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Angiotensin-converting enzyme (ACE) inhibitor transport in human intestinal epithelial (Caco-2) cells

David T. Thwaites, Megan Cavet, Barry H. Hirst & [1]Nicholas L. Simmons

Gastrointestinal Drug Delivery Research Centre, Department of Physiological Sciences, University of Newcastle upon Tyne, Medical School, Newcastle upon Tyne, NE2 4HH

1 The role of proton-linked solute transport in the absorption of the angiotensin-converting enzyme (ACE) inhibitors captopril, enalapril maleate and lisinopril has been investigated in human intestinal epithelial (Caco-2) cell monolayers.

2 In Caco-2 cell monolayers the transepithelial apical-to-basal transport and intracellular accumulation (across the apical membrane) of the hydrolysis-resistant dipeptide, glycylsarcosine (Gly-Sar), were stimulated by acidification (pH 6.0) of the apical environment. In contrast, transport and intracellular accumulation of the angiotensin-converting enzyme (ACE) inhibitor, lisinopril, were low (lower than the paracellular marker mannitol) and were not stimulated by apical acidification. Furthermore, [^{14}C]-lisinopril transport showed little reduction when excess unlabelled lisinopril (20 mM) was added.

3 pH-dependent [^{14}C]-Gly-Sar transport was inhibited by the orally-active ACE inhibitors, enalapril maleate and captopril (both at 20 mM). Lisinopril (20 mM) had a relatively small inhibitory effect on [^{14}C]-Gly-Sar transport. pH-dependent [^{3}H]-proline transport was not inhibited by captopril, enalapril maleate or lisinopril.

4 Experiments with BCECF[2',7',-*bis*(2-carboxyethyl)-5(6)-carboxyfluorescein]-loaded Caco-2 cells demonstrate that dipeptide transport across the apical membrane is associated with proton flow into the cell. The dipeptide, carnosine (β-alanyl-L-histidine) and the ACE inhibitors, enalapril maleate and captopril, all lowered intracellular pH when perfused at the apical surface of Caco-2 cell monolayers. However, lisinopril was without effect.

5 The effects of enalapril maleate and captopril on [^{14}C]-Gly-Sar transport and pH_i suggest that these two ACE inhibitors share the H^+-coupled mechanism involved in dipeptide transport. The absence of pH-dependent [^{14}C]-lisinopril transport, the relatively small inhibitory effect on [^{14}C]-Gly-Sar transport, and the absence of lisinopril-induced pH_i changes, all suggest that lisinopril is a poor substrate for the di/tripeptide carrier in Caco-2 cells. These observations are consistent with the greater oral availability and time-dependent absorption profile of enalapril maleate and captopril, compared to lisinopril.

Keywords: Proton-coupled transport; dipeptide; amino acid; ACE inhibitor; intestine; epithelium; Caco-2 cells; intracellular pH

Introduction

The intestinal di/tripeptide transport is an important membrane transport protein localized at the apical surface of the intestinal epithelium (Matthews, 1975; Matthews & Adibi, 1976) that plays a significant physiological role in the absorption of protein in the form of small peptides (2-3 amino acids in length). Unlike most other ion/solute co-transporters of the gastrointestinal tract, transport via the di/tripeptide carrier is coupled to the movement of protons (Ganapathy & Leibach, 1985). The driving force for this H^+-coupled carrier is provided by the acid microclimate (an area of low pH lying adjacent to the apical surface of the intestinal epithelium) as demonstrated both *in vivo* (McEwan *et al.*, 1988) and *in vitro* (Lucas *et al.*, 1975). Recently, the complementary DNA coding for a 707-amino acid peptide transporter (PepT1) was isolated from rabbit intestine using the *Xenopus laevis* expression cloning system (Fei *et al.*, 1994). The specificity of this cloned H^+-coupled transporter is similar to the specificity of the H^+-coupled di/tripeptide carrier in the human intestine epithelial cell line Caco-2 (Thwaites *et al.*, 1994a). Although the gastrointestinal epithelial cell wall represents a major barrier to drug delivery via the oral route, many peptide-like drugs have significant oral bioavailability (Humphrey, 1986; Humphrey & Ringrose, 1986). The H^+-coupled dipeptide carrier may play an important role in the oral absorption of a number of these peptide-like drugs including the angiotensin-converting enzyme (ACE) inhibitors, enalapril (Friedman & Amidon, 1989a), captopril (Hu & Amidon, 1988) and lisinopril (Friedman & Amidon, 1989b).

