modified Barth's medium (5 mM HEPES/NaOH pH 7.4, 100 mM NaCl, 1 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 000 U/mL penicillin and 10 mg/mL streptomycin). Five days post injection, the electrophysiological measurements were performed. Oocytes were placed in a flow-through chamber and continuously superfused (75 μL/s) with oocyte Ringer (ORi) buffer (10 mM Mes/Tris pH 6.5, 100 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 2 mM KCl) in the absence or presence of Ala-Pyrr and Pyrr-Ala at a concentration of 3 mM. Quick and reproducible solution exchanges were achieved using a small tubelike chamber (0.1 mL) combined with fast superfusion (*21*−*24*). Microelectrodes with resistances between 0.8 and 1.4 MΩ were made of borosilicate glass and filled with 3 M KCl. Whole-cell currents were recorded and filtered at 100 Hz using a two-electrode voltage-clamp amplifier (OC-725C, Hamden, CT) and sampled at 85 Hz. Oocytes were voltage clamped at a membrane potential of −60 mV.

Data Analysis. Results are given as mean ± SEM. The concentration of the unlabeled compound necessary to inhibit 50% of [^{14}C]Gly-Sar carrier-mediated uptake were determined by nonlinear regression using the logistical equation for an asymmetric sigmoid (allosteric Hill kinetics): $Y = \text{Min} + (\text{Max} - \text{Min})/(1 + (X/\text{IC}_{50})^P)$ where Max is the initial Y value, Min the final Y value and the power P represents Hill's coefficient (SigmaPlot program, Systat, Erkrath, Germany) (*20*). Inhibitory constants (K_i) were calculated from the IC_{50} values according to the method developed by Cheng and Prusoff (*25*). Log $p_{O/W}$ values were calculated using ChemDraw Pro 5.0 (Cambridgesoft, Cambridge, MA).

Oocyte data were analyzed using the Superpatch 2000 program (Julius-Bernstein-Institute of Physiology, SP-Analyzer by T. Böhm, Halle, Germany). Statistical values of oocyte experiments are expressed as mean ± SEM from measurements of 7−9 oocytes each from two batches of oocyte preparation. Currents induced by application of substances were calculated as the difference of the currents measured in the presence and absence of these substances.

RESULTS AND DISCUSSION

Inhibition of [^{14}C]Gly-Sar Uptake into HeLa Cells Transfected with hPEPT1. In a previous study (*16*) we observed that Ala-Pyrr and Pyrr-Ala are able to inhibit the uptake of [^{14}C]Gly-Sar into Caco-2 cells with high affinity. To show unequivocally that this inhibition occurs at the PEPT1 protein, in the present study measurements at the cloned human PEPT1 were performed. After heterologous expression of hPEPT1 in HeLa cells, inhibition of peptide uptake by increasing concentrations of pyrraline and its dipeptide derivatives was studied (**Figure 2**). From the inhibition curves, IC_{50} values, i.e. the inhibitor concentration necessary to inhibit the carrier-mediated [^{14}C]Gly-Sar uptake by 50%, were calculated and converted into K_i values. Ala-Pyrr and Pyrr-Ala inhibited the [^{14}C]Gly-Sar uptake with K_i values of 0.27 ± 0.02 mM and 0.08 ± 0.01 mM, respectively, whereas pyrraline showed no affinity to hPEPT1 (**Table 1**). According to our classification (*18*, *19*) Ala-Pyrr and Pyrr-Ala can be considered as high affinity ligands (inhibitors or substrates) of human PEPT1. The K_i values are very similar to those obtained at Caco-2 cells (**Table 1**) (*16*). For comparison, the enzymatically stable reference dipeptide Gly-Sar displayed a medium affinity with a K_i value of 0.64 ± 0.02 mM. Nontransfected HeLa cells do not possess endogenous peptide transport activity. Hence, from these results we conclude that the inhibitory effect of Ala-Pyr and Pyrr-Ala is due to direct interaction with the hPEPT1 protein.

Table 1. Inhibition Constants (K_i) of Gly-Sar, Ala-Pyrr, Pyrr-Ala and Pyrraline at hPEPT1-Transfected HeLa Cells and at Caco-2 Cells Expressing hPEPT1 Constitutively[a]

	K_i (mM)	
compound	hPEPT1 HeLa	hPEPT1 Caco-2[b]
Gly-Sar	0.64 ± 0.02	0.74 ± 0.01
pyrraline	>10	>10
Ala-Pyrr	0.27 ± 0.02	0.19 ± 0.01
Pyrr-Ala	0.08 ± 0.01	0.03 ± 0.01

[a] Uptake of [^{14}C]Gly-Sar was measured at pH 6.0 for 10 min at increasing concentrations of unlabeled pyrraline and its dipeptide derivatives. K_i values were derived from the competition curves shown in **Figure 2**. $n = 4$. [b] Hellwig et al. (*16*).

