Differential Recognition of ACE Inhibitors in Xenopus Laevis Oocytes Expressing Rat PEPT1 and PEPT2

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Received December 21, 1999; accepted February 3, 2000

Purpose. To examine the mechanism of inhibition of glycylsarcosine (GlySar) transport by quinapril and enalapril, and whether or not angiotensin converting enzyme (ACE) inhibitors are transported by PEPT2 as well as by PEPT1.

Methods. Xenopus laevis oocytes were cRNA-injected with rat PEPT1 or PEPT2 and the transport kinetics of radiolabeled GlySar were studied in the absence and presence of quinapril and enalapril. The two-microelectrode voltage-clamp technique was also performed to probe the electrogenic uptake of captopril, quinapril and enalapril.

Results. Kinetic analyses demonstrated that quinapril inhibited the uptake of GlySar in a noncompetitive manner in Xenopus oocytes injected with PEPT1 or PEPT2 (Ki = 0.8 or 0.4 mM, respectively). In contrast, a competitive interaction was observed between GlySar and enalapril (Ki = 10.8 mM for PEPT1 or 4.3 mM for PEPT2). Most significantly, captopril and enalapril, but not quinapril, induced inwardly-directed currents in both PEPT1- and PEPT2-expressed oocytes.

Conclusions. These results are unique in providing direct evidence for the substrate recognition and transport of some ACE inhibitors by the high- and low-affinity oligopeptide transporters. Our findings point to differences between PEPT1 and PEPT2 in their affinity to, rather than in their specificity for, ACE inhibitors.

KEY WORDS: ACE inhibitors; PEPT1; PEPT2; captopril; enalapril; quinapril.

INTRODUCTION

Expression and molecular cloning studies have resulted in the identification of two distinct peptide transporters in rabbit (1–3), rat (4–6) and human (7,8). The localization of PEPT1 (in intestine and kidney) and PEPT2 (in kidney) are consistent with their physiological roles in absorbing small peptides arising from digestion of dietary proteins in the small intestine, as well as in reabsorbing filtered peptides generated by luminal peptidases in the kidney (9–11). More recently, a peptide/histidine transporter (PHT1) which is weakly homologous to PEPT1 and PEPT2 has been cloned from rat brain (12). Although expressed in the brain and eye, PHT1 is not found in the intestine or kidney, and its physiological role remains to be elucidated.

The transport of small peptides and peptidomimetics by PEPT1 and PEPT2 are phenomenologically similar in that both processes are energized by an electrochemical proton gradient. However, there are marked differences in substrate affinity such that PEPT1 is a high-capacity, low-affinity transporter and PEPT2 is a low-capacity, high-affinity transporter. Although PEPT1 and PEPT2 have overlapping substrate specificities, differences have been reported in their ability to transport some angiotensin converting enzyme (ACE) inhibitors (1,2). As a result, it is generally believed that ACE inhibitors, lacking a free α-amino group, are not substrates for the renal peptide carrier PEPT2 (13). Conflicting with this belief are more recent studies in which several ACE inhibitors were found to interact with the renal carrier and with different mechanisms of interaction (14,15). In particular, quinapril inhibited the uptake of glycylsarcosine (GlySar) in a noncompetitive manner in rabbit renal brush border membrane vesicles, while enalapril's inhibition was of a competitive type.

In view of these findings, and because of both peptide transporters being present in kidney (16,17), the mechanism of inhibition of glycylsarcosine transport by quinapril and enalapril was further studied in Xenopus *laevis* oocytes injected with the cRNA of rat PEPT1 and PEPT2. Electrophysiology experiments were also performed in the same oocyte expression system with captopril, quinapril and enalapril. Overall, we demonstrate for the first time that some ACE inhibitors (i.e., captopril and enalapril, but not quinapril) are transported by PEPT2, as well as by PEPT1, in a concentration-dependent and electrogenic fashion.

METHODS

Materials

[^{14}C]Glycylsarcosine (GlySar; 119 mCi/mmol) was purchased from Amersham (Chicago, IL). Captopril was obtained from Sigma Chemical Co (St. Louis, MO). Quinapril was a gift from Parke-Davis (Ann Arbor, MI) and enalapril from Merck (Rahway, NJ). Collagenase A was purchased from Boehringer Mannheim (Indianapolis, IN). Other chemicals were obtained from standard sources and were of the highest quality available.

Isolation of Rat PEPT1 and PEPT2 Clones

A rat duodenal cDNA library (18) was screened using a 0.62 kb PEPT1 probe generated by specific PCR primers. In a similar manner, a rat kidney cortex λgt10 cDNA library (19) was screened using a specific 0.52 kb PEPT2 probe. The full-length PEPT1 and PEPT2 cDNAs were isolated and subcloned into PTLN2 plasmid. Sequence analyses have confirmed their complete identity with rat PEPT1 (4) and PEPT2 (6), and the clones have been used subsequently in stoichiometry (20) and immunolocalization studies (17).

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ABBREVIATIONS: ACE, angiotensin converting enzyme; BBMV, brush border membrane vesicles; GlySar, glycylsarcosine; Hepes, N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid); Mes, 2-(N-morpholino)ethanesulfonic acid; Tris, tris (hydroxymethyl)-aminomethane.