The Caco-2 cell system is a suitable model system for intestinal epithelial permeability studies (Hidalgo *et al.*, 1989). This human intestinal epithelial cell line expresses functional H^+-coupled dipeptide carriers at both apical and basolateral membranes (Thwaites *et al.*, 1993a,b). The transepithelial transport of the orally-absorbed cephalosporin cephradine (Inui *et al.*, 1992) and the anti-cancer agent, bestatin (Saito & Inui, 1993) across Caco-2 cell monolayers are mediated via this H^+-coupled transcellular route of absorption. These observations confirm the suitability of this human model system to determine the role of the peptide carrier in the absorption of peptide-like drugs.

The aim of this investigation, therefore, was to identify the role of the intestinal di/tripeptide carrier in the transepithelial transport of three orally-active ACE inhibitors (enalapril maleate, captopril and lisinopril) using a human intestinal epithelial model system (Caco-2).

Methods

Cell culture

Caco-2 cells (passage number 95-114) were cultured in DMEM (with 4.5 g l^{-1} glucose), with 1% non-essential amino acids, 2 mM L-glutamine, 10% (v/v) foetal calf serum

[1] Author for correspondence.

and gentamicin (60 μg ml^{-1}). Cell monolayers were prepared by seeding at high density (4.4–5.0 × 10^5 cells cm^{-2}) onto tissue culture inserts [Transwell polycarbonate filters (Costar)]. Cell monolayers were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. Cell confluence was estimated by microscopy and determination of transepithelial electrical resistance measured at 37°C.

Transport experiments

Uptake and transport experiments with Gly-Sar, lisinopril, mannitol and proline were performed 16–30 days after seeding and 18–24 h after feeding. Transepithelial flux measurements were performed as described previously (Thwaites *et al.*, 1993a). Briefly, the cell monolayers (24.5 mm in diameter) were washed by sequential transfer through 4 beakers containing 500 ml of modified Krebs buffer (all mmol l^{-1}): NaCl 137, KCl 5.4, $CaCl_2$ 2.8, $MgSO_4$ 1.0, NaH_2PO_4 0.3, glucose 10, HEPES/Tris 10 (pH 7.4, 37°C) and placed in 6-well plates, each well containing 2 ml of modified Krebs buffer. Krebs buffer (pH 7.4), 2 ml, was placed in the upper filter cup (apical solution) and the filters were incubated for 10 min at 37°C. The experimental composition of the buffers in the apical and basal chambers were identical except where stated otherwise. For Na^+-free experiments, NaCl was replaced by choline chloride and NaH_2PO_4 was omitted. The pH 5.5, 6.0 and 6.5 buffers were prepared by replacement of 10 mM HEPES with 10 mM MES and adjustment to the required pH using Tris base. Radiolabelled substrates (0.5 μCi ml^{-1}) were added to the apical chamber (Gly-Sar (36 μM) or lisinopril (66 μM), and in each case an equivalent concentration of mannitol). In experiments involving high (20 mM) concentrations of substrates iso-osmolarity was maintained by addition of mannitol to control samples. Fluxes in the absorptive (apical-to-basal, J_{a-b}) direction were determined for 1 h and are expressed as pmol cm^{-2} h^{-1}. The transcellular portion of Gly-Sar transport can be determined by subtraction of the passive (paracellular) component (estimated by mannitol flux). However, since [^{3}H]-mannitol is routinely used in our experiments the paracellular component is likely to be overestimated (i.e. the results in Figure 2 suggest that 20 mM cold Gly-Sar reduces [^{14}C]-Gly-Sar transport below zero). At the end of the incubation period cell monolayers were washed by sequential transfer through 4 beakers containing 500 ml volumes of Krebs buffer (pH 7.4) at 4°C to remove any loosely-associated radiolabel, and removed from the insert. Cell monolayer-associated radiolabel was determined by scintillation counting. Cellular accumulation of Gly-Sar, mannitol, lisinopril and proline are expressed as μM. Cell height was determined by confocal microscopy and this value was used in the determination of intracellular volume. Results are expressed as mean ± s.e.mean.

Experiments with proline were performed in Na^+-free conditions to eliminate any Na^+-coupled proline transport. Flux measurements were performed in two ways. Firstly, bidirectional proline (0.2 μCi ml^{-1}, 50 μM) fluxes were performed as described previously (Thwaites *et al.*, 1993c). Alternatively, the apical-to-basal (J_{a-b}) transport of [^{3}H]-proline (0.2 μCi ml^{-1}, 50 μM) was determined and compared with [^{14}C]-mannitol flux (0.2 μCi ml^{-1}, 50 μM). All other conditions were as described above.

