Epidermal growth factor regulates fatty acid uptake and metabolism in Caco-2 cells

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Darimont, Christian, Nathalie Gradoux, and Alain De Pover. Epidermal growth factor regulates fatty acid uptake and metabolism in Caco-2 cells. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G606–G612, 1999.—Epidermal growth factor (EGF) has been reported to stimulate carbohydrate, amino acid, and electrolyte transport in the small intestine, but its effects on lipid transport are poorly documented. This study aimed to investigate EGF effects on fatty acid uptake and esterification in a human enterocyte cell line (Caco-2). EGF inhibited cell uptake of [14C]palmitate and markedly reduced its incorporation into triglycerides. In contrast, the incorporation in phospholipids was enhanced. To elucidate the mechanisms involved, key steps of lipid metabolism were investigated. The amount of intestinal fatty acid-binding protein (I-FABP), which is thought to be important for fatty acid absorption, and the activity of diacylglycerol acyltransferase (DGAT), an enzyme at the branch point of diacylglycerol utilization, were reduced. EGF effects on DGAT and on palmitate esterification occurred at 2–10 ng/ml, whereas effects on I-FABP and palmitate uptake occurred only at 10 ng/ml. This suggests that EGF inhibited palmitate uptake by reducing the I-FABP level and shifted its utilization from triglycerides to phospholipids by inhibiting DGAT. This increase in phospholipid synthesis might play a role in the restoration of enterocyte absorption function after intestinal mucosa injury.

fatty acid-binding protein; diacylglycerol acyltransferase; acyl-coenzymeA synthetase; inflammatory bowel disease

The healing action of EGF is thought to result mainly from the stimulation of enterocyte migration and proliferation (15, 16). In addition, a number of effects have been reported that may play a role in the restoration of the epithelial absorption function after injury, e.g., increases in brush-border surface area (11) and in glucose (12), galactose (30), glycine (30), and electrolyte (12) transport processes. In contrast, EGF inhibited lipoprotein secretion in Caco-2 cells (24). The cytokines tumor necrosis factor-α (22) and interleukin-1β and -6 (25), which are secreted during inflammation, have also been reported to affect lipoprotein secretion. It was proposed that cytokines could decrease lipid absorption in the early stages of inflammation when cells are not morphologically damaged. Furthermore, the effect of the key cytokine, interleukin-6, has been suggested to be mediated by EGF (24). Although the significance of this effect in wound healing is unknown, the possibility that it could protect enterocytes by diverting metabolic processes away from lipid secretion and provide a stimulus for cell regeneration can be entertained (24).

The pathways of triglyceride synthesis are illustrated in Fig. 1. Fatty acids are delivered to acyl-CoA synthetase (ACS) by cytosolic fatty acid-binding proteins (FABP) and incorporated into lipids by different acyltransferases. Two FABP isoforms, the liver (L-FABP) and the intestinal (I-FABP), are expressed in enterocytes. These proteins bind long-chain fatty acids with submicromolar affinities and are thought to play an important role in fatty acid absorption and esterification, but their precise roles are still unknown (2, 29).

Diacylglycerol acyltransferase (DGAT) is at the branch point of diacylglycerol utilization; hence, its regulation may affect the balance between phospholipid and triglyceride syntheses. Two pathways lead to diacylglycerol: the phosphoglycerate and the monoacylglycerol pathways. The former is predominant in the small intestine but is virtually absent in Caco-2 cells due to the low activity of monoacylglycerol acyltransferase (34).

The key steps examined in the present study are common to the two pathways.

The presence of two FABP isoforms in enterocytes is intriguing. It has been postulated (1, 2, 19, 28) that I-FABP targets the delivery of fatty acids from the brush-border membrane to specific sites of lipid metabolism, whereas L-FABP is important for the basic cellular economy of fatty acids ("house-keeping" function). In a previous study, we showed that the patterns of regulation were different for I-FABP and L-FABP in Caco-2 cells (7). Thus we were interested to see whether EGF effects on lipid metabolism could be related to a specific regulation of I-FABP. The results show that EGF produced an inhibition of palmitic acid uptake by Caco-2 cells that could be due to a specific downregulation of I-FABP. Furthermore, EGF appeared to shift metabolic pathways from triglyceride to phospholipid synthesis by inhibiting DGAT. It is proposed that these
Effects play an important role in the restoration of enterocyte absorption function after intestinal mucosa injury.