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Uptake Studies in Xenopus Oocytes

Xenopus *laevis* oocytes were prepared as described previously (21), except that oocytes were defolliculated by incubating them in calcium-free Barth's solution containing collagenase (3 mg/ml) at 18°C for 3–3.5 hours. Capped cRNAs of rat PEPT1 and PEPT2 were synthesized by *in vitro* transcription and mature oocytes were injected with 50 nl of each transporter (10 ng for PEPT1 or 15 ng for PEPT2). Control oocytes were injected with 50 nl of deionized water. Oocytes were maintained at 18°C in Barth's solution supplemented with 50 μg/ml gentamicin, and 100 μg/ml each of penicillin and streptomycin. Uptake studies were carried out 3 days after injection. The research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

Oocytes were incubated with radiolabeled GlySar (± ACE inhibitors) for 1 hour at room temperature in a buffer that contained (in mM): NaCl, 100; KCl, 2; $MgCl_2$, 1; $CaCl_2$, 1; Mes, 3; Tris, 3; Hepes, 3; adjusted to pH 6.0 with Tris. Following several rinses with ice-cold washing solution (i.e., uptake solution Tris adjusted to pH 7.5), individual oocytes were dissolved in 250 μl of 10% SDS, mixed with 2.5 ml of scintillation cocktail, and the radioactivity measured by liquid scintillation counting. Uptake rates of GlySar into water-injected oocytes were always subtracted from uptake rates in oocytes expressing rat PEPT1 or PEPT2. These experimental conditions were optimized based on the results of preliminary studies. Likewise, dipeptide uptake was linear over a 1-hour time period (data not shown).

Electrophysiology Studies in Xenopus Oocytes

Steady-state currents were evaluated using a conventional two-microelectrode voltage-clamp method, as described previously (19,20). Uptake solutions (as described for inhibition studies, pH 6.0) were used for extracellular perfusion at approximately 1.5 ml/min. After 3–5 minutes of membrane potential stabilization following microelectrode impalements, the oocyte membrane potential was clamped at −50 mV. When recording currents at a holding potential, digitalization at 0.5 s/sample and filtering at 20 Hz were used. Substrate-induced currents were then evaluated, at room temperature, as the difference between currents recorded before and after substrate addition.

Data Analysis

Unless otherwise specified, results are reported as mean ± SD. In an experiment, each data point reflects 4–9 oocytes obtained from at least two different donors. Statistical differences among treatment groups were evaluated by analysis of variance (ANOVA) and pairwise comparisons were made using Tukey's test ($\alpha = 0.05$). All statistical computations were performed using SYSTAT v5.03 (Systat, Inc., Evanston, IL). Nonlinear as well as linear regression analyses were conducted using Scientist v2.01 (MicroMath Scientific Software, Salt Lake City, UT) and a weighting factor of unity. The quality of the fit was determined by evaluating the coefficient of determination (r^2), the standard deviation of parameter estimates, and by visual inspection of the residuals.

The saturation kinetics of GlySar (± inhibitors) have been described previously (14,15). Briefly,

$$v = \frac{Vmax \cdot C}{Km + C} \quad (1)$$

where v represents the uptake rate, Vmax is the maximal rate of uptake, Km is the Michaelis constant, and C is the substrate (GlySar) concentration. Quinapril inhibited the uptake of GlySar in a noncompetitive manner (see Results section). For transport in the presence of a noncompetitive inhibitor, the Lineweaver-Burk transformation gives:

$$\frac{1}{v} = \frac{1}{Vmax} \cdot \left(1 + \frac{I}{Ki}\right) + \frac{Km}{Vmax} \cdot \left(1 + \frac{I}{Ki}\right) \cdot \frac{1}{C} \quad (2)$$

where Ki is the inhibition constant and I is the inhibitor concentration. Likewise, for noncompetitive inhibition the Dixon plot gives:

$$\frac{1}{v} = \frac{\left(1 + \frac{Km}{C}\right)}{Vmax \cdot Ki} \cdot I + \frac{1}{Vmax} \cdot \left(1 + \frac{Km}{C}\right) \quad (3)$$

In contrast, enalapril inhibited the uptake of GlySar in a competitive manner (see Results section) and, as a result, the Lineweaver-Burk and Dixon plots become:

$$\frac{1}{v} = \frac{1}{Vmax} + \frac{Km \cdot \left(1 + \frac{I}{Ki}\right)}{Vmax} \cdot \frac{1}{C} \quad (4)$$

$$\frac{1}{v} = \frac{Km}{Vmax \cdot Ki \cdot C} \cdot I + \frac{1}{Vmax} \cdot \left(1 + \frac{Km}{C}\right) \quad (5)$$

Ki was estimated for quinapril and enalapril by two different approaches: i) by linear regression of Lineweaver-Burk plots expressed by Equations 2 and 4, respectively and ii) by linear regression of Dixon plots expressed by Equations 3 and 5, respectively.