Short-time uptake of Gly-Sar

Cell monolayers were washed by sequential transfer through 4 beakers containing 500 ml Krebs solution (pH 7.4) at 37°C and placed in fresh 6-well plates containing 2 ml pre-warmed Krebs (pH 7.4) in both apical and basolateral compartments. The filters were incubated at 37°C for 30 min. Uptake was initiated by replacing the apical solution with an experimental solution (pH 6.0, 37°C) containing [^{14}C]-Gly-Sar (0.5 μCi ml^{-1}, 36 μM) and [^{3}H]-mannitol (0.5 μCi ml^{-1}, 36 μM). After a 30 s incubation the apical solution was rapidly aspirated and the cell monolayer was washed by sequential transfer through 4 beakers containing 500 ml ice-cold Krebs (pH 7.4). Cell-associated radioactivity was determined as above. Residual extracellular marker activity (as determined with mannitol) associated with the cell monolayers was small (0.023 ± 0.001% (n = 36) of total label). All uptakes were corrected for this small residual component (Thwaites *et al.*, 1994b).

Intracellular pH measurements

For intracellular pH (pH_i) measurements (Thwaites *et al.*, 1993a), Caco-2 cells grown to confluence (15 days after seeding) on 12 mm diameter Transwell polycarbonate filters (Costar) were loaded by incubation with BCECF-AM (5 μM), in both apical and basal chambers, for 40 min at 37°C. After loading, the inserts were placed in a 24 mm diameter perfusion chamber mounted on the stage of an inverted fluorescence microscope (Nikon Diaphot). Perfusion of the apical and basolateral chambers was accomplished by a compressed air-driven system (flow rate 5 ml min^{-1}, at 37°C). Intracellular H^+ concentration was quantified by fluorescence (excitation at 440/490 nm and emission at 520 nm) from a small group of cells (5–10) with a photon counting system (Newcastle Photometric Systems). Intracellular BCECF fluorescence was converted to pH_i by comparison with values from an intracellular calibration curve using nigericin (10 μM) and high K^+ solutions (Thomas *et al.*, 1979; Watson *et al.*, 1991). Results are expressed as ΔpH_i min^{-1} [mean ± s.e.mean (n)]. The rate of change of pH_i (ΔpH_i min^{-1}) was calculated by linear regression using Photon Counter System 4.7 (Newcastle Photometric Systems). Changes in ΔpH_i min^{-1} (due to a change in the composition of the superfusate) were determined by linear regression, by comparison of the linear portions of the trace over 30–50 s (15–25 data points) periods before and after the change in composition (Thwaites *et al.*, 1994a).

Materials

[^{3}H]-mannitol (specific activity 30 Ci mmol^{-1}) was obtained from NEN. [^{14}C]-Gly-Sar (L-glycyl[1-^{14}C]-sarcosine (specific activity 14 mCi mmol^{-1})), D-[1-^{14}C]-mannitol (specific activity 57 mCi mmol^{-1}), L-[U-^{14}C]-proline (specific activity >250 mCi mmol^{-1}) and L-[2,3-^{3}H]-proline (specific activity 20–40 Ci mmol^{-1}) were from Amersham. [^{14}C]-lisinopril (specific activity 8.4 mCi mmol^{-1}) was a gift from Zeneca (UK). Lisinopril was a gift from Zeneca (UK) and Merck (UK). Carnosine (β-alanine-L-histidine) was from Peptide Inst. Inc. (Japan). Gly-Sar, captopril, enalapril maleate and L-proline were from Sigma. BCECF, cell culture media, supplements and plastic were supplied by Life Technologies. All other chemicals were from Merck.

Statistics

Results are expressed as mean ± s.e.mean (n). Statistical analysis was performed using one way analysis of variance ANOVA or Student's paired t test.

Results

The transepithelial transport and cellular uptake of the ACE inhibitor lisinopril were determined and compared with transport and uptake of the dipeptide, Gly-Sar and the paracellular marker, mannitol (Figure 1). Figure 1 demonstrates stimulation of transepithelial transport and intracellular accumulation of the dipeptide, Gly-Sar across Caco-2 cell monolayers, by lowering apical medium pH, as reported previously (Thwaites *et al.*, 1993a). At both apical pH 6.0 and 7.4, Gly-Sar transport was significantly greater

($P<0.001$) than transport of the paracellular marker mannitol (Figure 1a). In contrast the apical-to-basal transport of [^{14}C]-lisinopril was significantly lower ($P<0.001$) than mannitol transport (Figure 1a). Apical-to-basal transport (J_{a-b}) of lisinopril was not stimulated on lowering apical pH (Figure 1, Table 1). The dipeptide, Gly-Sar, showed marked intracellular accumulation above medium levels (36 μM) (Figure 1b,) at both apical pH 6.0 (352.7 ± 18.4 μM, $n=20$) and pH 7.4 (271 ± 18.0 μM, $n=19$). Lisinopril uptake was significantly reduced compared to Gly-Sar uptake ($P<0.001$) and was

