Role of actin in EGF-induced alterations in enterocyte SGLT1 expression

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Chung, Brian M., Jason K. Wong, James A. Hardin, and D. Grant Gall. Role of actin in EGF-induced alterations in enterocyte SGLT1 expression. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G463–G469, 1999.—Na+-glucose cotransporter (SGLT1) expression and the role of actin in epidermal growth factor (EGF)-induced alterations in glucose transport and brush-border surface area were examined in New Zealand White rabbit jejunal loops. In separate experiments, EGF or EGF concurrent with cytochalasin D, an inhibitor of actin polymerization, was administered to the experimental loop and compared with its vehicle control. SGLT1 expression was measured by Western blot in brush-border membrane vesicles (BBMV) after 5-min and 1-h exposure. Glucose kinetics were determined by a rapid filtration technique, and brush-border surface area was examined by electron microscopy after 1-h exposure. The effect of cytochalasin D alone on BBMV glucose kinetics and brush-border surface area was also assessed. EGF resulted in a significant increase in BBMV SGLT1 expression (P < 0.05), glucose maximal uptake (Vmax; P < 0.001), and absorptive brush-border surface area (P < 0.001). These effects were abolished with concurrent cytochalasin D treatment. Cytochalasin D alone had no effect on glucose transport or brush-border surface area. The findings suggest that EGF acutely upregulates jejunal brush-border surface area and the Vmax for jejunal glucose uptake via the recruitment and insertion of SGLT1 from an internal pool into the brush border by a mechanism that is dependent on actin polymerization.

epidermal growth factor; sodium-glucose cotransporter; glucose transport

EPIDERMAL GROWTH FACTOR (EGF) is a 53-amino acid peptide derived from numerous sources in the gastrointestinal tract, including saliva, bile, Paneth cells (34), and Brunner’s glands of the duodenum (26). EGF is a potent mitogen and has been shown to promote DNA synthesis (6, 26) and transcription of RNA leading to protein synthesis (6). EGF has also been shown to regulate small intestinal transport function. EGF increases intestinal nutrient and ion absorption both in vivo and in vitro (32). This effect is associated with increases in both brush-border surface area and total absorptive surface area, which appears to be due to the recruitment of a pool of preformed microvillus membrane (13). In addition, our laboratory as well as others (14, 16) have demonstrated an increase in maximal uptake (Vmax) for glucose transport in jejunal brush-border membranes after EGF treatment. These findings suggest EGF stimulates jejunal glucose transport via the insertion of new membrane and transport proteins into the brush border.

For EGF to exert its biological effects it must first bind to a specific transmembrane receptor. This receptor has three main regions: an extracellular ligand binding domain, a transmembrane segment, and an intracellular domain possessing intrinsic tyrosine kinase activity (2, 26). The binding of EGF to its receptor induces dimerization of bound receptors, resulting in activation of the EGF receptor complex by autophosphorylation on tyrosine residues (38, 42). The activated EGF receptor complex has been shown to be associated with the actin cytoskeleton (10, 35, 43), as well as to act on many intracellular substrates, including four enzymes linked to the cytoskeleton: diacylglycerol kinase, phosphoinositol kinase, phosphoinositol 4-phosphate kinase, and phospholipase Cγ (33).

Activation of the EGF receptor has been reported to lead to increased F-actin content in cultured cells (35), and stimulation of cells with EGF has been shown to induce serine phosphorylation on actin filaments (44). The brush border of intestinal epithelial cells, an evolutionary adaptation designed to increase surface area for digestion and absorption of ions and nutrients, appears to be a primary site of action of EGF in the small intestine. A major component of the brush border is actin, which comprises the core of each microvillus. Actin is also known to be involved in the regulated cycling and insertion of membrane proteins, including transport proteins (11, 28, 41). Thus we hypothesized that EGF increases glucose absorption by increasing the insertion of membrane and Na+-glucose cotransporter protein (SGLT1) into the brush border through a mechanism involving the polymerization of actin.

