Epidermal Growth Factor Receptor of the Intestinal Enterocyte

LOCALIZATION TO LATEROBASAL BUT NOT BRUSH BORDER MEMBRANE*

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Interaction of epidermal growth factor (EGF) with its specific receptor (EGFR) was explored in the intact rat small intestine and in highly purified isolated enterocyte membrane preparations. Despite the fact that the EGF ligand is known to be present at physiological concentration at what is the intestinal lumen, no significant binding of the ligand to the brush border surface was observed. Instead, binding of EGF to the EGFR was confined to other membrane populations, and correlation of ligand interaction with the laterobasal membranes (LBM) was nearly perfect (p < 0.001) across a special equilibrium gradient enriched in brush border and LBM but devoid of intracellular membranes. Specific binding to another minor population of intracellular membranes that migrated to a position less dense than typical endoplasmic reticulum-Golgi vesicles on equilibrium gradients was also observed. Immunocytochemical exposure of intestine to EGF antibody confirmed the localization of the EGFR to LBM and intracellular membranes. As estimated from the intensity of the staining, there may be immunologically active but nonbinding receptor species in the intracellular membrane compartment. Thus, despite the secretion of EGF into the intestinal lumen, the growth and maturational effects of EGF probably result from a specific interaction between EGF and EGFR solely at the laterobasal surface of the enterocyte. The functional role of the intracellular membrane species of EGFR, which remains to be established, may involve a source of inactive receptor that can be readily recruited and transferred to the LBM surface under changing environmental conditions.

Epidermal growth factor (EGF),1 a 6-kDa peptide, binds to a single-chain transmembrane glycoprotein receptor and induces growth and differentiation of epithelial and mesenchymal cells. Named originally for its stimulatory effects on proliferation and keratinization of epidermis (Carpenter and Cohen, 1979), EGF has since been shown to have actions on many other cell types (Scheving et al., 1980; Yeh et al., 1981), yet its biological role in the small intestine has only recently been the subject of active investigation (Chabot et al., 1983; Dembinski et al., 1982; Scheving et al., 1980).

Although specific cell surface EGF receptors have been identified by radioligand binding studies on isolated cells from intestinal crypts and villi (Forgue-Lafitte et al., 1980; Gallo-Payet and Hugon, 1985), the subcellular location of intestinal EGFR in situ is uncertain. Enterocytes of the small intestine are morphologically and functionally polarized columnar cells possessing distinct intracellular (LBM) and brush border (BB) surface membranes. Each surface membrane has access to EGF, the LBM surface being continuously exposed to EGF circulating in the blood (Carpenter and Cohen, 1979) and the BB being directly in contact with EGF secreted by Brunner’s duodenal glands, crypt Paneth cells, and goblet cells (Heitz et al., 1978; Poulsen et al., 1986). Because isolated villus cells bind EGF, it seemed likely that they would preferentially display EGF receptors on their brush border surface. Indeed, receptors for EGF were recently found in partially purified BB, LBM, and microsomal intestinal membrane preparations (Gallo-Payet and Hugon, 1985). However, the effects of intraintestinally infused exogenous EGF on intestinal growth and function are controversial (Dembinski et al., 1982; Fitzpatrick et al., 1987; Goodlad et al., 1987; Skov et al., 1984; Ushen et al., 1986).

By use of biochemical and immunocytochemical approaches, we have localized the EGFR receptor in the rat small intestine. Although the EGFR receptor was present on villus cells, no significant binding of EGF to purified BB membranes of the polarized enterocyte could be detected. Specific receptor interaction with radiolabeled EGF was confined to the LBM and to a special population of intracellular membranes.

EXPERIMENTAL PROCEDURES

Enzyme and Protein Assays—Enzymes established to be highly enriched in particular enterocyte membrane fractions were used as markers for these fractions. Mannosidase II (for Golgi), potassium-stimulated phosphatase (for LBM), and sucrase (for BB) were assayed as previously described (Ahnen et al., 1982; Cezard et al., 1979; Nguyen et al., 1987; Tulsiani et al., 1982).