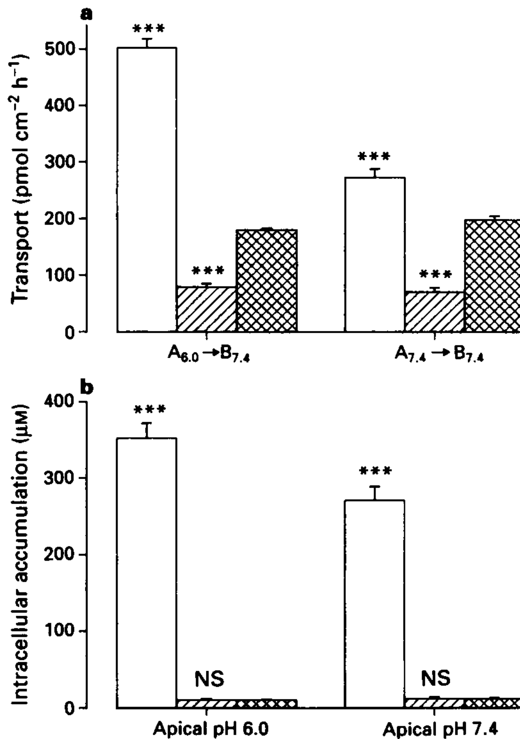

Figure 1 Transepithelial transport and cellular uptake of a dipeptide (Gly-Sar), and ACE inhibitor (lisinopril) and the paracellular marker mannitol. Apical-to-basal (J_{a-b}) transport (a) and cellular accumulation (across the apical membrane, b) of [^{14}C]-Gly-Sar (open columns), [^{14}C]-lisinopril (hatched columns) and [^{3}H]-mannitol (cross-hatched columns) across Caco-2 cell monolayers in the presence and absence of a transepithelial pH gradient. Basolateral pH was maintained at pH 7.4. Results are expressed as mean ± s.e.mean ($n=5.21$); ***$P<0.001$; NS$P>0.05$, significance of difference from mannitol data. For abbreviations, in this and subsequent legends, see text.

Table 1 Acidic apical media do not stimulate either transport or intracellular accumulation of lisinopril

pH of apical medium	*Apical to basal transport* J_{a-b} (pmol cm^{-2} h^{-1})	*Intracellular accumulation* *Lisinopril* (μM)	*Mannitol* (μM)
7.4	70.7 ± 7.3 (9)	12.0 ± 2.0 (15)	11.8 ± 1.3 (15)
6.5	64.2 ± 5.4 (4)	18.4 ± 0.4 (9)	13.0 ± 1.3 (9)
6.0	79.5 ± 5.8 (11)	10.6 ± 1.4 (15)	10.4 ± 1.0 (10)
5.5	85.7 ± 4.1 (7)	12.5 ± 0.6 (10)	11.4 ± 1.0 (10)

Apical-to-basal (J_{a-b}) transport and cellular accumulation (across the apical membrane) of [^{14}C]-lisinopril (66 μM) and [^{3}H]-mannitol (66 μM) across Caco-2 cell monolayers in the presence and absence of a transepithelial pH gradient. Basolateral pH was maintained at pH 7.4. Results are expressed as mean ± s.e.mean n value given in parentheses. One way analysis of variance (ANOVA) for transport data $F=1.67$ $P=0.20$, not significant. Note that neither lisinopril nor mannitol show accumulation above medium values, even at the most acidic apical pH.

similar in magnitude to cell monolayer-associated mannitol at acidic apical pH (Figure 1, Table 1). [^{14}C]-lisinopril transport J_{a-b} at 66 μM (apical pH 6.0, basolateral pH 7.4) was not substantially reduced from 73.4 ± 2.6 pmol cm^{-2} h^{-1} ($n=4$) when excess unlabelled lisinopril (20 mM) was added (to 63.0 ± 1.9 pmol cm^{-2} h^{-1} ($n=4$)). This lack of competitive inhibition contrasts with that seen for [^{14}C]-Gly-Sar transport with unlabelled Gly-Sar (Figure 2). The extracellular concentration of lisinopril did not affect intracellular levels of [^{14}C]-lisinopril at 1 h across the apical membrane (11.6 ± 1.1 μM ($n=4$) at 66 μM (extracellular lisinopril) and 9.5 ± 0.4 μM ($n=4$) at 20 mM extracellular lisinopril). Under identical experimental conditions lisinopril J_{a-b} was 34.2 ± 6.9 pmol cm^{-2} h^{-1} ($n=3$) at 66 μM (extracellular lisinopril) and 45.8 ± 6.0 pmol cm^{-2} h^{-1} ($n=5$) at 20 mM (extracellular lisinopril). Uptake of lisinopril across the basolateral membrane was 12.3 ± 0.5 μM ($n=4$) at an extracellular concentration of 66 μM and 9.5 ± 0.5 μM ($n=5$) at 20 mM extracellular lisinopril.

