Epidermal growth factor inhibits glycylsarcosine transport and hPepT1 expression in a human intestinal cell line

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Nielsen, Carsten Uhd, Jan Amstrup, Bente Steffansen, Sven Frokjær, and Birger Brodin. Epidermal growth factor inhibits glycylsarcosine transport and hPepT1 expression in a human intestinal cell line. Am J Physiol Gastrointest Liver Physiol 281: G191–G199, 2001.—The human intestinal cell line Caco-2 was used as a model system to study the effects of epidermal growth factor (EGF) on peptide transport. EGF decreased apical-to-basolateral fluxes of [14C]glycylsarcosine ([14C]Gly-Sar) up to 50.2 ± 3.6% (n = 6) of control values. Kinetic analysis of the fluxes showed that maximal flux (Vmax) of transepithelial transport decreased from 3.00 ± 0.17 nmol·cm−2·min−1 in control cells to 0.50 ± 0.07 nmol·cm−2·min−1 in cells treated with 5 ng/ml EGF (n = 6, P < 0.01). The apparent Michaelis-Menten constant (Km) was 2.71 ± 0.31 mM (n = 6) in control cells and 1.89 ± 0.28 mM (n = 6, not significantly different from control) in EGF-treated cells. Similarly, apical uptake of [14C]Gly-Sar decreased in cells treated with EGF, with an ED50 value of 0.36 ± 0.06 ng/ml (n = 6) EGF and a maximal inhibition of 80 ± 0.02% (n = 6). Vmax decreased from 2.61 ± 0.4 to 1.06 ± 0.1 nmol·cm−2·min−1 (n = 3, P < 0.05), whereas Km remained constant. Basolateral Gly-Sar uptake showed no z

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In mammalian small intestine, intact di- and tripeptides are transported from the intestinal lumen into the cells via a H+–peptide cotransporter, PepT1. The absorption of several important orally pharmacologically active compounds such as β-lactam antibiotics, angiotensin-converting enzyme, and renin inhibitors also depends to some extent on human (h)PepT1-mediated transport (8, 17, 19, 21). The transporter has been cloned from human (23), rat (29), rabbit (3, 10) mouse (11), and HEPES were from Sigma (St. Louis, MO). 14C-labeled Gly-Sar with a specific activity of 49.94 mCi/mmol and 12 putative membrane-spanning domains and

IN MAMMALIAN SMALL INTESTINE, intact di- and tripeptides are transported from the intestinal lumen into the cells via a H+–peptide cotransporter, PepT1. The absorption of several important orally pharmacologically active compounds such as β-lactam antibiotics, angiotensin-converting enzyme, and renin inhibitors also depends to some extent on human (h)PepT1-mediated transport (8, 17, 19, 21). The transporter has been cloned from human (23), rat (29), rabbit (3, 10) mouse (11), and Caco-2 (36) cells. hPepT1 consists of 708 amino acids and has a core molecular mass of ~79 kDa (23). PepT1 is exclusively located in the apical membrane of mature enterocytes in the absorptive epithelium covering the villi (26). Transport of peptides from the cytosol to the blood is mediated via a not yet fully characterized di-/tri-peptide transport system located in the basolateral membrane (28, 34).

Little is known about regulation of PepT1 transport activity. Two potential sites for protein kinase C phosphorylation have been identified in hPepT1 (23), and it has been shown that peptide transporter activity is downregulated in Caco-2 cells exposed to phorbol esters (4). Peptide transporter activity is also influenced by cAMP (25), insulin (33), the σ-receptor ligand (+)-pentazocine (13), and luminal dipeptides (30, 32, 36).