Membrane Fractionation—Male Sprague-Dawley rats (250–300 g) maintained on Purina Rat Chow and exposed to alternate 12-h periods of a light and dark environment were provided with food and water ad libitum. Three h before the onset of the dark period, prior to the usual circadian feeding phase, animals were anesthetized with either diethyl ether or CO_2 for 2 min and then killed by decapitation. In some experiments, rats were injected intraperitoneally with 2 μCi of ^125I-EGF (150–170 Ci/mmol, Du Pont-New England Nuclear) alone or in combination with a 1000 μM excess of unlabeled EGF (Bethesda Research Laboratories) at 2 or 10 min before killing. Segments of jejenum and proximal ileum (about 20 cm in length) were quickly excised, rinsed once with cold 1.4% NaCl, and placed on ice. All subsequent steps in membrane purification took place at 4°C. Intestinal segments were rinsed via a Pasteur pipette with 1.4% NaCl.

Segments of intestine were excised over a 15-cm blunt-ended glass
rod, and the enterocytes were either scraped free from the mucosal surfaces using a glass slide or dispersed and harvested as described by Nguyen et al. (1987). In initial experiments, the mucosa from 60-cm jejunal segments was scraped directly into a Waring Blender containing 80 ml of binding buffer (20 mM HEPES, pH 7.4) and homogenized for 10 sec in a 15-mm, 15-ml polycarbonate tube with a 2-mm, 8-leaf tissue stirrer at medium speed, and the homogenate was then centrifuged at 38,000 × g for 20 min to remove cytosol. Phase-contrast microscopic examination revealed the absence of intact cells and the presence of membrane fragments, including some intact nuclei and brush borders. The pellet from initial centrifugation was resuspended in 6 ml of binding buffer and divided into two equal 3-ml aliquots for incubation with 5 × 10^-11 M 125I-EGF alone or with a 1000-fold excess of unlabeled EGF (for nonspecific binding). The membranes were then layered directly onto a 25-60% sorbitol gradient containing binding buffer and centrifuged in an SW 27 rotor at 85,000 × g for 16 h. Fractions (2 ml each) were collected with a Beckman Auto Densi Flow II C instrument and the radioactivity determined. Assays for enzymes known to be enriched in various membrane fractions were performed on all fractions (cf. enzyme assays above).

In other experiments, comprehensive separation of enterocyte membrane populations was carried out as previously detailed in this laboratory by Ahnen et al. (1982) and Nguyen et al. (1987). Appreciable enrichment of BB (15-fold), LBM (15-fold), and of a combined endoplasmic reticulum (ER)-Golgi (10-fold) was achieved. Highly purified LBM, free of contamination with ER and Golgi membranes, was available from authentic sections. Membrane fractions were examined for their capacity to interact specifically with EGF.

**EGF Binding Studies**—For binding studies, up to 200 μg of membrane protein from the various subcellular fractions were incubated with 125I-EGF (final concentration 5.67 × 10^-10 M) in 0.5 ml of 20 mM Hepes buffer, pH 7.5, containing 0.1% bovine serum albumin and 50 mM MgCl₂ in a 12.5-mm polypropylene tube for 1 h at 22°C. This buffer had been centrifuged at 38,000 × g for 20 min and prefiltered through a Nalgene 0.20-μm membrane prior to use. After the incubation, the bound EGF was separated from the unbound by centrifugation at 150,000 × g for 15 min. Binding of EGF to the lumen was less than 1% of the initial amount added. Nonspecific binding of 125I-EGF, measured in the presence of 5 × 10^-5 M unlabeled EGF, was 1% of the total binding. Specific binding was calculated by subtracting nonspecific binding from total binding. Kinetics of the EGF binding to membranes was derived by plotting the data by the method of Scatchard (1949).

**Immunocytochemistry**—Segments of duodenum, jejunum, and ileum were rapidly removed and freeze-substituted as described (Alquati, 1972; Michael et al., 1984). Briefly, tissues were quick-frozen in chloroform/lithium fluoride (Freon 22) at -70°C, transferred to a solution of chloroform/methanol (1:1, v/v) at -80°C, and mantained in this solution for 2 weeks. After warming to 4°C, the freeze-substituted tissues were washed in cold chloroform before infiltration with paraffin and embedding into blocks. The tissue was then cut into 5-μm sections and mounted onto glass slides for immunoperoxidase staining or immunogold staining.