Excess unlabelled Gly-Sar (20 mM) inhibited transcellular [^{14}C]-Gly-Sar transport (Figure 2a). The three ACE inhibitors also inhibited [^{14}C]-Gly-Sar transport, but to varying degrees in the order enalapril > captopril > lisinopril (Figure 2). However, the effect of these substrates on the steady-state uptake of [^{14}C]-Gly-Sar showed a different pattern (Figure 2b). Gly-Sar and enalapril maleate both significantly ($P<0.001$) reduced [^{14}C]-Gly-Sar uptake. Lisinopril also reduced uptake of the dipeptide but the inhibitory effect was less marked ($P<0.05$). However, unlike its effect on transport, captopril did not inhibit [^{14}C]-Gly-Sar uptake at steady-state (there was in fact a small increase). This unexpected effect of captopril is likely to represent an inhibitory effect on the exit of [^{14}C]-Gly-Sar across the basolateral membrane since the initial uptake of the dipeptide (measured at 30 s) was significantly reduced ($P<0.001$) in the presence of cap-

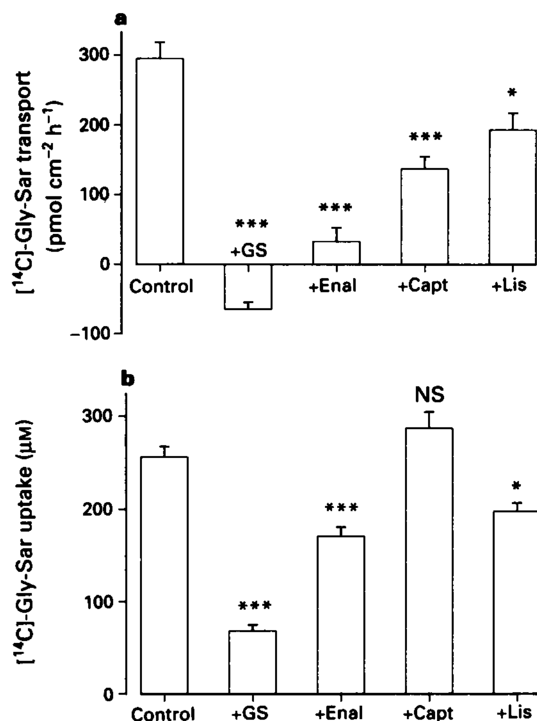

Figure 2 pH-dependent dipeptide (Gly-Sar) transport and cellular uptake in the presence of cold Gly-Sar and three ACE inhibitors. Apical-to-basal (J_{a-b}) [^{14}C]-Gly-Sar (36 μM) transport (a) and cellular uptake across the apical membrane (b) in the presence and absence of 20 mM cold Gly-Sar (GS), enalapril maleate (Enal), captopril (Capt), and lisinopril (Lis) (apical pH 6.0, basolateral pH 7.4). Results are mean ± s.e.mean ($n=9-16$); ***$P<0.001$; *$P<0.05$; NS$P>0.05$ versus control data.

topril (Figure 3). The inhibitory effects of the compounds (all at 20 mM) on the initial uptake of [^{14}C]-Gly-Sar (Figure 3) showed a similar order of potency (Gly-Sar > enalapril maleate > captopril > lisinopril) as observed with transepithelial [^{14}C]-Gly-Sar transport (Figure 2a). Lisinopril showed no significant inhibition of initial [^{14}C]-Gly-Sar uptake.

The Caco-2 cell line expresses an apically-localized H^+-coupled amino acid transporter (Thwaites *et al.*, 1993c) that is involved in transepithelial proline transport. A possible role for this H^+-coupled transporter in ACE inhibitor transport was investigated (Figure 4). Figure 4a shows that 20 mM excess unlabelled proline markedly reduced transport of [^{3}H]-proline whereas the dipeptide, Gly-Sar, had a smaller inhibitory effect (which most probably represents an effect on the driving force (the H^+ gradient) rather than the transport itself). The three ACE inhibitors failed to inhibit [^{3}H]-proline transport (Figure 4a). However, the levels of proline uptake in the presence of this group of substrates showed a different pattern with only proline inhibiting [^{3}H]-proline uptake (Figure 4b). The most striking effect is that of enalapril maleate (Figure 4b) which more than doubled the uptake of [^{3}H]-proline (1091 ± 98 μM (n = 10) compared to 430 ± 31 μM (n = 11) under control conditions).