RESULTS

Concentration-Dependent Uptake of GlySar

The initial rate uptake of radiolabeled GlySar was evaluated as a function of increasing concentrations of unlabeled dipeptide in cRNA-injected rat PEPT1 or PEPT2 Xenopus oocytes. As shown in Fig. 1, these studies demonstrate that GlySar has a low-affinity for PEPT1 (Km = 237 ± 30 μM, Vmax = 1263 ± 239 pmol/oocyte/hr; $r^2 \geq 0.991$) relative to PEPT2 (Km = 22.3 ± 1.2 μM, Vmax = 212 ± 35 pmol/oocyte/hr; $r^2 \geq 0.990$) and that the differences in affinity are substantial (i.e., 10-fold). Moreover, a linear transformation of the data (Fig. 1 inserts) indicates that GlySar interacts with a single transporter. Overall, the results are consistent with PEPT1 being a high-capacity, low-affinity symporter whereas PEPT2 transports small peptides with low-capacity but high-affinity. These findings plus dose-response analyses with quinapril and enalapril (data not shown) were then used to guide subsequent experiments probing the mechanism of inhibition for each ACE inhibitor (as described below).

Inhibition Studies

In Xenopus oocytes expressing rat PEPT1 or PEPT2, quinapril resulted in changes in the slope and y-intercept of the

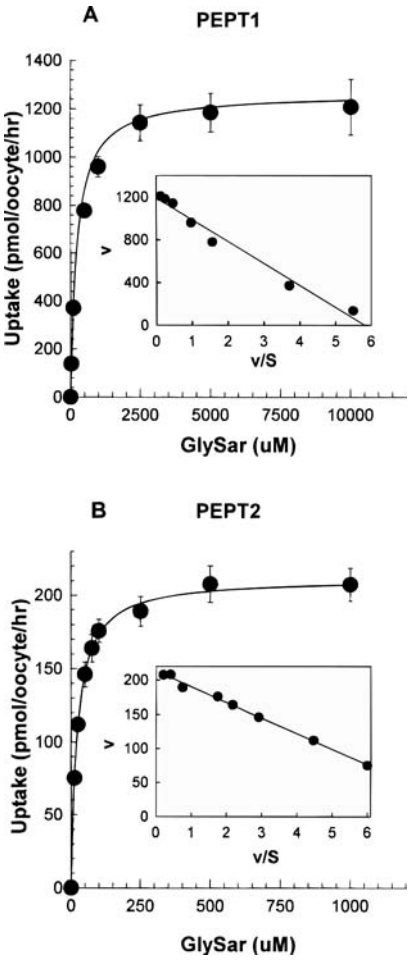

Fig. 1. Saturable uptake of GlySar in Xenopus oocytes expressing rat PEPT1 (1A) or PEPT2 (1B). Uptake studies were carried out 3 days after oocytes were cRNA-injected with 10 ng of PEPT1 or 15 ng of PEPT2. The one-hour uptake of radiolabeled GlySar (5.9 μM) ± unlabeled dipeptide (0–10 mM for PEPT1 or 0–1.0 mM for PEPT2) was determined at room temperature in pH 6.0 buffer. All data were corrected for the uptake in water-injected oocytes, and are expressed as mean ± SE from 3 separate experiments. The line was generated using fitted mean parameters (see text), as determined by nonlinear regression analysis. The inset shows a Woolf-Augustinsson-Hofstee transformation of the data [GlySar uptake, v (pmol/oocyte/hr) vs. GlySar uptake/concentration, v/S (μl/oocyte/hr)].

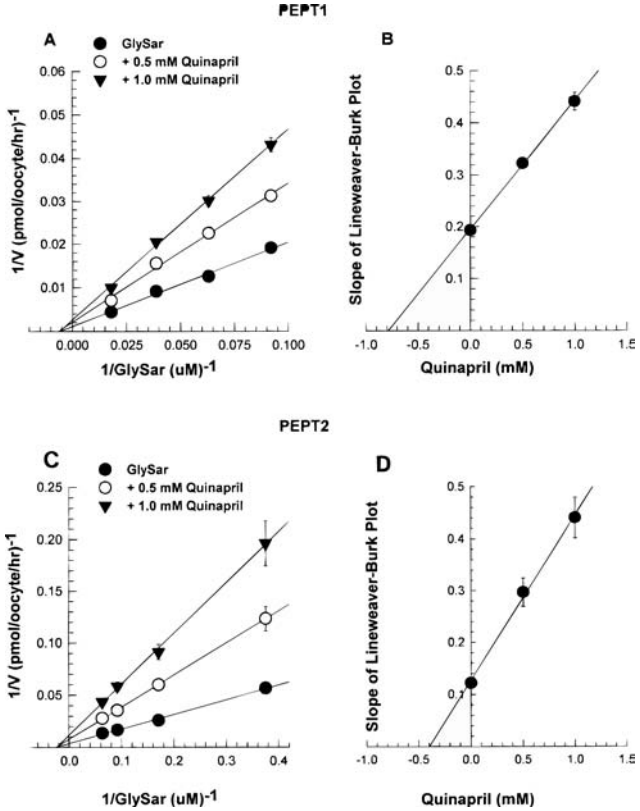

Fig. 2. Lineweaver-Burk analyses of the effect of quinapril on GlySar uptake in Xenopus oocytes expressing rat PEPT1 (2A and 2B) or PEPT2 (2C and 2D). Uptake studies were carried out 3 days after oocytes were cRNA-injected with 10 ng of PEPT1 or 15 ng of PEPT2. The one-hour uptake of radiolabeled GlySar (2.7–55.9 μM) ± quinapril (0–1.0 mM) was determined at room temperature in pH 6.0 buffer. All data were corrected for the uptake in water-injected oocytes, and are expressed as mean ± SE from 3 separate experiments. Lines were generated using fitted mean parameters (see Table I), as determined by linear regression analysis.