Overall, the information on hormonal and growth factor-mediated regulation of peptide transport activity is sparse. In the present study we used the Caco-2 cell line to investigate the effects of treatment with epidermal growth factor (EGF) on peptide transport and hPepT1 expression. EGF is a peptide growth factor consisting of 53 amino acids that stimulates the proliferation of epidermal cells and a variety of other epithelial and nonepithelial cell types (12). The present results showed a decrease in transcellular transport and apical uptake of the nonhydrolyzable peptide glycylsarcosine (Gly-Sar), a decrease in total hPepT1 protein content, and a decrease in hPepT1 mRNA after long-term EGF treatment of Caco-2 cells. We conclude that EGF downregulates Gly-Sar transport in Caco-2 cells by decreasing hPepT1 mRNA and thereby hPepT1 expression.

MATERIALS AND METHODS

Materials. Caco-2 cells were obtained from the American Type Culture Collection (Manassas, VA). Cell culture media and human recombinant lyophilized EGF were purchased from Life Technologies (Hejø Taastrup, Denmark). Hanks’ balanced salt solution (HBSS) was obtained from Life Technologies. BSA, 2-(N-morpholino)ethanesulfonic acid (MES), and HEPES were from Sigma (St. Louis, MO). 14C-labeled Gly-Sar with a specific activity of 49.94 mCi/mmol and

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[3H]mannitol with a specific activity of 51.50 mCi/mmol were from NEN (Boston, MA). Restriction enzymes PstI, SacI, and EcoRI were from Amersham Pharmacia Biotech (Little Chalfont, UK). Rabbit anti-hPepT1, raised against a peptide corresponding to the last 15 carboxy-terminal amino acid residues of the human peptide transporter hPepT1, was a generous gift from Dr. Wolfgang Sadee (San Francisco, CA) (15). Horseradish peroxidase-conjugated anti-rabbit IgG was purchased from Bio-Rad (Heracles, CA). Alexa 488-conjugated goat anti-rabbit IgG, Alexa 488-conjugated phalloidin, and propidium iodide were from Molecular Probes (Eugene, OR).

Cell culture. Caco-2 cells at passage 20 were seeded in culture flasks and passaged in DMEM supplemented with 10% fetal bovine serum, penicillin-streptomycin (100 U/ml and 100 μg/ml, respectively), 1% L-glutamine, and 1% non-essential amino acids. When cells reached passages 30–50, they were seeded onto tissue culture-treated Transwells (4.7 cm², 0.4-μm pore size) at a density of 10⁵ cells/cm². Monolayer cultures were grown in an atmosphere of 5% CO₂-95% air, at 37°C. Growth media were replaced every other day. EGF was dissolved in sterile water to a concentration of 1 μg/ml and stored in aliquots at −20°C. On the day of use, EGF was dissolved in the culture medium and added to the monolayers (at both the apical and the basolateral sides), except when used examinations of the sidedness (i.e., apical vs. basolateral) of the inhibitory effect of EGF on dipeptide transport. Transepithelial electrical resistance (TEER) was measured during growth in tissue resistance measurement chambers (Endohm) with a volthmometer (EVOM), both of which were from World Precision Instruments (Sarasota, FL). Dipeptide transport activity reached a steady maximal level at days 24–30. Transport experiments were subsequently performed on days 26–28 after seeding; isolation of protein and RNA was performed on day 24.

Transport experiments. Transport of [14C]Gly-Sar was measured in HBSS supplemented with 0.05% BSA. Apical media were buffered with 10 mM MES, and pH was adjusted to 6.0; basolateral media were buffered with 10 mM HEPES and adjusted to pH 7.4. The cell monolayers were rinsed once in prewarmed HBSS and placed on a shaking plate heated to 37°C. The cells were allowed to equilibrate for 1 h in the relevant experimental solutions without radiotopes. The experiment was initiated by adding fresh apical buffer containing varying amounts of Gly-Sar (0–5 mM), 0.5 μCi/well [14C]Gly-Sar, and 0.5 μCi/well [3H]mannitol. Twenty-microliter samples were taken from the apical solution at t = 0, 60, and 120 min. One hundred-microliter samples were taken at 15-min intervals from the basolateral solution and replaced with fresh buffer (t = 0–120 min). Samples were transferred to counting vials, scintillation fluid was added (Ultima Gold; Packard, Canberra, Australia), and radioactivity was counted in a liquid scintillation analyzer. Fluxes of Gly-Sar and mannitol were constant after 60 min. The steady-state flux values of Gly-Sar and mannitol were therefore obtained as the means of the flux values at 90, 105, and 120 min.