METHODS

Animal model. New Zealand White rabbits (800–1,000 g) were used. Experimental procedures followed standards set by the Canadian Council of Animal Care. Animals were anesthetized with halothane, a laparotomy was performed, jejunum was isolated, and two blind 10- to 15-cm loops, separated by a 1-cm segment, were tied off 5 cm distal to the ligament of Treitz. Three separate experimental conditions were examined: either EGF (60 ng/ml; Sigma, St. Louis, MO) in 1.8–2.0 ml of Krebs buffer ([(mM)] 140 Na+, 127.5 Cl−, 25 HCO3−, 10 K+, 1.25 Ca2+, 1.1 Mg2+, 2 H2PO4−, pH 7.4); EGF (60 ng/ml) and cytochalasin D (2 μM; Sigma) in 1.8–2.0 ml of Krebs buffer, or cytochalasin D (2 μM) alone in 1.8–2.0 ml of Krebs buffer. Experimental solutions were added alternately to either the proximal or distal loop, and vehicle alone (0.1% DMSO in 1.8–2.0 ml Krebs buffer) was added to the remaining loop. Cytochalasins have previously been shown to effectively inhibit actin polymerization (4, 7, 31). After a 1-h
incubation, loops were removed and tissue was prepared for glucose transport measurements and electron microscopy. Brush-border membrane vesicle glucose transport. For the glucose transport studies the mucosa was scraped and brush-border membrane vesicles (BBMV) prepared by a calcium chloride precipitation method as previously described (20). BBMV were stored in liquid nitrogen until day of assay. All measurements were normalized to membrane protein, as determined by the method of Lowry et al. (19). Basolateral contamination was assessed by comparing Na\(^+-\)K\(^-\)-ATPase activity in the initial homogenate and the microvillus membrane preparation. Sucrase activity was determined by the method of Dalquist (9). Basolateral contamination was assessed by comparing Na\(^+-\)K\(^-\)-ATPase activity in the initial homogenate and the microvillus membrane preparation by the method of Kelly et al. (19).

BBMV uptake of \(\Delta\)-glucose was measured using a rapid filtration technique and a 5-s time course as previously described (18). All chemicals were obtained from Sigma except for the \(\Delta\)-[\(\text{H}\)]glucose, which was obtained from DuPont NEN Research products (Mississauga, Ontario). Vesicles were resuspended to a final concentration of 8–15 mg protein/ml in (in mM) 100 KCl, 300 mannitol, and 10 Tris-HEPES (pH 7.5). Valinomycin (4 µM) was added immediately before transport measurements to voltage clamp the vesicle preparations.

Glucose transport was measured by mixing 10 µl membrane vesicles with 50 µl reaction buffer containing a concentration of mannitol ranging from 0 to 100 mM, 100 mM NaSCN, 10 mM Tris-HEPES, 100 mM KCl (pH 7.5), 4 µM \(\Delta\)-[\(\text{H}\)]glucose, and varying concentrations of unlabeled \(\Delta\)-glucose ranging from 0 to 100 mM. Experiments were performed at 22°C. Glucose transport was stopped by addition of 5 ml ice-cold stop solution [(in mM) 100 NaCl, 100 mannitol, 10 Tris-HEPES, 100 KCl, pH 7.5]. The solution was then rapidly filtered through a 0.45-µm filter (Millipore/Continuous Water Systems, Bedford, MA) and washed with 5 ml of stop solution. Filters were placed in 10 ml scintillation fluid and counted in a liquid scintillation counter. Brush-border membrane kinetic analysis was performed by nonlinear regression techniques as previously described (25). Data are expressed as nanomoles of \(\Delta\)-[\(\text{H}\)]glucose taken up per minute per milligram protein.

Brush-border surface area. Jejunal brush-border surface area was determined from three animals for each of the three treatment groups. After 1 h, EGF, EGF-cytosoladin D, cytosaladin D alone, or control loops were removed and fixed in 5% glutaraldehyde in phosphate buffer [(in M) 0.2 Na\(_2\)PO\(_4\), 0.2 Na\(_2\)HPO\(_4\), pH 7.3], osmolality of 300 mosmol/kgH\(_2\)O at 20°C. Tissue was postfixed in 1% OsO\(_4\) for 2 h, dehydrated in graded alcohols, cleared with propylene oxide, and infiltrated with and embedded in Spurr’s low-viscosity medium (J. B. EM Services, Dorval, Quebec). Ultrathin sections were obtained and double-stained with uranyl acetate in 50% ethanol and 0.4% lead citrate. Micrographs were obtained from the midvillus region of the sections as determined by a low-magnification observation of complete villi. Duplicate measurements from each micrograph were obtained, and jejunal midvillus brush-border area was calculated as previously described (5). To avoid observer bias, the study was performed under blinded conditions.

Brush-border membrane SGLT1 expression. Animals were anesthetized, and two blind jejunal loops were prepared as described above. Two separate experimental conditions were examined: either EGF (60 ng/ml) in 1.8–2.0 ml of Krebs buffer was administered into one loop and 1.8–2.0 ml of Krebs buffer was added to the other loop. Experimental solutions were added alternately to either the proximal or distal loop, and vehicled (0.1% DMSO) alone was added to the remaining loop. In separate experiments, loops were removed after 5 min or 1 h, and the mucosa was harvested. BBMV were prepared as described above.