Lineweaver-Burk plots, but not the x-intercept values (Figs. 2A and 2B for PEPT1; Figs. 2C and 2D for PEPT2). As a result, kinetic analyses (Table I) revealed a significant decrease in the apparent Vmax of GlySar as the concentrations of quinapril increased (918 pmol/oocyte/hr for control vs. 525 and 387 pmol/oocyte/hr for PEPT1; 188 pmol/oocyte/hr for control vs. 107 and 61 pmol/oocyte/hr for PEPT2). On the other hand, the apparent Km for GlySar did not change significantly when studied with quinapril (169 to 177 μM for PEPT1; 23.1 to 26.3 μM for PEPT2). These results demonstrate that the interaction between quinapril and GlySar for both rat PEPT1 and PEPT2 was noncompetitive. Further evidence for a noncompetitive inhibition was provided by Dixon plot analyses. As shown in Figs. 3A and 3B (for PEPT1) and Figs. 3C and 3D (for PEPT2), the curves (1/uptake vs. inhibitor) intersect on the x-axis, and the slopes of these same curves vs. 1/GlySar have a y-intercept that is significant. Regardless of the method used (Table I), the Ki values of quinapril were comparable, approximating 0.8 mM for PEPT1 and 0.4 mM for PEPT2.

In Xenopus oocytes expressing rat PEPT1 or PEPT2, the effect of enalapril was in sharp contrast to that of quinapril. Thus, enalapril addition revealed changes in the slope and x-intercept of Lineweaver-Burk plots, but not in the y-intercept of the curves (Figs. 4A and 4B for PEPT1; Figs. 4C and 4D for PEPT2). Kinetic analyses (Table II) were consistent with a competitive inhibition mechanism, in which the apparent Km of GlySar increased significantly in the presence of enalapril (195 μM for control vs. 275 and 397 μM for PEPT1; 20.3 μM for control vs. 29.0 and 41.0 μM for PEPT2) with no change in apparent Vmax values (615 to 649 pmol/oocyte/hr for PEPT1; 89.5 to 93.9 pmol/oocyte/hr for PEPT2). A competitive inhibition of GlySar by enalapril was also supported by Dixon plot analyses (Figs. 5A and 5B for PEPT1; Figs. 5C and 5D for PEPT2). For both transporters, the curves (1/uptake vs. inhibitor) intersect above the x-axis and the slopes of these same curves vs. 1/GlySar form a line that goes right through the origin. Ki estimates for enalapril were consistent between the

Table I. Noncompetitive Inhibition of GlySar Uptake by Quinapril in Xenopus Oocytes Expressing Rat PEPT1 and PEPT2

	PEPT1		PEPT2	
GlySar Parameters Lineweaver-Burk	Vmax,app (pmol/ oocyte/hr)	Km,app (μM)	Vmax,app (pmol/ oocyte/hr)	Km,app (μM)
GlySar (control)	918 ± 27	177 ± 6	188 ± 16	23.1 ± 5.1
+ Quinapril 0.5 mM	525 ± 14[a]	169 ± 3	107 ± 22[c]	24.8 ± 3.1
+ Quinapril 1.0 mM	387 ± 38[b]	170 ± 20	61 ± 9[d]	26.3 ± 4.8
Quinapril Parameters	Ki (mM)		Ki (mM)	
Lineweaver-Burk	0.81 ± 0.17		0.42 ± 0.09[e]	
Dixon	0.81 ± 0.10		0.41 ± 0.12[e]	

Note: Values are mean ± SD from 3 separate experiments. Vmax,app is equal to Vmax divided by (1 + I/Ki) and Km,app is equal to Km.
[a] Significantly different from control and 1.0 mM inhibitor for rat PEPT1.
[b] Significantly different from control and 0.5 mM inhibitor for rat PEPT1.
[c] Significantly different from control and 1.0 mM inhibitor for rat PEPT2.
[d] Significantly different from control and 0.5 mM inhibitor for rat PEPT2.
[e] Significantly different from Ki for rat PEPT1.