Glycylsarcosine uptake experiments. Uptake of [14C]Gly-Sar was initiated for transport experiments. However, the cells were allowed to equilibrate for 15 min in apical and basolateral solutions without Gly-Sar, after which the experiment was started by adding fresh apical buffer containing the relevant Gly-Sar concentration (0–5 mM) and 0.5 μCi/well [14C]Gly-Sar or fresh basolateral buffer containing the relevant Gly-Sar concentration (0–10 mM) for basolateral uptake experiments. Apical uptake of Gly-Sar into the cells was terminated after 5 min and basolateral uptake after 15 min by gentle suction of the uptake medium followed by four washes of the monolayer with ice-cold HBSS. The polycarbonate filters were cut from the Transwell supports and placed into scintillation vials. Two milliliters of scintillation fluid was added, and the radioactivity was counted in a liquid scintillation analyzer.

Kinetic analysis. Uptake of Gly-Sar as a function of apical or basolateral Gly-Sar concentration was fitted to a Michaelis-Menten-type equation

\[ V = \frac{V_{\text{max}} \cdot [S]}{K_m + [S]} \]  

where V is flux (nmol·cm⁻²·min⁻¹), \( V_{\text{max}} \) is maximal flux (nmol·cm⁻²·min⁻¹), \( K_m \) is the Michaelis-Menten constant (mM), and \([S]\) is Gly-Sar concentration (mM). Transport of Gly-Sar as a function of apical Gly-Sar concentration was fitted to an expression combining the Michaelis-Menten equation with a passive linear component

\[ V = \frac{V_{\text{max}} \cdot [S]}{K_m + [S]} + k \cdot [S] \]  

where k is the slope of the linear component (cm/min). The linear component represents paracellular permeability, whereas the saturable component represents the carrier-mediated transcellular component.

ED50 values for EGF-mediated inhibition of Gly-Sar fluxes were determined using the equation

\[ F = \frac{a - d}{1 + \frac{x}{c}} + d \]  

where a is maximal flux, d is minimal flux ([EGF] = ∞), c is ED50, and F is the flux at [EGF] = x (9).

Protein extraction, Western blotting, and immunoprecipitation. Caco-2 cells cultured for 24 days in either the absence or the presence of 5 ng/ml EGF in the culture media were lysed in lysis buffer containing 10 mM Tris-HCl, pH 7.4, 0.25 M sucrose, 1 mM EDTA, 0.25 mM EGTA, 2% NP-40, and 2% NP-40, and a Complete Protease Inhibitor Cocktail Tablet (Boehringer Mannheim, Mannheim, Germany). Protein content in lysates free of cellular debris was measured using Bio-Rad protein assay dye reagent concentrate according to the manufacturer's instructions. Equal amounts of cell lysate were dissolved in 2× Laemmli buffer (22) and subjected to SDS-PAGE. Subsequently, the proteins were transferred to nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Immunoreactive proteins were made visible using horseradish peroxidase-conjugated anti-rabbit IgG (Bio-Rad) and enhanced chemiluminescence reagents according to the manufacturer's instructions (Biological Industries). Immunoprecipitates were collected by adding 100 μl of a 10% protein A-Sepharose 4B slurry (Pharmacia, Uppsala, Sweden) for 1 h followed by brief centrifugation. The precipitates were washed three times in 10 mM Tris-HCl, pH 7.4, 62.5 mM sucrose, 0.25 mM EDTA, 0.25 mM ECTA, 0.5% NP-40 containing a Complete Protease Inhibitor Cocktail Tablet and dissolved in 2× Laemmli buffer, and proteins were separated by SDS-PAGE. The proteins were transferred to nitrocellulose membranes, and proteins were made visible as described above. The band densities on immunoblots were measured with a densitometer (Imagestation 440 CF, Eastman Kodak). All densitometry was performed on exposures within the linear range of the film and the densitometer.