The transapical transport of the dipeptide, Gly-Sar, is associated with the movement of protons across the cell wall that can be detected by the resultant acidification of the intracellular environment (Thwaites *et al.*, 1993a). Figure 5 clearly indicates that after exposure to the dipeptide carnosine (β-Ala-His) at 20 mM at the apical surface (apical pH 6.5, basolateral pH 7.4) the intracellular pH became more acidic due to substrate-induced H^+ flow into the cells. The inclusion of carnosine (20 mM) in the apical superfusate significantly increased ($P < 0.001$) the initial rate of intracellular acidification (ΔpH_i min^{-1}) from 0.009 ± 0.003 pH units min^{-1} (n = 5) to 0.115 ± 0.013 pH units min^{-1} (n = 5). Similar effects were noted with enalapril maleate and captopril (Figure 5). Enalapril maleate (20 mM) increased ΔpH_i min^{-1} from 0.009 ± 0.003 pH units min^{-1} (n = 5) to 0.115 ± 0.017 pH units min^{-1} (n = 5) whereas captopril increased ΔpH_i min^{-1} from 0.013 ± 0.004 pH units min^{-1} (n = 4) to 0.074 ± 0.026 pH units min^{-1} (n = 4). The ACE inhibitor lisinopril, however, failed to induce a significant ($P > 0.05$) change in intracellular pH (0.021 ± 0.002 pH units min^{-1}, n = 3) compared to the effect of exposure to pH 6.5 superfusate alone (0.017 ± 0.008 pH units min^{-1}, n = 3). The rate of intracellular acidification (ΔpH_i min^{-1}) observed in the presence of enalapril maleate or captopril were both significantly greater than that measured in the presence of lisinopril.

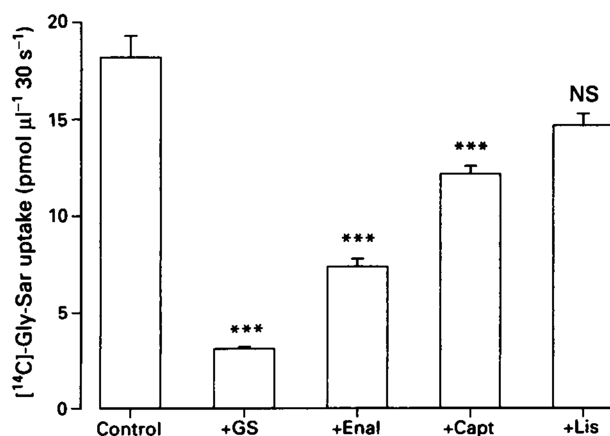

Figure 3 Short-time uptake of Gly-Sar into Caco-2 cell monolayers in the presence or absence of cold compounds. pH-dependent (apical pH 6.0, basolateral pH 7.4) [^{14}C]-Gly-Sar uptake across the apical membrane of Caco-2 cell monolayers in the presence and absence of 20 mM cold Gly-Sar (GS), enalapril maleate (Enal), captopril (Capt), and lisinopril (Lis). Uptake was measured over a 30 s period. Results are expressed as mean ± s.e.mean (n = 6, n = 3 for lisinopril); ***$P < 0.001$; NS$P > 0.05$, significance of difference from control data.

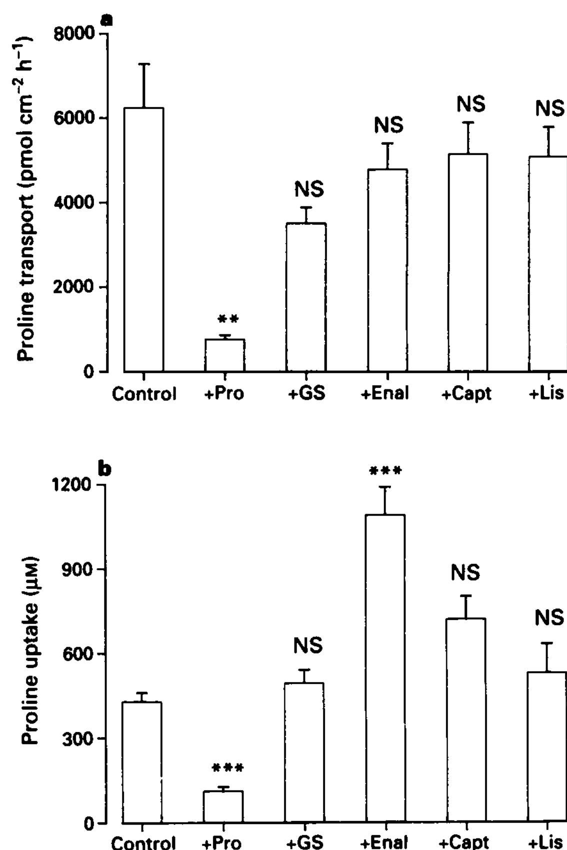

Figure 4 pH-dependent (Na^+-independent) amino acid (L-proline) transport and cellular accumulation in the presence of proline, Gly-Sar and three ACE inhibitors. pH-dependent (apical pH 6.0, basolateral pH 7.4), Na^+-independent proline net transport (a) and cellular uptake across the apical membrane (b) in the presence and absence of 20 mM cold L-proline (Pro), Gly-Sar (GS), enalapril maleate (Enal), captopril (Capt) and lisinopril (Lis). Results are expressed as mean ± s.e.mean (n = 7–11); ***$P < 0.001$; **$P < 0.05$; NS$P < 0.05$ versus control data.