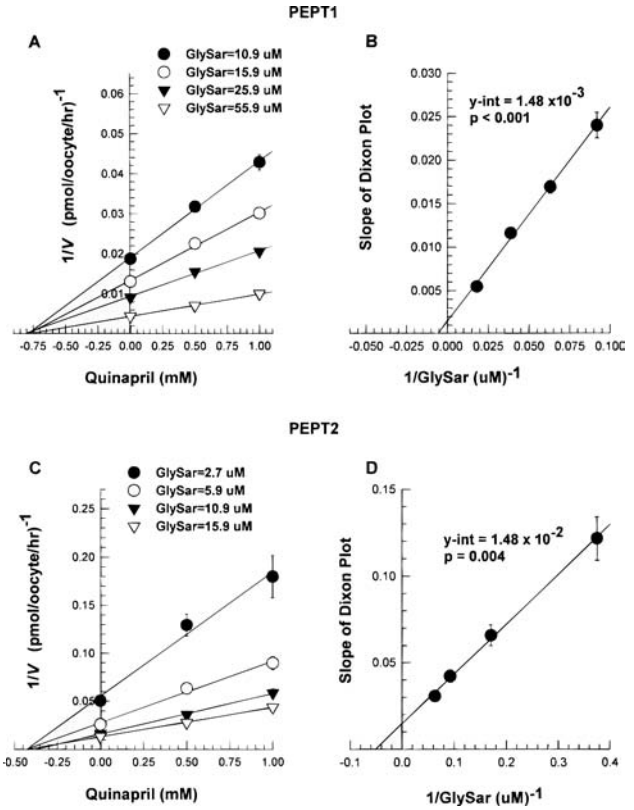

Fig. 3. Dixon plots of the effect of quinapril on the kinetics of GlySar uptake in Xenopus oocytes expressing rat PEPT1 (3A and 3B) or PEPT2 (3C and 3D). Uptake studies were carried out 3 days after oocytes were cRNA-injected with 10 ng of PEPT1 or 15 ng of PEPT2. The one-hour uptake of radiolabeled GlySar (2.7–55.9 μM) ± quinapril (0–1.0 mM) was determined at room temperature in pH 6.0 buffer. All data were corrected for the uptake in water-injected oocytes, and are expressed as mean ± SE from 3 separate experiments. Lines were generated using fitted mean parameters (see Table I), as determined by linear regression analysis.

two methods tested, and were about 10.8 mM for PEPT1 and 4.3 mM for PEPT2.

Electrophysiology Studies

The electrogenic uptake of ACE inhibitors (i.e., captopril, quinapril, enalapril) was evaluated in rat PEPT1-and PEPT2-expressed oocytes under voltage-clamp conditions. As shown in Fig. 6, inwardly-directed currents were induced by 10 mM captopril in both PEPT1-and PEPT2-expressed oocytes. Application of 1.2 mM quinapril in the presence of 10 mM captopril substantially decreased these currents. In addition, quinapril alone did not evoke inward currents. These data demonstrate that quinapril is a non-transported inhibitor of both peptide transporters. Note that quinapril induced small outward currents in the absence of external substrate for both PEPT1 and PEPT2 (Fig. 6). In fact, in the absence of external substrate, PEPT2 was shown to mediate small uncoupled proton influxes (proton leak, see reference 20) that were inhibited by 1.2 mM of quinapril. Taken together, quinapril inhibited both cotransport and uncoupled proton leak mediated by PEPT1 or PEPT2. In contrast, enalapril was capable of inducing concentration-dependent currents for both rat PEPT1 (Vmax = 55.2 ± 5.9 nA, Km = 15.7 ± 3.3 mM; $r^2 \geq 0.989$) and PEPT2 (Vmax = 13.9 ± 3.9 nA, Km = 1.2 ± 0.2 mM; $r^2 \geq 0.969$), as shown in Fig. 7, indicating that enalapril is a transportable substrate. Moreover, enalapril appears to have a greater affinity for PEPT2 as compared to PEPT1 ($p < 0.001$). For all studies, ACE inhibitors had no significant effect on control (water-injected) oocytes.

DISCUSSION

Conflicting data have been reported regarding the selective transport of ACE inhibitors by PEPT1 and PEPT2, and the reason underlying this apparent selectivity. Thus, it is generally believed that in contrast to the intestinal transporter PEPT1, ACE inhibitors and β-lactam antibiotics without an α-amino side chain are not substrates for the renal homologue PEPT2. This opinion was based largely on inhibition and electrophysiology studies in Xenopus oocytes expressing rabbit PEPT1 or PEPT2. In the first study (1), the transport of 1 mM radiolabeled cefadroxil (in pH 6.5 buffer) was cis-inhibited by 10 mM of enalapril or captopril, but not lisinopril, in rabbit PEPT1 cRNA-injected oocytes. This finding was confirmed by a rapid depolarization of the membrane potential in oocytes being bathed with 2 mM of captopril. However, in the second study (2), 5 mM of captopril or enalapril failed to cis-inhibit the uptake of 25 μM radiolabeled cefadroxil (in pH 6.0 buffer) in oocytes expressing rabbit PEPT2. Subsequent to these experiments, studies in rabbit renal brush border membrane vesicles (BBMV) have demonstrated that a wide variety of ACE inhibitors can interact with the high-affinity peptide carrier in kidney (14,15). It was also observed that while quinapril was a noncompetitive inhibitor of GlySar, enalapril inhibited the uptake of GlySar in a competitive manner. These latter studies suggested that enalapril was a substrate for PEPT2 and that quinapril could inhibit but was not, in itself, transported. However, renal BBMV