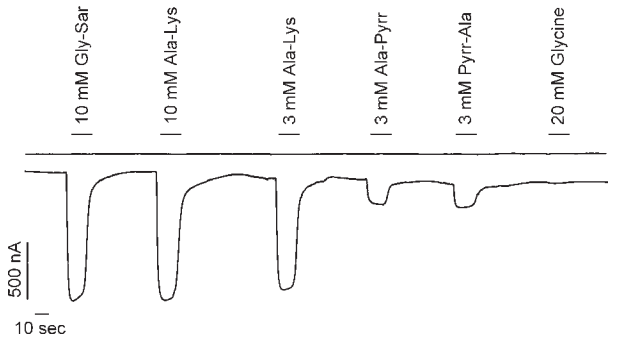

Figure 3. hPEPT1-mediated inward currents at hPEPT1-cRNA injected *Xenopus laevis* oocytes. Currents induced by Ala-Pyrr, Pyrr-Ala, Ala-Lys, Gly-Sar and glycine in water- (upper trace) and hPEPT1-cRNA-injected oocytes (lower trace). Currents were recorded at pH 6.5 by two-electrode voltage-clamp at a membrane potential of −60 mV.

Inward Directed Currents Generated by Ala-Pyrr and Pyrr-Ala at *X. laevis* Oocytes Expressing hPEPT1. Inhibition of [^{14}C]Gly-Sar uptake does not necessarily mean that Ala-Pyrr and Pyrr-Ala themselves are transported by hPEPT1. Using the two-electrode voltage-clamp technique we investigated whether Ala-Pyrr and Pyrr-Ala generate inward directed currents at *X. laevis* oocytes expressing hPEPT1. Such currents at *X. laevis* oocytes occur when a substrate is cotransported by PEPT1 with H^+ in an electrogenic manner. **Figure 3** shows that Ala-Pyrr and Pyrr-Ala (3 mM) generated inward currents of 272 ± 14 nA and 234 ± 12 nA, respectively. Gly-Sar (10 mM) and Ala-Lys (3 mM and 10 mM) generated inward currents of 1033 ± 68 nA, 818 ± 46 nA and 983 ± 44 nA, respectively. Glycine (20 mM, control), which is not transported by PEPT1, showed no currents. Also no currents were observed for either of the test compounds in water-injected oocytes (**Figure 3**).

The currents generated by these pyrraline-containing dipeptides are smaller than those triggered by Gly-Sar or Ala-Lys (**Figure 3**) or by other native dipeptides (*26*) but they are significant. We consider currents as significant when they are (i) transporter specific, i.e., when no signals are obtained using the same substrate concentration in control oocytes, (ii) at least 5% of the currents elicited by reference substrates (dipeptides) and (iii) statistically significantly different from zero (*27*). All three criteria are met. We conclude therefore that Ala-Pyrr and Pyrr-Ala are actively transported by the human PEPT1 in an electrogenic manner, i.e., an H^+-symport. Translocation of substrates by PEPT1 comprises at least the following steps: (i) binding of H^+ and the substrate, (ii) conformational change of the resulting complex, (iii) release of the substrate and H^+ into the cell, and (iv) conformational change of the protein to the initial stage the binding site facing outward. The rate-limiting step of the translocation is still not known. With the exception of the last step, the return of the empty carrier, different PEPT1 substrates might affect these steps differently depending on their specific chemical properties. Modification of lysine to pyrraline leads to an increase in hydrophobicity. The hydrophobicity as expressed by the log $p_{O/W}$ value of Ala-Pyrr and Pyrr-Ala ($\log p_{O/W} = -1.11$) is in the range of the side-chain modified peptides Ala-Lys(Ac) and Ala-Lys(Bu) (*26*) ($\log p_{O/W} = -1.88$ and -0.81, respectively). In most cases, the affinity of such dipeptides to PEPT1 equals or surpasses that of the unmodified peptides (*16*, *26*). The reduced transport rate of Ala-Pyrr and Pyrr-Ala compared to the unmodified dipeptides is very consistent with the results obtained for side-chain modified Lys-Ala and Ala-Lys or Orn-Ala and Ala-Orn derivatives. Inward currents elicited by these substances were reduced by almost 50% as compared to the unmodified peptide (*26*). Space-demanding hydrophobic peptide side-chains can impede the actual transport, i.e. the conformational change of loaded PEPT1. It appears therefore that binding of the substrate is not the rate-limiting step for PEPT1 mediated translocations. This view is strongly supported by the fact that the competitive PEPT1 inhibitors developed previously by side-chain modification of dipeptides (*18*, *26*), even though they display with K_i values as low as 2 μM affinities much higher than those of PEPT1 substrates, do not elicit any currents. Nonetheless, the currents of Pyrr-Ala and Ala-Pyrr are still slightly lower than expected. Hypothetically, the formyl group of pyrraline could react, e.g., with lysine residues to form Schiff's bases. Such bonds might be able to temporarily stabilize the carrier/substrate complex and delay translocation, thereby reducing the currents measured.