RNA isolation, cDNA synthesis, PCR, and enzyme digestion. Total RNA was isolated from Caco-2 cells, either unstimulated or stimulated with 5 ng/ml EGF for 24 days, using
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RESULTS

EGF-treated Caco-2 cells display dose-dependent decrease in transepithelial transport and apical uptake of [14C]Gly-Sar. Caco-2 cells were grown in the presence of varying amounts of EGF in the culture medium for

Fig. 1. Transepithelial transport (A) and apical uptake (B) of 14C-labeled glycylsarcosine ([14C]Gly-Sar) in Caco-2 cell monolayers cultured for 26–28 days and treated with varying concentrations of epidermal growth factor (EGF) throughout the culture period. Experiments were performed on a shaking plate at 37°C in Hanks’ balanced salt solution (HBSS) + 0.5% BSA; apical solutions were buffered to pH 7.4 with 10 mM 2-N-morpholineethanesulfonic acid. A: apical-to-basolateral steady-state fluxes of [14C]Gly-Sar (0.5 μCi/well) at varying EGF concentrations. Total Gly-Sar concentration ([Gly-Sar]) in the apical solution was 2.5 mM. Samples were taken from the basolateral solution at 15-min intervals, and radioactivity was determined. Steady-state flux values were calculated as the means of the flux values at 90, 105, and 120 min. Each bar represents mean ± SE of 6 individual passages; experiments were performed in duplicate within each passage. The flux values at 2, 5, and 20 ng/ml were significantly lower than that of the control (0 ng/ml); **P < 0.02. B: Caco-2 monolayers were incubated with [14C]Gly-Sar (0.5 μCi/well) for 5 min; total [Gly-Sar] in the apical solution was 1 mM. Cells were washed 4 times in ice-cold HBSS and solubilized. Radioactivity was determined, and uptake was calculated. Each bar represents mean ± SE of 6 individual passages; experiments were performed in duplicate within each passage. The flux values at 2, 5, and 20 ng/ml were significantly lower than that of the control (0 ng/ml); **P < 0.02.
in the basolateral solution. Transepithelial fluxes for this concentration range (Fig. 3A) were used to generate transport kinetic constants. This yielded a \( V_{\text{max}} \) in control cells of \( 3.00 \pm 0.17 \) nmol\( \cdot \)cm\(^{-2}\)\( \cdot \)min\(^{-1} \) compared with \( 0.50 \pm 0.07 \) nmol\( \cdot \)cm\(^{-2}\)\( \cdot \)min\(^{-1} \) in the EGF-treated cells \( (n = 6) \), different from control cell, \( P < 0.01 \). Apparent \( K_m \) was \( 2.71 \pm 0.31 \) mM in the control cells and \( 1.89 \pm 0.28 \) mM in the EGF-treated cells \( (n = 6) \), not significantly different from control). The \( k \) value (i.e., slope of the linear component) was \( 5 \times 10^{-5} \pm 2 \times 10^{-5} \) cm/min \( (n = 6) \) in the EGF-treated cells but absent in control cells. Figure 3B shows the apical uptake of Gly-Sar in controls and cells treated with 5.0 ng/ml EGF. Data were fitted to the Michaelis-Menten equation (Eq. 1), and kinetic constants were obtained. The control curve showed saturable kinetics, with an apparent \( K_m \) of \( 0.66 \pm 0.3 \) mM and a \( V_{\text{max}} \) of \( 2.61 \pm 0.4 \) nmol\( \cdot \)cm\(^{-2}\)\( \cdot \)min\(^{-1} \) (\( n = 3 \)). The corresponding curve for cellular uptake of Gly-Sar in cells treated with EGF showed an apparent \( K_m \) of \( 0.57 \pm 0.2 \) mM and a \( V_{\text{max}} \) of \( 1.06 \pm 0.1 \) nmol\( \cdot \)cm\(^{-2}\)\( \cdot \)min\(^{-1} \) (\( n = 3 \)). The decrease in maximal uptake capacity was \( \sim 60\% \) without any change in \( K_m \). As seen in Fig. 3C, the basolateral uptake control curve showed saturable kinetics, with
an apparent $K_m$ of 13.09 ± 1.05 mM and a $V_{\text{max}}$ of 1.21 ± 0.10 nmol·cm⁻²·min⁻¹ ($n = 3$). The corresponding curve for cellular uptake of Gly-Sar in cells treated with 5.0 ng/ml EGF showed an apparent $K_m$ of 15.83 ± 1.10 mM and a $V_{\text{max}}$ of 1.98 ± 0.10 nmol·cm⁻²·min⁻¹ ($n = 3$). There were thus no significant differences in kinetic parameters for basolateral uptake between control and EGF-treated cells. This indicates that EGF decreases transepithelial peptide transport by decreasing the apparent $V_{\text{max}}$ of apical peptide uptake, suggesting that the EGF treatment reduced the population of active peptide transporters in the apical membrane of the Caco-2 cell monolayers.