Western blot. BBMV proteins were separated on denaturing 8% SDS-PAGE minigels according to the method of Laemmli (22). Briefly, BBMV were diluted 1:1 in 2× Laemmli SDS protein sample buffer (S-2401, Sigma) and boiled for 5 min at 100°C. BBMV were then loaded onto 8% SDS minigels (25 µg protein/lane) and separated using a constant current. High-range molecular weight markers (V-5251, Promega, Madison, WI) were concurrently run to determine molecular weights. Separated proteins were transferred overnight onto nitrocellulose paper (0.45 µM, Trans-Blot transfer medium, Bio-Rad, Mississauga, Ontario) with a low-ionic transfer buffer at constant current. The finished blots were rinsed in 0.05% Tween 20 Tris-buffered saline (TTBS), blotted dry on filter paper, and stored at −20°C until staining.

Blots were probed with a polyclonal antibody raised against a proposed extracellular loop of the rabbit SGLT1 sequence (8327–1109, Cedarlane Laboratories, Hornby, Ontario) (15). In preliminary experiments, a densitometric analysis of serial dilutions of the peptide against which the antibody was raised was performed. Optical density was linear for concentrations of the peptide ranging from 1 to 2.25 pmol. All samples measured in this study fell within the linear portion of the curve. Immuno reactive bands were developed using a modification of the immunogold-silver process described by Fowler (12). Briefly, blots were thawed and rehydrated with TTBS. Blots were blocked for 1 h with 5% wt/vol BSA (A-3803, Sigma). Blots were then incubated for 4 h with a 1:40 vol/vol dilution of the antibody in 1% BSA-TTBS and subsequently washed three times for 10 min. Blots were then probed with a secondary antibody (G-7402, Sigma) diluted 1:300 vol/vol in gelatin buffer (0.1% BSA, 0.4% gelatin, TTBS) for 1.5 h. Finally, blots were washed three times with TTBS and once in distilled water and then incubated with silver enhancer (RPN 492, Cedarlane Laboratories, Hornby, Ontario) for 20–30 min to amplify the gold signal. Immunoreactive bands were assessed by two-dimensional scanning densitometry with incident illumination on a Scanalytics SCPI Masterscan densitometer, using Camscan (Scanalytics, Fairfax, VA). The image was analyzed by RFLPScan 1.01 and expressed as units of integrated optical density.

Statistical analysis. Data are expressed as means ± SE, and statistical analyses were performed by Student’s t-test or ANOVA with repeated measurements. Western blots were analyzed by paired t-test. Statistical comparison of kinetic curves was performed as previously described (27). Significance levels were set at 0.05.

RESULTS

Glucose kinetics. Jejunal BBMV prepared from EGF-, EGF-cytosoladin D-, and cytosaladin D-treated loops were compared with control vesicle preparations. All BBMV preparations used in this study demonstrated at least a 10-fold increase in sucrase activity compared with their respective mucosal homogenates and <3% basolateral contamination as determined by Na\(^+-\)K\(^-\)-ATPase. Kinetic parameters for the uptake of glucose into brush-border vesicles were calculated and are presented in Table 1. Luminal exposure of tissue to 60
The control values for the various treatment groups examined did not significantly differ; thus all control data were pooled. The increase in brush-border surface area was mostly due to a significant increase in microvillus height. Microvillus width and density did not significantly differ between treatment groups (Fig. 2). Concurrent addition of the inhibitor of actin polymerization, cytochalasin D, abolished the increase in brush-border surface area seen with EGF treatment (36.9 ± 2.2 μm², n = 22). When the effect of cytochalasin D alone was assessed, cytochalasin D exhibited no significant effect on any brush-border parameter, including brush-border surface area (35.2 ± 1.7 μm², n = 26). Consistent with the findings of Madara et al. (24), treatment with cytochalasin D (either EGF-cytochalasin D or cytochalasin D alone) appeared to result in the slight opening of tight junctions compared with control (data not shown).

Brush-border SGLT1 expression. All BBMV preparations utilized for Western blots had a >10-fold increase in sucrose activity compared with initial homogenates and <3% basolateral contamination as assessed by Na⁺-K⁺-ATPase activity. BBMV protein, sucrase, and Na⁺-K⁺-ATPase activity did not differ between any of the groups after either 5 min or 1 h as shown in Table 2.

Probing with the polyclonal antibody showed a single immunoreactive band at 70–75 kDa. This is in agreement with previous reports for the molecular mass of the rabbit intestinal SGLT1 (15). Immunoreactive bands are shown in Figs. 3 and 4. Densitometric analysis demonstrated that 5-min (Fig. 3, P < 0.01) or 1-h (Fig. 4, P < 0.05) luminal exposure to EGF (60 ng/ml) significantly increased BBMV SGLT1 band density over saline-exposed controls. Concurrent administration of cytochalasin D with EGF for 1 h completely abolished the increase in BBMV SGLT1 band density (Fig. 4).