hPEPT1-Mediated Transport of Ala-Pyrr and Pyrr-Ala into Transfected HeLa Cells. To establish PEPT1-mediated uptake of pyrraline dipeptides unequivocally, we studied their transport in a second, independent and direct approach. Unlabeled pyrraline, Ala-Pyrr and Pyrr-Ala (1 mM, 30 min) were added to HeLa cells transfected with the empty vector pcDNA3 (control) or with pcDNA3-hPEPT1. Intracellular contents were analyzed by HPLC. The uptake of free pyrraline shows no difference between HeLa cells expressing hPEPT1 and mock cells (**Figure 4**). In contrast, when added as dipeptide, the concentration of intracellular pyrraline is 4- to 7-fold higher in hPEPT1-transfected HeLa cells than in cells transfected with the empty vector. Neither Ala-Pyrr nor Pyrr-Ala could be detected in the cells. We conclude that Ala-Pyrr and Pyrr-Ala are indeed transported by hPEPT1 into the cells. Inside the cells, both dipeptides are hydrolyzed by intracellular peptidases to free pyrraline and alanine.

Transport by hPEPT1 could explain the high oral absorption rates of certain AGEs observed *in vivo* (*14*, *15*). In these studies, healthy volunteers were first asked to renounce processed food

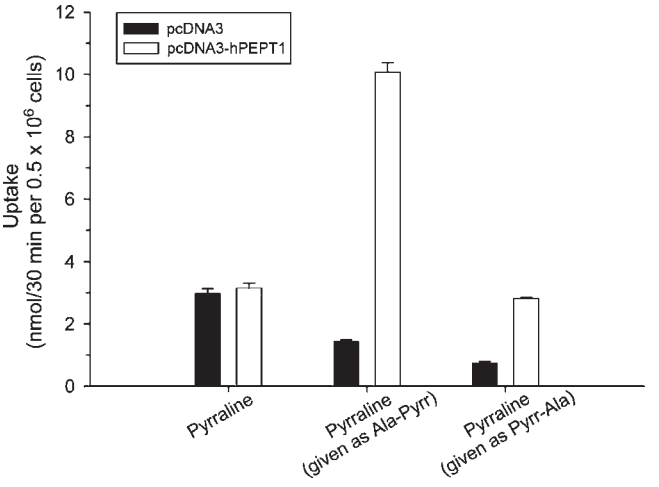

Figure 4. Uptake of pyrraline and its dipeptide derivatives into HeLa cells transfected with pcDNA3 (empty vector) or pcDNA3-hPEPT1. Uptake of pyrraline, Ala-Pyrr and Pyrr-Ala (1 mM) was measured for 30 min at pH 6.0 and 37 °C. The cell contents were analyzed by RP-HPLC. $n = 3-5$.

for 2 days. Then, one single meal with protein-bound pyrraline, which had been formed during processing, was administered. Urine of the volunteers was collected and analyzed for pyrraline and other glycation compounds throughout the study. 50−100% of the administered pyrraline were excreted *via* the urine, providing circumstantial evidence for (i) the ability of intestinal proteases and peptidases to hydrolyze glycated proteins into absorbable peptides and/or amino acids, and (ii) the actual transport. Other glycation compounds did not show such high excretion rates.

In order to demonstrate that pyrraline can indeed be released from glycated proteins, β-casein glycated with 3-DG was subjected to a two-step simulated gastrointestinal digestion. In a preliminary study, the digested sample was analyzed by HPLC−UV−ESI-MS showing that most of the pyrraline is peptide bound. Specific peptides like Ser-Pyrr and Val-Pyrr could be detected. From what we know about the structural requirements of PEPT1 substrates (*18*), we believe that all possible pyrraline dipeptides, probably with the exception of Pro-pyrraline, are transported.

Under physiological conditions, at the intestinal microclimate pH of 6.5 to 6.8 (*17*), hPEPT1 will transport such peptides even uphill against a concentration gradient and accumulate them in enterocytes where they are hydrolyzed. It should be noted that the peptide transporter hPEPT1 is also expressed in the kidney where it is located in the apical membrane of the epithelial cells of renal tubule. At this epithelium, hPEPT1 mediates the reabsorption of its substrates from the primary filtrate into the blood (for review see refs *17*−*19*). Even though our data imply that also the renal PEPT1 would be able to reabsorb Ala-Pyrr and Pyrr-Ala from the primary filtrate, it is very doubtful that these compounds ever appear in the blood and hence in the urine in intact form. Transport of Ala-Pyrr and Pyrr-Ala by renal hPEPT1 (or hPEPT2 (*17*, *19*)) is therefore probably irrelevant under physiological conditions.

In summary, this study established for the first time the transport of an advanced glycation end product by a peptide transporter. PEPT1 is most likely responsible for the intestinal absorption of pyrraline dipeptides after food intake. This finding necessitates further, intensified studies on potentially harmful effects of glycation products in the human body. Moreover, with Ala-Pyrr and Pyrr-Ala we identified new peptide transporter substrates.

ABBREVIATIONS USED

Ala-Pyrr, alanylpyrraline; Pyrr-Ala, pyrralylalanine; Gly-Sar, glycylsarcosine; PEPT1, proton-coupled peptide transporter 1; AGE, advanced glycation end product; HPLC, high pressure liquid chromatography; 3-DG, 3-deoxyglucosulose.

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