**Effects of EGF on Gly-Sar transport are caused by long-term stimulation.** In the experiments described above, EGF was present in the culture medium throughout the growth period. We investigated the time course of the EGF-mediated inhibition of peptide transport using Caco-2 cells grown for 26 days. Cells were exposed to EGF at 5 ng/ml for various time intervals before the experiment. A significant decrease in peptide transport was observed after 5 days of treatment ($n = 3$; Fig. 4). The inhibition of Gly-Sar flux was maximal when monolayers had been treated for >15 days, indicating that the decrease in peptide transport was caused by long-term treatment with EGF. Mannitol fluxes did not change significantly as a function of time of EGF treatment ($n = 3$; Fig. 4, inset).

**Effect of EGF on peptide transport is mediated via basolateral receptors.** EGF receptors are present on both apical and basolateral membranes of Caco-2 cells (2). A series of experiments were performed to investigate whether the observed effects were caused by apical stimulation, basolateral stimulation, or both. EGF (5 ng/ml) was added to either the basolateral or the apical solution throughout the culture period. Cells grown in the absence of EGF and cells cultured with EGF on both sides were used as controls (Fig. 5). Cells treated with EGF in the apical solution displayed a Gly-Sar flux identical to the control Gly-Sar flux with-
transcellular peptide transport thus appears to be mediated solely via the basolateral receptors. No statistical significant changes in mannitol fluxes were observed under the different experimental conditions (Fig. 5, inset).

Expression of hPepT1 mRNA and hPepT1 protein in EGF-treated Caco-2 cells. The localization of hPepT1 in 26-day-old Caco-2 cell monolayers is shown in Fig. 6. The peptide transport protein was primarily localized in the apical membrane and in vesicles just below, with little or no staining in the basolateral membranes. The degree of expression of hPepT1 varied between individual cells, as judged by both the vertical $x$-$y$ image (Fig. 6A) and the horizontal $x$-$z$ image (Fig. 6B). mRNA levels of hPepT1 were analyzed by RT-PCR to investigate whether there was a relationship between the decrease in transepithelial of Gly-Sar and the expression of hPepT1. Total RNA was obtained from untreated monolayers or monolayers treated with 5 ng/ml EGF throughout the culture period. RNA isolation and RT-PCR were carried out as described in MATERIALS AND METHODS. Agarose gel electrophoresis demonstrated the presence of PCR products of the expected sizes (hPepT1 ~643 bp, G6PDH ~404 bp; Fig. 7A). mRNA levels were quantified using $^{32}$P-labeled cDNAs followed by polyacrylamide gel electrophoresis and imaging as described in MATERIALS AND METHODS. Figure 7B shows hPepT1 mRNA levels in controls and EGF-treated cells normalized to the internal control G6PDH. The level of hPepT1 mRNA was significantly reduced (to ~65%; $P < 0.05$, $n = 3$) in cells grown in the presence of EGF compared with controls. Protein levels of hPepT1 were visualized using Western blotting and anti-hPepT1 antibody. Figure 8 shows a blot of total cell lysate (lanes 1 and 2) and cell protein immunoprecipitated with anti-hPepT1 (lanes 3 and 4). In all lanes, a prominent band at ~80 kDa was apparent, corresponding to hPepT1. Quantification of the bands from total cell lysate showed a 35 ± 3% ($n = 3$) decrease in band intensity in EGF-treated cells compared with controls.