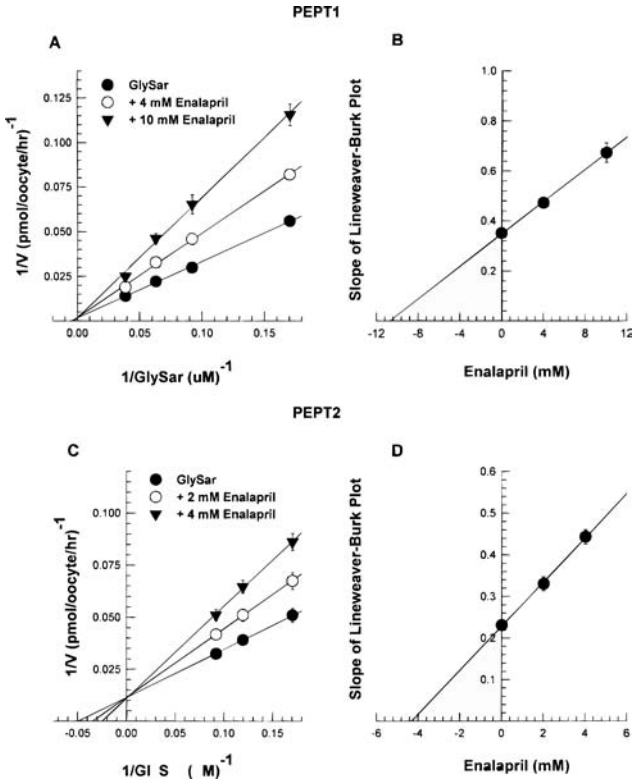

Fig. 4. Lineweaver-Burk analyses of the effect of enalapril on GlySar uptake in Xenopus oocytes expressing rat PEPT1 (4A and 4B) or PEPT2 (4C and 4D). Uptake studies were carried out 3 days after oocytes were cRNA-injected with 10 ng of PEPT1 or 15 ng of PEPT2. The one-hour uptake of radiolabeled GlySar (3.0–25.9 μM) ± enalapril (0–10 mM) was determined at room temperature in pH 6.0 buffer. All data were corrected for the uptake in water-injected oocytes, and are expressed as mean ± SE from 3 separate experiments. Lines were generated using fitted mean parameters (see Table II), as determined by linear regression analysis.

Table II. Competitive Inhibition of GlySar Uptake by Enalapril in Xenopus Oocytes Expressing Rat PEPT1 and PEPT2

	PEPT1		PEPT2	
GlySar Parameters Lineweaver-Burk	Vmax,app (pmol/ oocyte/hr)	Km,app (μM)	Vmax,app (pmol/ oocyte/hr)	Km,app (μM)
GlySar (control)	615 ± 40	195 ± 16		
+ Enalapril 4 mM	629 ± 6	275 ± 2[a]		
+ Enalapril 10 mM	649 ± 52	397 ± 19[b]		
GlySar (control)			89.9 ± 5.1	20.3 ± 0.9
+ Enalapril 2 mM			89.5 ± 4.1	29.0 ± 1.7[c]
+ Enalapril 4 mM			93.9 ± 5.0	41.0 ± 0.4[d]
Enalapril Parameters	Ki (mM)		Ki (mM)	
Lineweaver-Burk	10.8 ± 0.8		4.29 ± 0.54[e]	
Dixon	10.8 ± 0.9		4.33 ± 0.49[e]	

Note: Values are mean ± SD from 3 separate experiments. Vmax,app is equal to Vmax and Km,app is equal to Km multiplied by (1 + I/Ki).
[a] Significantly different from control and 10 mM inhibitor for rat PEPT1.
[b] Significantly different from control and 4 mM inhibitor for rat PEPT1.
[c] Significantly different from control and 4 mM inhibitor for rat PEPT2.
[d] Significantly different from control and 2 mM inhibitor for rat PEPT2.
[e] Significantly different from Ki for rat PEPT1.

experiments are limited in that they do not allow for the individual evaluation of PEPT1 and PEPT2. In addition, due to the unavailability of radiolabeled ACE inhibitor, direct evidence for transport is lacking.

In the present study, the differential mechanism of dipeptide inhibition by quinapril and enalapril was clearly demonstrated in Xenopus oocytes injected with rat PEPT1-or PEPT2-cRNA. Quinapril noncompetitively inhibited the transport of GlySar in oocytes expressing PEPT1 (Ki ≈ 0.8 mM) while enalapril was a competitive inhibitor under similar experimental conditions (Ki ≈ 10.8 mM). Likewise, quinapril was a noncompetitive inhibitor of GlySar transport in oocytes expressing PEPT2 (Ki ≈ 0.4 mM) while enalapril displayed a competitive inhibition under similar conditions (Ki ≈ 4.3 mM). These results demonstrate that ACE inhibitors exhibit low-affinity interactions with both oligopeptide transporters, and that not all ACE inhibitors (e.g., quinapril) are substrates for PEPT1 and/or PEPT2.