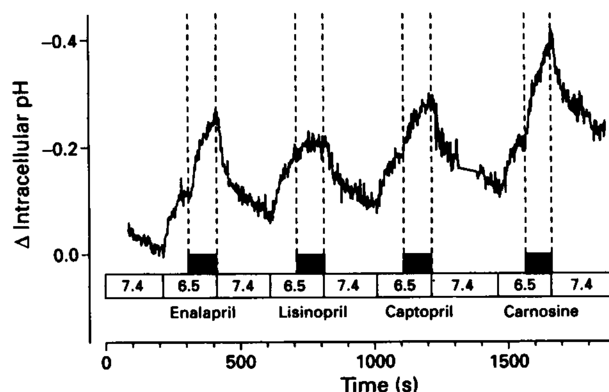

Figure 5 Intracellular pH measured in BCECF-loaded Caco-2 cell monolayers. The effect on intracellular pH of apical exposure to enalapril maleate, lisinopril, captopril or the dipeptide carnosine. All substrates (20 mM) were superfused across the apical surface at pH 6.5. Basolateral pH was maintained at pH 7.4. The figure is a representative trace of 3–5 separate experiments.

Discussion

Oral bioavailability is crucial to the use of angiotensin-converting enzyme (ACE) inhibitors in the clinical treatment of systemic hypertension and congestive heart failure (Humphrey, 1986; Humphrey & Ringrose, 1986). Captopril demonstrates high oral bioavailability (62%) in man with a peak plasma concentration after 1 h (Duchin *et al.*, 1982). Enalapril maleate is a monoethyl ester prodrug of the diacid enalaprilat. This prodrug (enalapril maleate) needs to undergo deesterification *in vivo* to produce its active form, enalaprilat (Wyvratt & Patchett, 1985). Studies in man indicate that absorption of enalapril maleate (61% oral availability) is rapid (peak plasma levels between 0.5–1.5 h after administration) and that enalapril maleate/enalaprilat is recovered intact (94%) from urine and faeces (apart from the bioactivation which is post-absorptive) (Ulm *et al.*, 1982). In animal experiments similar observations were made (Tocco *et al.*, 1981). Enalapril maleate had 61% oral bioavailability in the dog (whereas enalaprilat was 11%) and 34% in the rat (Tocco *et al.*, 1981). Lisinopril is similar in structure to enalaprilat but the alanine residue of enalaprilat has been replaced by a lysine residue (Wyvratt & Patchett, 1985). In human studies, the absorption of lisinopril was low (29%) and slow (peak plasma concentration 6–8 h after administration) compared to enalapril maleate (Ulm *et al.*, 1982). Lisinopril was, however, resistant to metabolism (97% was recovered intact in the urine and faeces, Ulm *et al.*, 1982). The effects of enalapril maleate and lisinopril are both longer lasting than the effects of the less stable captopril (Ulm *et al.*, 1982).

The structures of the first three orally-active ACE inhibitors (captopril, enalapril maleate and lisinopril) are similar or analogous to the dipeptide Ala-Pro (Wyvratt & Patchett, 1985) which is the C-terminal dipeptide of bradykinin-potentiating peptide 5_a ($BPP5_a$), a potent inhibitor of ACE activity isolated from snake venom (Cheung *et al.*, 1980; Wyvratt & Patchett, 1986). Studies in rat jejunum using the single-pass perfusion method have suggested that the significant levels of absorption of these three ACE inhibitors across the intestinal epithelial wall is due to transport via the intestinal di/tripeptide carrier (Hu & Amidon, 1988; Friedman & Amidon, 1989a,b).

We have investigated whether captopril, enalapril maleate and lisinopril are substrates for proton-coupled solute transport using the human intestinal epithelial Caco-2 cell model. This human intestinal epithelial cell line expresses H^+-coupled dipeptide transporters at both apical and basolateral membranes (Thwaites *et al.*, 1993a,b). The specificity of the dipeptide transporter at the apical surface of Caco-2 cells (Thwaites *et al.*, 1994a) is similar to the cloned dipeptide transporter from rabbit intestine (PepT1) when expressed in *Xenopus laevis* oocytes (Fei *et al.*, 1994). Similarly, dipeptide transport is rheogenic in both Caco-2 cell monolayers (Thwaites *et al.*, 1993d) and PepT1-expressing oocytes (Fei *et al.*, 1994). The importance of this H^+-coupled transcellular route in uptake and transepithelial transport of the orally-absorbed cephalosphorins cephalexin (Dantzig & Bergin, 1990) and cephradine (Inui *et al.*, 1992), and the anti-cancer agent, bestatin (Saito & Inui, 1993) has been demonstrated using this cell line. Although it is clear that this model has proved useful in demonstrating both pH-dependency and H^+-coupling of dipeptide/aminocephalosporin transport the present studies are the first to investigate ACE inhibitor transport. An additional advantage of using the Caco-2 cell system rather than a cloned membrane transporter expressed in oocytes is that alternative transport pathways and their relative importance may be studied in the same cell system.