DISCUSSION

The present study demonstrates, for the first time, that a growth factor (EGF) decreases hPepT1-medi-
ated transport of Gly-Sar in a dose-dependent manner. EGF caused a decrease in transepithelial transport of Gly-Sar in Caco-2 cells treated with EGF throughout the culture period. Apical and transepithelial kinetic data showed a decrease in $V_{\text{max}}$ without significant changes in $K_m$. EGF influenced the apical peptide transport step but did not regulate the peptide transport activity in the basolateral membrane. Using semi-quantitative RT-PCR we demonstrated a decrease in hPepT1 mRNA, and Western blotting indicated a decreased expression of hPepT1. Together, these data suggest that EGF decreased the number of hPepT1 transporters in the apical membrane of the Caco-2 cell monolayers. PCR data indicated that this was caused by a decrease in hPepT1 mRNA, thereby causing a decrease in the expression of the hPepT1 gene product.

Studies concerning the regulation of hPepT1 expression are sparse. Evidence indicates that protein kinase C (4), cAMP (25), and insulin (33) are involved in the regulation of PepT1. However, for these stimuli the regulation is on the level of transport function or protein recruitment to the plasma membrane. Both EGF and insulin act on tyrosine kinase receptors. The effect of insulin on hPepT1 was an insertion of preformed hPepT1 transporters after 1 h of insulin stimulation (33), whereas the downregulation of hPepT1 transport activity was caused by a stimulation of EGF for 5 days. This is probably because EGF acts at the level of gene expression whereas the effects of insulin are caused by short-term stimulation of protein kinases, which in turn phosphorylates cellular targets, eventually leading to the observed insertion of transporters. A study by Shiraga et al. (30) clearly shows that hPepT1 can be regulated at the level of gene expression by specific amino acids and peptides. Thamotharan et al. (32) showed that increased hPepT1 expression and increased levels of hPepT1 mRNA could be caused by stimulation of luminal peptides, and Walker et al. (36) showed that a similar upregulation of hPepT1 was mediated in part via an increase in hPepT1 mRNA stability. The downregulation of sucrase-isomaltase (SI) in Caco-2/15 cells after long-term EGF treatment showed that EGF acts primarily at the pretranslational level by influencing SI gene transcription and/or SI mRNA stability (6).

The basolateral transport activity has been shown by some groups to be coupled to protons (34, 35), whereas others have shown the transport process to be independent of or only slightly dependent on protons (28, 31), suggesting the existence of distinct apical and basolat-
eral transporters. In this study we showed a differential regulation of apical and basolateral peptide transport activity by EGF. Whereas apical peptide transport activity was markedly downregulated after EGF treatment, basolateral transport parameters remained unchanged. Furthermore, hPepT1 was found (using a hPepT1 antibody and laser scanning confocal microscopy) to be located in the apical membrane of the Caco-2 cells (Fig. 6). Similar results were found by Walker et al. (36), who showed that anti-hPepT1 immunostaining was present predominantly in the apical membrane in Caco-2 cells (using an anti-hPepT1 antibody distinct from the one used in our study). Our data thus add to the body of evidence indicating that two different transporters might be involved in transcellular transport of intact di-/tripeptides and certain peptidyl compounds (for references, see Refs. 28, 31, and 34–36).