Direct evidence for transport of some ACE inhibitors by either rat PEPT2 or PEPT1 was afforded by the electrophysiology experiments performed with captopril, quinapril and enalapril. In this regard, convincing evidence was provided for the electrogenic and concentration-dependent uptake of captopril and enalapril by both oligopeptide transporters. On the other hand, quinapril was not a viable substrate but an inhibitor capable of reducing the captopril-evoked currents in oocytes expressing PEPT1 and PEPT2. In the absence of external substrate, an inward quinapril-sensitive proton current (i.e., proton leak) was also observed in oocytes expressing PEPT2 and, to a lesser extent, PEPT1. This finding, together with a noncompetitive mechanism of inhibition, suggests that quinapril may adversely affect the binding and/or translocation of at least one proton with the transporter, as described previously for the rat PEPT2 protein (20). Given the large degree of structural similarity between quinapril and enalapril, it is curious why these two ACE inhibitors are handled so differently by the H^+ -coupled peptide symporters. While enalapril has a proline moiety at the carboxyl end of its peptidomimetic structure, this group is replaced in quinapril by tetrahydroisoquinolinecarboxylic acid. Although speculative, it is possible that additional steric or hydrophobic interactions may occur, because of this substitution, such that quinapril binds to PEPT1 and PEPT2 in such a way that cotransport is conformationally unfavorable. For the same reason, captopril can be transported by both peptide transporters since it too has a proline group at its carboxyl terminus. Ultimately, confirmation of this issue will require a deeper analysis of the structure-activity relationship of these compounds.

ACE inhibitors are important therapeutic agents for treating patients with hypertension and cardiovascular diseases. Since these drugs are administered orally and have local (i.e., renal) actions in addition to systemic effects, it is crucial to understand the mechanisms by which ACE inhibitors are transported across brush border membranes in the intestine and kidney. At usual therapeutic doses, ACE inhibitors reach mM concentrations in the intestine and, in doing so, achieve levels at which they may compete with other substrates for PEPT1 uptake. At these same doses, ACE inhibitors are likely to be present in the renal tubular lumen at low μM concentrations

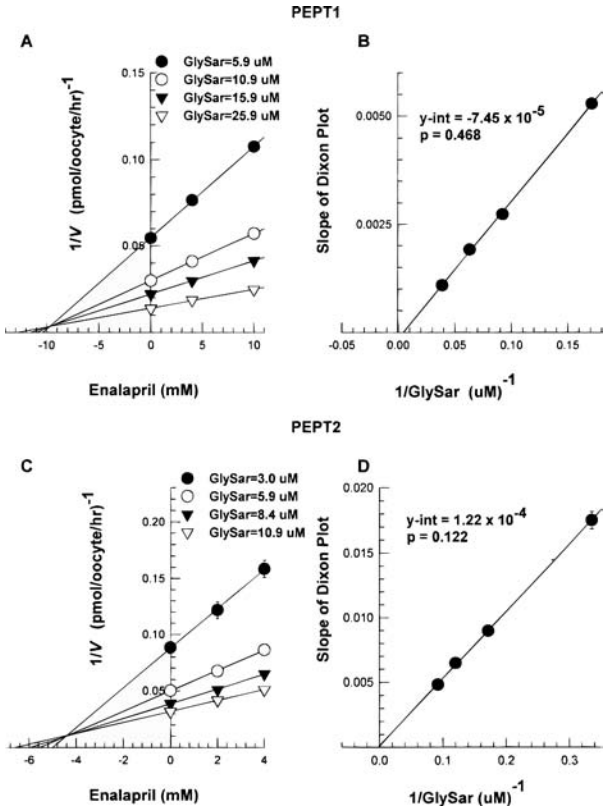

Fig. 5. Dixon plots of the effect of enalapril on the kinetics of GlySar uptake in Xenopus oocytes expressing rat PEPT1 (5A and 5B) or PEPT2 (5C and 5D). Uptake studies were carried out 3 days after oocytes were cRNA-injected with 10 ng of PEPT1 or 15 ng of PEPT2. The one-hour uptake of radiolabeled GlySar (3.0–25.9 μM) ± enalapril (0–10 mM) was determined at room temperature in pH 6.0 buffer. All data were corrected for the uptake in water-injected oocytes, and are expressed as mean ± SE from 3 separate experiments. Lines were generated using fitted mean parameters (see Table II), as determined by linear regression analysis.