Paraffin-embedded tissue sections were processed for immunostaining for the EGF receptor by modification of the immunogold-silver staining method of Holgate et al. (1983) and Danscher and Norgaard (1983). Sections were de-waxed in xylene, rehydrated in graded ethanol series (100% to 70%), and soaked in Tris-buffered saline (0.15 M Tris, 0.15 M NaCl, pH 7.6). Lugol's iodine solution (0.05% w/v) iodine crystals in absolute ethanol) was applied for 5 min, and removed by thorough rinsing in deionized water, followed by treatment in 2.5% (w/v) sodium thiosulfate for 3 min. After a brief rinse in water, slides were dehydrated for 5 min in 0.5% Tris, 0.1 M NH₄Cl, pH 7.6. Five antibodies were then used. The primary antibody, mouse monoclonal 29.1.1 IgG (from ICN ImmunoBiologics), was diluted 1:5000 in Tris-buffered saline and applied for 18 h at room temperature. This well characterized monoclonal, originally raised against the human EGF receptor in A431 epidermoid carcinoma cells, binds with high affinity to a cytoplasmic portion of the receptor distinct from the EGF binding domain on both human and rodent cells. A well characterized rabbit polyclonal antibody against mouse epidermal growth factor receptor (generously provided by Eileen D. Adamson, Cancer Research Center, La Jolla, CA) was used at a concentration of 0.05 μg/ml and applied to different sections on the same dilution for the same length of time. This antibody has comparable affinity for both rat and mouse EGF receptor (Weller et al., 1987). Parallel experiments were conducted using a rabbit polyclonal monospecific antisemum to brush border rat sucrase and amino-oligopeptidase (both previously characterized in our laboratory) (Ahnen et al., 1982; Cezard et al., 1979) and to submandibular gland mouse epithelial growth factor (from ICN ImmunoBiologics).

Sections were washed for 30 min in Tris-buffered saline (50 mM Tris, pH 7.6) to remove cytosol. Phase-contrast microscopic examination revealed the absence of intact cells and the presence of membrane fragments, including some intact nuclei and brush borders. The pellet from initial centrifugation was resuspended in 6 ml of binding buffer and divided into two equal 3-ml aliquots for incubation with 5 × 10^-11 M 125I-EGF alone or with a 1000-fold excess of unlabeled EGF (for nonspecific binding). The membranes were then layered directly onto a 25-60% sorbitol gradient containing binding buffer and centrifuged in an SW 27 rotor at 85,000 × g for 16 h. Fractions (2 ml each) were collected with a Beckman Auto Densi Flow II C instrument and the radioactivity determined. Assays for enzymes known to be enriched in various membrane fractions were performed on all fractions (cf. enzyme assays above).
associated by buffered EDTA (see under "Experimental Procedures"), the specific ¹²⁵I binding increased to ~15%. These results suggested that the vast majority of active EGF cell surface receptors resides on the LBMs of the enterocyte and prompted us to define more precisely the EGFR localization with highly purified membrane preparations.

Properties of EGF Binding—Preliminary experiments with ¹²⁵I-EGF and purified intestinal LBMs revealed that maximal specific binding occurred at 22 °C after 60 min of incubation and remained stable for at least 90 min (data not shown), and binding experiments were carried out at 22 °C for 60 min in the subsequent studies detailed below. Specific binding of EGF was a linear function of the amount of membrane protein added from 30 to 300 µg/ml of assay, and nonspecific binding was less than 1% of the total binding. Specific binding was consistently 2–3 times higher when conducted with membranes in 20 mM Hepes buffer, pH 7.5, compared with either a 200 mM sodium-potassium buffer or Ringer's solution at the same pH. The addition of 5 mM MgCl₂ or CaCl₂ enhanced EGF binding in membranes prepared with EDTA buffers and therefore was included in binding studies with membranes that had been exposed to the chelator. The K₀ was determined by the method of Scatchard to be of high affinity, ~10⁻¹⁰ M ¹²⁵I-EGF (data not shown).