**MATERIALS AND METHODS**

**Materials.** Palmitate, EGF, homovanillic acid, and acyl-CoA oxidase were obtained from Sigma Chemical (St. Louis, MO). Horseradish peroxidase (grade II), ATP, and CoA were from Boehringer Mannheim. [14C]palmitic acid and [14C]palmitoyl-CoA were purchased from Amersham (Zurich, Switzerland) and DuPont NEN (Boston, MA), respectively.

**Cell cultures.** Cell cultures were performed essentially as described previously (7). Caco-2 cells were subcultured on plastic plates (Falcon, Becton Dickinson) coated with 30 µg collagen I/ml PBS (bovine skin type I collagen, Boehringer Mannheim). For differentiation, cells were seeded on 24.5-mm (uncoated) polycarbonate Transwell-Clear filter inserts (0.4 µm pore size; Costar, Cambridge, MA) in 10% FCS. At day 17 after confluence, cell differentiation was evaluated by measuring the activities of brush-border membrane enzymes [sucrase-isomaltase as previously described (23) and alkaline phosphatase using a commercially available kit (Sigma)]. For fatty acid transport experiments, the integrity of the monolayer was checked with phenol red.

Unless otherwise stated, the effects of EGF were studied after 48 h of incubation (from day 15 to day 17 after confluence) with increasing concentrations of the peptide. EGF was added to the apical and basolateral compartments in a serum-free medium (DMEM with 4.5 g/l glucose, 4 mM glutamine, 40 µg/ml gentamicin, and 1% nonessential amino acids) supplemented with 0.1% fatty acid-free BSA. Incubation was carried out at 37°C in 93% air-7% CO2, and the serum-free medium supplemented with EGF was replaced after 24 h.

Western blot analysis of Caco-2 cell lysates. The quantitative analysis by Western-blot of I-FABP and L-FABP concentrations in Caco-2 monolayer lysates was performed using sensitive and specific rabbit anti-human I-FABP and L-FABP antisera as previously described (7).

Fatty acid uptake and metabolism by Caco-2 cells. [14C]palmitic acid-sodium taurocholate micelles were prepared by mixing 100 µM palmitic acid and 10 µM [14C]palmitic acid (55 Ci/mol; Amersham) with the serum-free medium described above, supplemented with 8 mM sodium taurocholate (Fluka). This solution was equilibrated for 30 min at 37°C and added to the apical compartment. The same medium, but without fatty acid, was added to the basolateral compartment. Both compartments were previously washed with 2 ml warm PBS. After a 2-h incubation, the apical and basolateral media were removed, and the two compartments were washed twice with 0.75 ml of ice-cold PBS. The washes were combined with the media. Cells were scraped into 1 ml of ice-cold PBS and sonicated for 1 min; 50-µl aliquots were diluted in scintillation fluid (Irgascint A300; Novartis, Basel, Switzerland), and radioactivity was quantified using a LKB Wallac 1214 RackBeta liquid scintillation counter. Fatty acid uptake was determined from the specific activity of the incubation medium.

To determine fatty acid metabolites, lipids were extracted from Caco-2 cell lysates and basolateral medium according to the method of Bligh and Dyer (4). TLC was used to determine [14C]palmitic acid incorporation into lipid metabolites. Lipid
Because EGF receptors have been reported on both apical and basolateral membranes of Caco-2 cells and rat enterocytes (3, 14, 32), EGF was added to both compartments. Under these culture conditions, the presence of EGF did not affect the activities of two brush-border markers, sucrase-isomaltase and alkaline phosphatase (Table 1). This indicates that Caco-2 cells cultured in the presence of EGF for 48 h were well differentiated. Therefore, fatty acid uptake and esterification could be studied under these conditions.

Figure 2A shows no significant effect of EGF on [14C]palmitic acid uptake up to 5 ng/ml but a 19% decrease at 10 ng/ml. Figure 2B shows the incorporation of [14C]palmitate into phospholipids and triglycerides expressed as percentage of cell radioactivity. At 10 ng/ml, radioactivity decreased from 43.7 ± 3.6 to 26.7 ± 2.2% in triglycerides but increased from 44.6 ± 2.5 to 62.0 ± 1.4% in phospholipids. These opposite effects occurred already at 2 ng/ml EGF and were maximal at 5 ng/ml EGF did not significantly affect cell unesterified palmitic acid (control: 7.1 ± 1.0%; 10 ng/ml EGF: 6.3 ± 1.1%) and diacylglycerol (control: 4.5 ± 0.3%; 10 ng/ml EGF: 4.9 ± 1.1%).