The present studies provide no evidence for pH-dependent transport or accumulation of lisinopril (Figure 1) in the human intestinal cell line, Caco-2. Moreover, lisinopril permeability is lower than mannitol (Figure 1). Nor is lisinopril (20 mM) addition associated with H^+ flow across the apical membrane (Figure 5) as has been observed with both dipeptides (Gly-Sar) and aminocephalosporins (cephalexin) (Thwaites *et al.*, 1993a,b). Lisinopril (20 mM) failed to induce H^+ flow into the cells above levels seen with apical acidity alone. Clearly from these results it appears that the transport of Gly-Sar is via the transcellular route and lisinopril via the paracellular pathway. The weak inhibitory effects of 20 mM lisinopril on Gly-Sar transport and accumulation (Figure 2) suggest that this ACE inhibitor may be a weak non-transported inhibitor, although non-specific effects on cell monolayer-transporter integrity cannot be ruled out.

In contrast to lisinopril, the inhibitory actions of captopril and enalapril maleate on transepithelial Gly-Sar transport (Figure 2a) and the ability of these two ACE inhibitors to stimulate H^+-flow across the apical membrane (Figure 5) suggest that they may be transported across the apical membrane by the di/tripeptide carrier. However, although both captopril and enalapril maleate reduce the initial uptake (Figure 3) of the dipeptide, Gly-Sar, their effects on the steady-state levels of accumulation of Gly-Sar within the cell monolayers show a different pattern (Figure 2b). It is likely that the inhibitory action of captopril on transepithelial dipeptide transport consists of two components. Firstly, the results suggest that although Gly-Sar appears to have a higher affinity for the apical transporter (Figure 3), once inside the cell captropril has a higher affinity for the basolateral efflux mechanism. This accounts for the decrease in transport but increase in accumulation of Gly-Sar in the presence of captopril (Figure 2).

All three ACE inhibitors fail to inhibit the pH-dependent (Na^+-independent) transepithelial transport of the amino acid, proline (Figure 4). However, enalapril maleate caused a large increase in the accumulation of proline within the cell monolayer suggesting that enalapril maleate may inhibit the efflux of proline across the basolateral membrane. This effect of enalapril maleate was not observed with dipeptide accumulation (Figure 2).

These observations regarding the mechanisms involved in the absorption of the ACE inhibitors captopril, enalapril maleate and lisinopril show broad agreement with the available data from man (Duchin *et al.*, 1982; Ulm *et al.*, 1982) and suggest that the Caco-2 cell model may be useful in predictive studies for oral bioavailability. There are however differences with the present data and work using the rat jejunum as the main experimental model (Hu & Amidon, 1988; Friedman & Amidon, 1989a,b). The permeability of all three ACE inhibitors in rat jejunum show saturation and a decrease in permeability in the presence of high concentrations of dipeptides or the aminocephalosporin cephradine. Although our evidence from experiments in this human intestinal epithelial cell-line point to a role for the intestinal dipeptide carrier in the transport of enalapril maleate and captopril, the evidence also suggests that lisinopril is absorbed passively. A passive mechanism of lisinopril absorption is consistent with the slow rate of disappearance observed in the experiments with rat jejunum (Friedman & Amidon, 1989b) and in studies in man (Ulm *et al.*, 1982).

There are two steps involved in transport across the intestinal epithelial barrier. Firstly, movement across the apical membrane into the cell and, secondly, efflux across the basolateral membrane. For Gly-Sar transport it is likely that the rate limiting step for transepithelial transport is in fact exit from the cell across the basolateral membrane (Thwaites *et al.*, 1993b). Nothing is known about the mechanisms involved in ACE inhibitor transport across the basolateral membrane of the intestinal enterocyte. However, our observations in this study suggest that although both captopril and enalapril maleate share a basolateral efflux mechanism with the dipeptide Gly-Sar, the relative affinities for this mechanism are different from the apical dipeptide transporter. Perhaps, more importantly, the results also suggest

that enalpril maleate has a high affinity for a basolateral transporter associated with the Na^+-independent transport of amino acids such as proline. Clearly more work needs to be performed to define the exact mechanisms of exit of ACE inhibitors across the basolateral membrane.

In conclusion it appears that both captopril and enalopril maleate are substrates for the H^+/dipeptide transporter expressed in human intestinal cells. Lisinopril is not a transported substrate. It is likely that the Caco-2 cell system will be useful in predictive studies of oral bioavailability via the H^+-coupled dipeptide transporter.

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