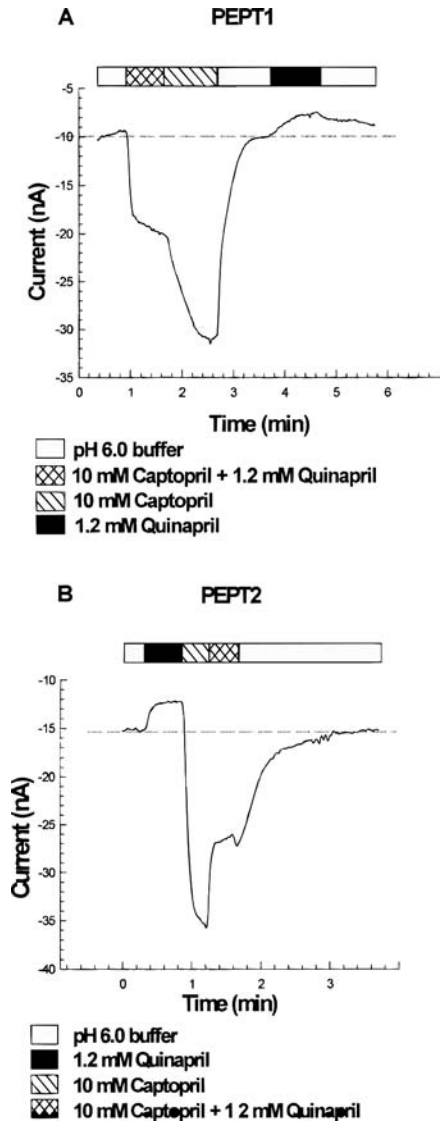

Fig. 6. Representative recording of steady-state currents evoked by captopril and/or quinapril in Xenopus oocytes expressing rat PEPT1 (6A) or PEPT2 (6B). Similar results were observed in additional oocytes (n = 4 for PEPT1 or PEPT2). Using the two-microelectrode voltage-clamp method, currents were measured at −50 mV in pH 6.0 buffer at room temperature. Studies were performed 3 days after oocytes were cRNA-injected with 10 ng of PEPT1 or 15 ng of PEPT2. Water-injected oocytes did not display a significant response in current to the addition of ACE inhibitors.

and, consequently, their ability to inhibit PEPT2-mediated reabsorption of peptides and peptide-like drugs is small. In contrast, peptide-bound amino acids (i.e., di- and tripeptides) are filtered at the glomerulus in high μM to mM concentrations (22) and, as a result, may compete with ACE inhibitors to minimize drug reabsorption in the kidney. However, the extent of ACE inhibitor uptake (i.e., reabsorption as well as absorption) is uncertain *in vivo*, as well as the role of oligopeptide transporters relative to other carrier-mediated and passive components. Further, a lack of information regarding the actual concentrations of naturally-occurring peptides at transporting sites, the residence times in a particular region during the sequential transport of substrate along the nephron, the modulating effect of peptidases in the luminal brush border, and the presence of both PEPT1 and PEPT2 in kidney make predictions difficult. Perhaps, with the development of transgenic knockout mice, more definitive answers can be obtained concerning the significance of PEPT1 and PEPT2 in influencing the physiological uptake of peptides and peptidomimetic drugs in the intestine and kidney.

In summary, our studies demonstrate for the first time the direct electrogenic uptake of captopril and enalapril in Xenopus oocytes expressing rat PEPT1 or PEPT2. This finding is novel in that it argues against a free α-amino group as being an absolute requirement for substrate recognition by the high-affinity transporter in kidney, PEPT2. We also demonstrate that not all ACE inhibitors (e.g., quinapril) are uniformly transported across the intestine or kidney by H^+-coupled peptide transporters. These observations point to differences between PEPT1 and PEPT2 in their affinity to, rather than in their specificity for, ACE inhibitors.

ACKNOWLEDGMENTS

We thank P. Fong for kindly providing the PTLN2 oocyte expression vector. This work was supported in part by Grant R01

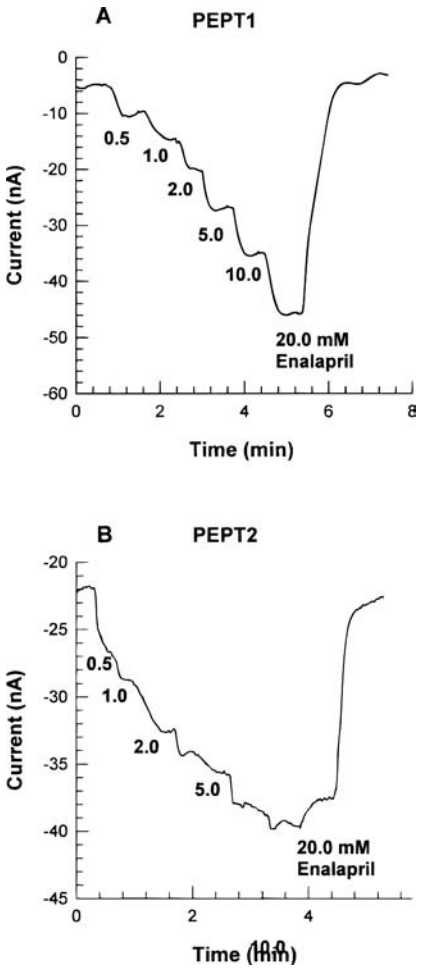

Fig. 7. Representative recording of dose-dependent currents evoked by enalapril in Xenopus oocytes expressing rat PEPT1 (7A) or PEPT2 (7B). Similar results were observed in additional oocytes (n = 4 for PEPT1 or PEPT2; see text for enalapril's transport parameters). Currents were measured at −50 mV and external pH 6.0 using enalapril at concentrations ranging from 0 to 20 mM. No significant currents due to enalapril addition were observed in water-injected oocytes.

GM35498 (to D.E.S.) and DK43171 (to M.A.H.) from the National Institutes of Health. X.-Z.C. is a recipient of the International Human Frontier Science Program, Long-Term Fellowship.

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