DISCUSSION

The results suggest that EGF acutely upregulates jejunal brush-border surface area and the Vₘₐₓ for jejunal glucose uptake via the recruitment and insertion of membrane rich in SGLT1 from an internal pool into the brush border. This process is dependent on actin filaments. EGF treatment resulted in a significant increase in the Vₘₐₓ for brush-border glucose transport, brush-border surface area, and brush-border SGLT1 content. Concurrent cytochalasin D administration inhibited EGF-induced increases in brush-border glucose transport, surface area, and SGLT1 content.
suggesting that these are linked phenomena and that actin polymerization is required for EGF-induced effects. Furthermore, previous studies have demonstrated that EGF-stimulated elevations in \( V_{\text{max}} \) are not associated with any alteration in brush-border membrane lipid composition, physical characteristics, or sucrase content (13), and no alteration in either affinity of the \( \text{Na}^+ \)-coupled cotransporter for glucose or brush-border membrane sodium permeability was observed after EGF treatment (14). As demonstrated, the response to EGF is very rapid (<5 min); EGF stimulated a significant increase in brush-border SGLT1 content. This is in keeping with our previous results demonstrating a rapid upregulation of transport (32) and absorptive surface area (13). Thus it seems likely that the

![Fig. 2](image)

Fig. 2. Microvillus height, width, density, and surface area of jejunal brush border from midvillus region in EGF-, EGF-cytochalasin D-, and cytochalasin D-treated tissue compared with control. Solid bars represent experimental group and open bars respective control values. Data are expressed as means ± SE. * \( P < 0.001 \). CYTO, cytochalasin D. Control data did not differ between any experimental groups and were therefore pooled.

Table 2. Jejunal BBMV protein, sucrase activity, and \( \text{Na}^+\text{-K}^+\)-ATPase activity

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<thead>
<tr>
<th></th>
<th>Protein, mg/ml</th>
<th>Sucrase, U/g protein</th>
<th>( \text{Na}^+\text{-K}^+)-ATPase, U/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5-min Incubation groups</strong></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>6.58 ± 0.53</td>
<td>18.6 ± 1.4</td>
<td>0.74 ± 0.02</td>
</tr>
<tr>
<td>EGF</td>
<td>6.37 ± 0.49</td>
<td>19.7 ± 1.0</td>
<td>0.74 ± 0.01</td>
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<tr>
<td><strong>1-h Incubation groups</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.31 ± 1.7</td>
<td>13.9 ± 1.1</td>
<td>0.84 ± 0.13</td>
</tr>
<tr>
<td>EGF</td>
<td>6.35 ± 1.55</td>
<td>15.6 ± 3.1</td>
<td>0.95 ± 0.30</td>
</tr>
<tr>
<td>EGF + cytochalasin D</td>
<td>7.48 ± 1.15</td>
<td>12.9 ± 1.1</td>
<td>0.50 ± 0.12</td>
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Values are means ± SD; \( n \) = no. of animals. There were no significant differences between any values for all treatment groups.

![Fig. 3](image)

Fig. 3. Na⁺-glucose cotransporter (SGLT1) expression in EGF and control brush-border membrane vesicle (BBMV) preparations exposed to EGF or saline for 5 min. Top: representative immunoblots of BBMV protein separated on 8% SDS gel, depicting single immunoreactive band corresponding to SGLT1 from EGF and vehicle control membrane preparations. Bottom: bar graph of integrated optical density expressed as means ± SE of BBMV harvested from EGF- and vehicle control-treated loops (\( n = 5 \)). Integrated optical density was significantly increased in BBMV harvested from EGF-treated loops compared with controls. * \( P < 0.01 \) compared with respective paired control BBMV preparations.
observed elevation in glucose $V_{\text{max}}$ after EGF treatment is due to the insertion of additional membrane containing transport proteins into the brush-border membrane. This effect appears to represent a general increase in membrane nutrient transport, inasmuch as previous studies have demonstrated a similar increase in brush-border proline uptake after EGF treatment (14). The inhibition of EGF-induced effects by cytochalasin D may reflect either an inhibition of actin polymerization. Concurrent treatment with cytochalasin D administration blocked increase in SGLT1 expression. *$P < 0.05$ compared with respective paired control BBMV preparations.

In summary, the findings in the current study suggest that luminal EGF acutely increases the expression of brush-border SGLT1 by a mechanism dependent on actin polymerization. Concurrent treatment with cytochalasin D, a specific inhibitor of actin polymerization, abolishes EGF-induced increases in apical surface area, glucose transport, and brush-border SGLT1 expression.
EFFECT OF EGF ON ENTEROCYTE SGLT1 EXPRESSION

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