Localization of the EGF Receptor in Rapidly Prepared Enteroctye Membrane Fractions—Because of the possibility that EGF receptors might migrate in the plane of the surface membrane between the BB and LBMs during incubation of everted sacs or dispersed enterocytes, we carried out rapid mucosal scraping (60 cm of intestine over 3 min) at 4 °C, cell lysis in 20 mM Hepes in a Waring Blender at maximal setting for 45 s, and centrifugation (50,000 × g, 30 min) followed by resuspension of the crude membranes in binding buffer with 10⁻¹¹ M ¹²⁵I-EGF alone or with 10⁻⁶ M unlabeled EGF to allow estimation of nonspecific binding. The membrane populations were then separated by equilibrium centrifugation in a 25–60% sorbitol gradient (see under "Experimental Procedures") and the collected gradient fractions analyzed for radioactivity and for the appropriate membrane marker enzymes (see under "Experimental Procedures").

As shown in Fig. 1, the specific binding of EGF was maximal in the fractions enriched in ER-Golgi and LBMs, but there was also a broad distribution extending into the lower density region (fractions 5–10, Fig. 1). Notably, no peak was seen in the region of the brush border membranes. Also, when ¹²⁵I-EGF was bound to dispersed enterocytes at 4 °C prior to homogenization (data not shown) or when ¹²⁵I-EGF was administered intraperitoneally 2 or 10 min before killing (Fig. 2, A and B), a similar distribution of the EGF receptor was seen after equilibrium centrifugation, but a greater percentage of radioactivity was associated with the low density vesicles (fractions 5–8).

Localization of the EGF Receptor in Highly Purified Membrane Fractions—Because EGF must interact initially with either the BB or LBMs of the enterocyte, we prepared highly purified membranes by a combination of differential and equilibrium centrifugation to define the localization of EGF binding to extracellular surfaces and intracellular membranes. Our conventional separation technique (Ahnen et al., 1982; Cezard et al., 1979) yields a gradient (P3) containing ER-Golgi, intracellular membranes, LBMs, and some BB. Treatment with 8 mM CaCl₂ yields a supernatant highly enriched in laterobasal and brush border membranes, but substantially free of ER-Golgi, that can then be separated by equilibrium centrifugation (Nguyen et al., 1987). We now show that this technique not only removes ER-Golgi but other low density vesicle populations that contain EGF receptors. Of particular importance is that the results of these experiments with purified membranes were consistent with the findings using crude membrane vesicles, even though the binding reaction was performed after the separation of the individual fractions on the equilibrium gradient, rather than prior to cell disruption or membrane separation.

In these studies, the starting material was enterocytes dispersed from the everted gut sac. When the P3 pellet was placed on top of the gradient and centrifuged to equilibrium, the ER-Golgi (marked by mannosidase II) and the LBM (by potassium-stimulated phosphatase) could only be partially resolved from one another. As illustrated in Fig. 3, the distributions of the ER-Golgi membrane vesicles and LBMs overlapped. Whereas the ER-Golgi activity peaked in fraction 11, and the LBM in fractions 12 and 14, the pattern of EGF binding activity was more complex. EGF binding activity was definitely identified between fractions 4 and 7 and then peaked in fractions 8, 10, and 12. The EGF-EGFR binding correlated with both ER-Golgi and LBMs, even though the binding reaction was performed after the separation of the individual fractions on the equilibrium gradient, rather than prior to cell disruption or membrane separation. When the P3 (ER-Golgi/LBM) pellet was placed on top of the gradient and centrifuged to equilibrium, the two predominant membrane populations were LBMs.
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Fig. 2. Distribution of \(^{125}\text{I}-\text{EGF}\) binding after intraperitoneal administration of the ligand. Pellets were prepared from intestinal scrapings from rats that had been injected intraperitoneally with \(^{125}\text{I}-\text{EGF}\) (2 \(\mu\text{Ci}\)) at 2 (A) or 10 (B) min prior to killing, as described under "Experimental Procedures." The crude membrane pellet was taken up in 3 ml of binding buffer and centrifuged as described in the legend to Fig. 1. 2-ml fractions were collected and assayed for the associated radioactivity and marker enzymes. The radioactivity (open squares) was primarily associated with the LBM marker enzyme (closed circles) and lower density vesicles. The peak in fraction 1 represents unbound radiolabeled ligand in the residual cytosol.