The effect of EGF on lipid secretion is illustrated in Fig. 3. Total radioactivity in the basolateral medium did not decrease significantly (Fig. 3A), but the radioactivity incorporated into triglycerides decreased from 30.5 ± 6.6% (control) to 10.4 ± 2.9% at 10 ng/ml, whereas the radioactivity in phospholipids increased from 21.1 ± 2.5 to 45.2 ± 6.4% (Fig. 3B).

Effects of EGF on FABP protein levels and on DGAT and ACS activities. To investigate the mechanism of the inhibitory effects of EGF on palmitic acid uptake and triglyceride synthesis, the effects of EGF on key steps of metabolic pathways were examined. I-FABP and L-FABP isoforms were selected because they have been proposed to play distinct roles in fatty acid uptake and metabolism (1, 7, 28). Figure 4A shows that EGF decreased I-FABP protein levels by 55% (from 0.27 ± 0.01 to 0.12 ± 0.01 µg/mg cytosolic protein) without significantly affecting L-FABP (Fig. 4B). The effect of EGF on the I-FABP level, like that on palmitic acid uptake, was significant only at 10 ng/ml.

The activity of ACS, which is a key enzyme providing fatty acyl-CoA to most enzymatic steps of lipid esterification, particularly the transformation of diacylglycerol into triacylglycerol (Fig. 1), was measured. EGF did not significantly affect ACS activity in differenti-
imated Caco-2 cells (Fig. 5). For comparison, the hepatocyte ACS inhibitor (20), α-bromopalmitate, decreased the activity of the Caco-2 cell enzyme by 70% (Fig. 5).

DGAT is an enzyme at the branch point of triglyceride and phospholipid synthesis (Fig. 1). It produces triglycerides by transferring the acyl chain of acyl-CoA to diacylglycerol. Figure 6 shows that EGF inhibited DGAT activity by 62% at 10 ng/ml, a significant effect being already produced at 5 ng/ml. DGAT was similarly inhibited after a 5-min incubation (see Fig. 6, inset: 75% inhibition at 10 ng/ml, P < 0.01). After such a brief exposure, effects on DGAT protein level are unlikely.

DISCUSSION

A role for EGF, which is likely due primarily to its mitogenicity, has been proposed in the healing of gastrointestinal ulcers (15, 16, 26). The inhibitory effect of EGF on lipoprotein secretion has also been postulated to contribute to wound healing, perhaps by diverting metabolic processes away from triglyceride secretion (24). However, it is unclear how enterocytes can benefit from such an effect. The purpose of the present report was to give insights into this aspect of EGF activity by studying key steps of triglyceride synthetic pathways in Caco-2 cells.

In this study, differentiated Caco-2 cells were treated for 48 h with EGF concentrations from 2 to 10 ng/ml (i.e., 0.3–1.6 nM). These concentrations were within the range reported in human (15) and rat (31) duodenal lumen, i.e., 1.5 and 0.2 nM, respectively. Furthermore, these concentrations have been reported to be active in Caco-2 cells. From 1 to 5 ng/ml, cell growth was dose...
dependently stimulated (3) and lipoprotein secretion was inhibited (24). The inhibition of lipoprotein secretion was observed after a 3-h and up to a 24-h treatment (24). For the present experiments, we chose a long time of incubation to observe possible effects of EGF on FABP protein levels. Indeed, these proteins have a slow turnover and a very long degradation half-life of ~3 days (2). Accordingly, polypeptide YY has been reported to increase I-FABP mRNA in intestinal hBRIE380i cells after 6 h but has been reported to significantly increase protein levels only after 3 days of incubation (10). In Caco-2 cells, we show that the activities of the brush-border enzymes, sucrase-isomaltase and alkaline phosphatase, were unchanged after 48-h EGF treatment, suggesting that differentiation was not affected. Much longer exposure (12 days) of Caco-2 cells with higher EGF concentrations (20–200 ng/ml) seems to be required for sucrase-isomaltase inhibition (6). Therefore, it is unlikely that EGF effects under the present conditions were due to a global change in cellular economy and metabolic status.

EGF produced different effects on fatty acid metabolism, depending on its concentration. The uptake of \([^{14}C]\)palmitate by Caco-2 cells was inhibited only at 10 ng/ml, whereas its distribution into metabolites was already affected at 2 ng/ml, suggesting distinct underlying mechanisms. There are several mechanisms that could account for the inhibition of fatty acid uptake. An attractive possibility is the reduction of intracellular

Fig. 4. Effects of EGF on intestinal and liver fatty acid-binding protein (I-FABP and L-FABP, respectively) levels in Caco-2 cells. Between days 15 and 17 after confluence, cells were incubated with increasing concentrations of EGF. I-FABP (top) and L-FABP (bottom) were quantified by Western blot analysis in Caco-2 cells homogenized at day 17. Data are means ± SE obtained in 4 separate experiments, and values statistically different from controls are indicated (***P < 0.05).