Previous studies have indicated that EGF receptors are present on both apical and basolateral membranes of Caco-2 cells (2). Estimates of the basolateral-to-apical receptor ratio range from 3 to 15 (for references, see Ref. 2). However, only basolateral stimulation with EGF mediated the decrease in peptide transport, as demonstrated in the present study. Only a few studies have examined from which side (apical vs. basolateral) EGF exerts its effect in Caco-2 cells. Bishop and Wen (2) showed that Caco-2 cell proliferation by EGF was mediated exclusively by basolateral EGF receptors (EGFR). EGF and related substances mediate their effects on epithelial cells in vivo through binding to high-affinity EGFR at their basolateral surface (for review, see Ref. 5). Caco-2 cells thus functionally resemble human enterocytes, in which only basolateral EGFR have been found (27).

The decrease in Gly-Sar transport displayed an inhibition constant fairly close to binding constant (Kd) values for EGF binding to the EGFR observed in other tissues. The ED50 value found in this study was 0.36 ng/ml (0.7 nM) for the apical uptake. Kd values have been estimated to be 0.67 nM in Caco-2 cells (18), 0.86 nM in human urothelial cells (24), 0.83 nM in the jejunal crypt cell line IEC-6 (1), and 2.31 nM in rat enterocytes (14). The concentrations at which EGF decreased peptide transport activity are thus in the physiological relevant range for EGF-EGFR interaction.

Despite the large body of literature describing the effects of EGF in various biological systems, its exact role in tissue development and differentiation still remains unclear. The overall result of EGFR activation is a change in the steady-state RNA concentration of a number of cellular genes (for references, see Ref. 16). In Caco-2 cells it appears that treatment with EGF blocks differentiation and keeps the cells in a proliferative stage. Daniele and Quaroni (7) showed that Caco-2 cells grown in the presence of EGF throughout the culture period showed a decrease in expression of the differentiation marker dipeptidyl peptidase IV compared with controls, and Cross and Quaroni (6) showed the same to be the case with respect to differentiation marker SI (6). In the present study we demonstrated that the differentiation marker hPepT1 displayed a decrease after EGF treatment. This was not caused only by a delayed differentiation of the cells, because cells treated without EGF for 21 days and then subsequently treated with EGF showed a significant decrease in Gly-Sar transport (Fig. 4), indicating an actual dedifferentiation.

It appears that the decrease in the expression of hPepT1 is part of a general pattern of effects after long-term stimulation of the basolateral EGF receptor in Caco-2 cells, namely, increased proliferation and a decrease in the number of differentiation-specific brush border marker proteins. However, a correlation of the present studies to the in vivo situation is required to fully understand the physiological role of EGF in downregulation of apical brush border proteins and enterocyte proliferation. In vivo, the luminal membrane will be more or less constantly exposed to EGF, because EGF is secreted from the gastrointestinal glands and is present in the intestinal lumen at fairly high concentrations (for references, see Ref. 12). Effective basolateral EGF concentrations are not easily estimated, because EGF acts in an autocrine/paracrine manner and serum concentrations (which are extremely low in adults) are not likely to reflect the effective concentrations encountered by the basolateral receptors on the enterocytes. Furthermore, EGF is released enzymatically from an extracellular domain of a large transmembrane precursor, which also is able to activate EGF receptors of neighboring cells while still anchored in the membrane. The exact role of EGF released in the lateral spaces by neighboring enterocytes or by other cell types still remains unclear, and there is a possibility that native enterocytes might experience a tonic stimulation by soluble EGF originating from the local environment and from EGF precursor molecules on neighboring cells.

In conclusion, the results of the present study provide evidence that long-term treatment of Caco-2 cell monolayers with EGF causes a decrease in transepithelial transport and apical uptake of Gly-Sar. We showed that this was due to a decrease in hPepT1 protein expression caused by the decrease in hPepT1 mRNA. Further studies are needed to investigate cellular events linking EGFR activation and hPepT1 expression in Caco-2 cells.

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