Fig. 3. EGF binding to purified intracellular and cell surface membranes. The P3 pellet obtained as described under "Experimental Procedures" was loaded onto the sorbitol gradients and centrifuged to equilibrium. Specific EGF binding (open squares) and the marker enzymes were measured in each collected fraction after removing the sorbitol. LBM was identified by K\(^+-\)stimulated phosphatase (closed circles). These patterns represent typical results obtained from a single experiment. Other details are given in the legend to Fig. 1.

Fig. 4. EGF binding activity to LBM-enriched fractions from dispersed enterocytes. The PB pellet devoid of ER-Golgi membranes (see under "Experimental Procedures") was loaded on top of a 25–60% sorbitol gradient and centrifuged to equilibrium. Other details are given in the legend to Fig. 3. Note the close correlation of EGF binding (open squares) with the LBM (closed circles).

In other experiments, crypt and villus tip cells were sequentially released by EDTA treatment, harvested, and examined for the ability to bind to \(^{125}\text{I}-\text{EGF}\) at the 55,000 \(\times\) g membrane pellet. Crypt specific binding was 8.3%/mg of protein compared to 4.0%/mg of protein for villus tip cells. When radio-labeled EGF was injected intraperitoneally 2 or 10 min before killing the rat, a crypt-villus gradient in the distribution of radiolabeled EGF persisted. Most of the binding appeared to correspond with the LBM, when membranes from the crypt pellet were separated on an equilibrium gradient. Finally, a similar preponderance of EGF receptors in the jejunal LBM was seen in Wistar rats, Biobreed Wistar Diabetic (BBW) rats, rats fasted 72 h, and those killed at different circadian phases (data not shown).

Immunohistochemical Localization of the EGF Receptor—The studies of radiolabeled EGF binding strongly suggested that the majority of EGF receptors in the enterocyte is localized to the laterobasal surface membranes. However, brush borders may harbor EGF receptors whose binding sites were occupied by endogenous EGF from the lumen and hence unavailable for radioligand binding. To examine this possi-
bility, we localized the receptor in freeze-substituted tissue using well characterized monoclonal and polyclonal receptor antibodies that recognize the rodent EGF receptor. For comparison, we used rabbit polyclonal antibodies to localize jejunal sucrase, aminooligopeptidase, and epidermal growth factor.

Fig. 5C shows the distribution of sucrase along the villus axis. As expected, this hydrolase localized primarily to the brush border of the mature enterocyte. The major immunoreactivity appeared above the crypt-villus junction. The lamina propria, blood vessels, and nuclei were negative. Similar results were obtained for the hydrolase aminooligopeptidase (not shown). Fig. 5, A and B, shows the distribution of the EGF receptor along the villus axis. In contrast to sucrase, immunoreactivity determined using the monoclonal antibody (Fig. 5A) was comparable in both crypt and villus cells with the greatest grain density being localized intracellularly in both an infra- and supranuclear position. Brush border staining was negligible. Studies with a rabbit polyclonal antibody against the EGF receptor revealed a similar staining pattern; however, the laterobasal staining was more intense (Fig. 5B), and there was also significant grain distribution over endothelial cells lining capillaries and lymphatics in the lamina propria as well as smooth muscle of the muscularis externa. Few grains were observed in the brush border. Although both the monoclonal and polyclonal antibodies specifically recognized the EGF receptor in A431 cells as determined by immunoblot analysis (data not shown), only the polyclonal antibody reacted strongly with the positive control section of liver (not shown). This suggests that the epitope to which the monoclonal antibody reacts may not be present in the liver.

The localization of EGF, the ligand, was confined in the intestinal lumen as well as a laterobasal surface exposed to blood and subepithelial cells such as lymphocytes and plasma cells. Since EGF is secreted directly into the intestinal lumen from several different cellular sites of origin, including Brunner’s glands, Paneth cells, and goblet cells, we expected the majority of EGF surface receptors to be localized to the luminal brush border membranes. Indeed, in a single previous study utilizing partially purified enterocyte brush border preparations, the majority of receptors appeared to be localized to the brush border rather than to LBM or microsomal membranes (Gallo-Payet and Hugon, 1985), a finding contrasting with the laterobasal distribution of enteric receptors for vasoactive intestinal peptide (Dharmasathaphorn et al., 1983) and insulin (Gingerich et al., 1987). Even more recently, specific microvillus receptors for EGF have been reported in fetal and adult but not newborn rats (Thompson, 1988). These receptors could be detected at 20 °C but not at 4 °C, and binding was relatively unaffected by pH. However, the membrane preparations used in both studies were not monitored for LBM or microsomal contamination.