Fig. 5. Effects of EGF on ACS activity in Caco-2 cells. After 48-h incubation with 10 ng/ml EGF or 10 µM bromopalmitate (BrP), ACS activity of cell homogenates was determined as described in MATERIALS AND METHODS. Data are means ± SE obtained in 4 separate experiments. Values statistically different from controls are indicated (**P < 0.05). NS, nonsignificant values.

Fig. 6. Effects of EGF on DGAT activity in Caco-2 cells. After 48-h or 5-min (inset) incubation with various EGF concentrations, DGAT activity of cell homogenates was determined as described in MATERIALS AND METHODS. Data are means ± SE obtained in at least 4 separate experiments. Values statistically different from controls are indicated (*P < 0.05; **P < 0.01).
fatty acid transport due to downregulation of cytosolic FABP. Indeed, measurements of FABP levels showed that 10 ng/ml EGF specifically inhibited the intestinal type of FABP. This isoform has been shown to stimulate fatty acid uptake in transfected embryonic stem cells (1). We previously reported that L-FABP represented 2% of cytosolic proteins in Caco-2 cells, such as in rat jejunum, but that I-FABP was only 1/50th of its jejunum level (7). Despite this low level, I-FABP may play a significant role in intracellular fatty acid transport in this cell line due to its high transport efficiency, presumably by a collisional mechanism, as suggested by experiments with liposomes (13). Thus I-FABP downregulation may contribute to the decrease in palmitic acid uptake produced by EGF in Caco-2 cells. Furthermore, because I-FABP is a major protein in the small intestine, stronger effects of EGF on lipid absorption could be expected in vivo.

We cannot exclude a possible contribution of membrane-bound FABPs such as plasma membrane-bound FABP (33) or the fatty acid transporter (27) in EGF effects, but their role in fatty acid uptake is highly controversial (36). The selective effect of EGF on L-FABP vs. L-FABP levels suggests a specific role for this isoform in enterocytes. Recently, another gut hormone, the polypeptide YY, has been reported to regulate I-FABP in hBRIE380i cells (10). The effect of EGF in the present study is the second example of a hormonal regulation of this protein by a peptide secreted in the intestinal lumen.

EGF effects on [14C]palmitate distribution into metabolites were observed at concentrations that did not affect uptake (2 ng/ml). Strikingly, the inhibition of radiolabel incorporation into triglycerides appeared to be “compensated” by an increase into phospholipids. These opposite effects of EGF led us to study the activity of DGAT, which is at the branch point of diacylglycerol utilization. Indeed, EGF dose dependently inhibited DGAT, the effect reaching 62% at 10 ng/ml. In contrast, EGF did not inhibit ACS, the enzyme that activates fatty acids before their incorporation into glycerol esters. Although other enzymes, in particular cytidyl transferase, were not studied, it is reasonable to conclude that DGAT inhibition can account for the opposite effects of EGF on triglyceride and phospholipid synthesis. It is noteworthy that the regulation of DGAT is still poorly documented. In hepatocytes, glucagon and 2-bromooctanoate have been reported to reduce DGAT (9, 21). In Caco-2 cells, palmitate and palmitate plus 2-monolein were ineffective (34). To our knowledge, the present data give the first description of a hormonal regulation of the key enzyme DGAT in intestinal cells.

In adipocytes, DGAT inactivation has been reported to result from tyrosine phosphorylation of the protein (18). The fast onset of EGF action (5 min) on DGAT activity in Caco-2 cells is consistent with such mechanisms. In contrast, no or very little tyrosine phosphorylation has been shown for FABP isoforms (29). Thus DGAT inhibition and I-FABP level reduction emphasize the complexity of EGF action on lipid metabolism in enterocytes.

The inhibition of triglyceride secretion, which is in agreement with a previous study (24), accompanied the reduction of its synthesis. Secretory processes are energy dependent so that inhibition of triglyceride secretion might be useful for enterocytes in a stress situation. The present results show, however, that a major effect of EGF may be rather to divert lipid metabolism from triglyceride to phospholipid synthesis. This effect on phospholipid synthesis might be related to the enhancement of brush-border surface area previously reported (11). It is therefore tempting to propose that the increase in phospholipid synthesis is a key factor in the restoration of cell absorption function after intestinal mucosa injury.

We thank A. Vladimirov for technical assistance and Dr. B. Fallier for critically reading the manuscript.

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Received 24 July 1998; accepted in final form 23 November 1998.

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