By use of highly purified membranes free of significant cross-contamination, we now report the virtual absence of brush border receptors for EGF, based on both binding to purified enterocyte membranes and immunohistochemical studies. Instead, specific enterocyte EGF receptors were identified on the LBM and in intracellular membranes having a slightly lower density than typical ER-Golgi on equilibrium gradients (Figs. 1–3). There was nearly a perfect correlation of the LBM membrane marker with these receptors (Fig. 4). These studies, while not totally excluding the possibility of a minute population of brush border receptors, suggest that the cell surface receptor is restricted or at the very least preferentially localized to the LBM. This finding may account for the inconsistent and controversial effects of intragastrically or luminally infused exogenous EGF on intestinal growth and function (Dembinski et al., 1982; Fitzpatrick et al., 1987; Goodlad et al., 1987; Ulshen et al., 1986) and also for why luminal EGF, although absorbed across the intestine in the neonatal rat, does not seem to be absorbed in the adult rat (Olsen et al., 1984; Skov et al., 1984).

Other well differentiated highly polarized cells such as those from eccrine sweat duct epithelium cells (Nanney et al., 1986), epidermal cells (Nanney et al., 1986), hepatocytes (Burwen et al., 1984; Dunn et al., 1986), and the Madin-Darby canine kidney cell line (Maratos-Flier et al., 1987) possess EGF receptors. Because all of these mature highly specialized cells rarely undergo mitosis, the presence of EGF receptors on surface membranes implies that the ligand may exert a regulatory role beyond its well accepted effect on cell replication and maturation (Scheving et al., 1987). Several polarized cell types display their surface EGF receptor either on LBM or apical membranes. Thus, in the placenta, the majority of EGF receptors present in syncytiotrophoblasts is associated with the brush border rather than the LBM (Venkateswara-Rao et al., 1986).
In contrast, hepatocytes and Madin-Darby canine kidney cells possess the EGF receptor principally on the lateral-basal membrane, a site from which some of the internalized ligand-receptor complexes can cross the cytoplasm to be subsequently discharged into the apical lumen (Burwen et al., 1984; Maratos-Flier et al., 1987). These findings are analogous to the intestinal enterocyte since a fraction of systemically administered EGF crosses the gastrointestinal epithelium and appears to be excrated into the lumen (Olsen et al., 1984). The possible mechanism of EGF movement from the intestinal lumen to the LBM receptor site of the enterocyte is considered further below.

Intracellular Membrane Location of EGF Receptor (Active and Cryptic)—The enterocytic intracellular low density vesicles that harbor the EGF receptor have not been characterized in our studies, but studies in other organ systems may provide insights. By use of the rat isolated perfused liver, Dunn et al. (1986) have also found two major peaks of high affinity EGF binding activity in purified membranes separated in equilibrium gradients; one peak was associated with the plasma membrane, and the other constituted a population of low density vesicles observed only after exogenous EGF was introduced into the system. This suggested that they may represent endosomes. Since the low density intestinal binding sites detected in the present study appeared within minutes after 125I-EGF (Fig. 2, A and B) was injected into the peritoneal cavity, they must represent either endosomes or a specialized surface population; yet other cell types containing immunoreactive EGF receptor, such as the endothelial cells within the lamina propria, do not substantially contaminate enterocyte preparations (Weiser et al., 1986), and enterocyte crypt cell plasma membranes migrate to an equilibrium density similar to enterocyte LBM. Moreover, because endogenous EGF is concentrated in jejunal sites (Paneth (Fig. 5D) and goblet cells), the spontaneous release of this ligand in vivo or during membrane preparation could induce the formation of similar receptor-containing endosomes. Indeed, when the circulating and tissue levels of endogenous EGF are barely detectable, such as in rats fasted for 50% (Luminal to LBM Transport)

Although the 125I-EGF binding experiments (Figs. 1–4) identified the majority of the enterocyte’s active receptor on the LBM, the immunohistochemical analysis revealed a prominent specific intracellular receptor pool that appears to possess few active EGF binding sites. Because the antibodies used identify epitopes distinct from the receptor’s ligand binding site, the majority of intracellular receptor species may be devoid of ligand binding sites. The predominant intracellular histochemical localization, while not quantitative, suggests that there may be even more receptors inside the enterocyte than on its surface (Fig. 5, A and B). A similar discrepancy between the pools of active and immunoreactive receptor has been recently reported in the A431 epidermoid carcinoma cell line where as many as 80–85% of EGF receptors were associated with several different intracellular organelles and less than 15% of the total immunologically reactive EGF receptors were capable of binding the EGF ligand (Carpentier et al., 1986). Cryptic receptors have also been observed in OCl5 embryonal carcinoma cells (Weller et al., 1987).

Possible Modes of Luminal to LBM Transport of EGF—Because EGF is secreted from Brunner’s duodenal glands and from crypt Paneth cells into the intestinal lumen, it seemed logical that the EGFR would be localized at the luminal surface where EGF binding could regulate replication and enterocyte function. In light of our results, the physiological significance of EGF within the intestinal lumen must be reexamined. Luminal infused exogenous EGF has been demonstrated in some but not in all cases to modulate intestinal growth and function. In one instance, intraintestinal infused EGF stimulated ornithine decarboxylase activity and DNA synthesis not only in the surgically isolated loop of ileum receiving infusate but also in jejunum that was denied access to the ligand from the luminal surface (Ulshen et al., 1986). This suggests that the effects of intraluminal EGF may be neuroendocrine in nature or involve its transport from the lumen across the enterocyte where it could have access to both the LBM receptor sites and to the extracellular compartment. Possibly, receptors are recruited or transported to the luminal surface under special physiological conditions, such as in the intestinal adaptation subsequent to surgical resection. Such an adaptive mechanism could involve other cell types (Paneth, tuft, neuroendocrine, or membranous epithelial (M) cells) which, being much less numerous than enterocytes, are difficult to analyze in the intact intestine and cannot be readily isolated.

Whatever the exact mechanism whereby luminal EGF interacts with the intestine, our experiments do bring into focus an alternative hypothesis: intraluminal EGF may gain access to its receptor at the LBM surface by virtue of local movement. Under normal conditions, the tight junctions effectively seal the lateral surfaces of adjacent enterocytes, restricting the movement of molecules and particles between luminal and perilateral spaces. However, there is evidence that the luminal-laterobasal boundary is not inviolable and that luminal contents may gain access to the LBM. Recent electron microscopic studies of normal intestine indicate that under special circumstances small gaps can arise in either crypt or villus cells, thereby exposing luminal contents to lateral surface membranes. In crypt cells, these gaps arise because tight junctions disassemble and re-form during cell division (Marchal and Madara, 1987; Tice et al., 1979). Acetylcholine triggering of crypt goblet cell secretion produces a similar breakdown in goblet cell tight junctions, creating luminal-pericellular communications (Marchal and Madara, 1987). Since most of the intestinal EGF is normally secreted by Brunner’s glands into ducts that empty into the duodenal crypts or by Paneth and goblet cells, these intercellular gaps may provide a conduit for luminal EGF to reach its LBM receptor. In villus cells, gaps arise when enterocytes at the villus tips are shed into the intestinal lumen. Pathological conditions causing erosions or ulcerations and a loss of integrity of the tightly connected blanket of enterocytes may assure the interaction of lateral membranes of crypt or villus cells with luminal molecules (Olsen et al., 1984; Skov et al., 1984). Thus, the seemingly paradoxical distribution of receptor and ligand in vivo may reflect the existence of a regulatory barrier between luminal EGF and its LBM surface receptor. Definition of the mechanisms whereby this barrier is modulated may be crucial for our understanding of the maintenance of physiological and pathological enterocyte function.

REFERENCES

2 L. A. Scheving and G. M. Gray, unpublished observations.
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