Apical and basolateral EGF receptors regulate gastric mucosal paracellular permeability

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Chen, Monica C., Jeffrey Goliger, Nigel Bunnett, and Andrew H. Soll. Apical and basolateral EGF receptors regulate gastric mucosal paracellular permeability. Am J Physiol Gastrointest Liver Physiol 280: G264–G272, 2001.—Previous studies found that monolayers formed from canine oxyntic epithelial cells in primary culture displayed remarkable resistance to apical acidification and both mitogenic and migratory responses to epidermal growth factor (EGF) treatment. In our present studies, we found that EGF increased transepithelial resistance (TER) but not short-circuit current in these monolayers. Parallel effects of EGF on decreasing mannitol flux and increasing TER implicate direct regulation of paracellular permeability. EGF acting at either apical and basolateral receptors rapidly increased TER, but the apical response was sustained whereas the basolateral response was transient. 125I-labeled EGF binding revealed specific apical binding, but receptor numbers were 25-fold lower than on the basolateral surface. Both apical and basolateral EGF activated tyrosine phosphorylation of EGF receptors (EGFR), β-catenin, and cellular substrate as evident on confocal microscopy. Although apical EGF activated a lesser degree of receptor autophosphorylation than basolateral EGF, phosphorylation of β-catenin was equally prominent with apical and basolateral receptor activation. Together, these findings indicate that functional apical and basolateral EGFR exist on primary canine gastric epithelial cells and that these receptors regulate paracellular permeability. The sustained effect of apical EGFR activation and prominent phosphorylation of β-catenin suggest that apical EGFR may play a key role in this regulation.

Apical and basolateral EGF receptors regulate gastric mucosal paracellular permeability. The sustained effect of apical EGF activation and functional consequences of apical EGFR remain unclear. Model systems are limited for in vivo studies of EGF family. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
investigate the presence and functions of apical EGFR. In
previous studies (4), we found these monolayers to dis-
play marked resistance to apical but not basolateral acid-
ification, thus mimicking an important property of mu-
cosal defense in vivo (27). We (4) further showed that
apical acidification increased transepithelial resistance
(TER) and decreased mannitol flux, indicating that the
paracellular pathway was a critical point of resistance to
apical acid. We now show that functional EGFR are
located on the apical, as well as basolateral, surfaces of
primary gastric mucosal cells and that both apical and
basolateral EGFR mediate ligand-induced decreases in
paracellular permeability and tyrosine phosphorylation
of apical junction proteins.

Fig. 1. Epidermal growth factor (EGF)-mediated regulation of paracellular permeability. Canine oxyntic cells were
cultured on Transwell filters until confluent [transepithelial resistance (TER) > 1,000 Ω·cm²] and then switched to
serum-free medium for an additional 6 h as described in METHODS. A: EGF (5 nM) was added to either the apical
(AP) or basolateral (BL) surface at t = 0. Control monolayers were treated with vehicle only. TER was measured
with an EVOM at indicated times after addition of EGF. Data represent means ± SE from 5 experiments using
separate cell preparations. B: mannitol flux was measured in parallel monolayers pretreated for 30 min (EGF
added at t = 0) with 20 nM EGF on the apical or basolateral surface; control monolayers were pretreated with
vehicle only. The initial TER response to EGF or vehicle was recorded and 3Hmannitol was added to the apical
solution at t = 0, and aliquots of basolateral solution were assayed for radioactivity at indicated times. Mannitol
flux is expressed as counts·minute (cpm)−1·aliquot−1. Data are means of 3 replicate wells and are representative
of experiments using 3 separate cell preparations. First-order linear regressions were performed with Sigma Plot
software to calculate average rates of flux (all values for r² > 0.98). C: mannitol flux was measured as described in
B with parallel monolayers pretreated for 120 min with 20 nM EGF added at t = 0 on the apical or basolateral
surface; control monolayers were pretreated with vehicle only. The initial TER response to EGF or vehicle was
recorded and 3Hmannitol was added to the apical solution at t = 2. D: average initial flux rates (over the first 2.5 h
after adding mannitol) for the individual monolayers depicted in B were plotted against the reciprocal of their TER
measured at t = 0 (30 min after the addition of EGF or vehicle). The linear relationship (r² = 0.85) indicates that
EGF-dependent changes in TER are predominantly due to changes in paracellular permeability.
METHODS

Materials. Materials for cell culture were obtained from sources outlined previously (6). Transwell inserts were from Costar (Cambridge, MA). 125I-labeled EGF and 125I-labeled TGF-α were purchased from Biomedical Technology (Stoughton, MA), and human recombinant EGF was from R&D Systems (Minneapolis, MN). Anti-EGFR antibody MAb528 (Ab-1) was purchased from Oncogen Science (Cambridge, MA). An epithelial voltohmmeter (EVM) Millicell-ERS was from Millipore (Bedford, MA). Affinity-purified rabbit anti-ZO-1 was from Zymed Laboratories (San Francisco, CA). Anti-phosphotyrosine (monoclonal IgG2a) and agarose-conjugated anti-phosphotyrosine were from Upstate Biotechnology (Lake Placid, NY). A monoclonal antibody phosphotyrosine-PY 20 used in the Western blot and anti-β-catenin (mouse IgG1) were from Transduction Laboratories (Lexington, KY). Other chemicals were from Sigma Chemical (St. Louis, MO).

Tissue dispersion, cell separation, and culture. Enzyme-dispersed canine oxyntic mucosal cells were separated by elutriation and cultured on collagen-coated Transwell filter inserts (6 or 12 well; Costar), as described previously (4–6). When cells became confluent cultures, they contained predominantly chief cells (25%), as indicated by immunologic detection of pepsinogen and H2- catenin–positive cells, and occasional endocrine cells. Cells were cultured in DMEM-Ham’s F-12 (1:1) plus 20 mM HEPES, 100 μg/ml amikacin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2% calf serum. Cultures were fed with the same medium at the indicated time intervals were sampled to determine the radioactive activity.

125I-EGF binding. Binding studies were performed on monolayers with TER >2,500 Ω·cm² as described previously (5). 125I-EGF was added either apically or basolaterally, as indicated, in the absence or presence of various dilutions of either cold EGF or monoclonal EGFR antibody MAb528. Unless otherwise mentioned, MAb528 at the concentration of 20 nM (1:25 dilution of the stock) was used in the studies. Binding was performed at 37°C for 45 min. At the end of binding, aliquots from the counterlateral side of the 125I-EGF label were taken to determine the amount of label crossing the monolayers. After extensive washing of the monolayers, membranes were cut off from the inserts, and the radioactivity was counted in a gamma counter. The total binding was determined after subtracting the membrane blank (tracer incubated with filter without cells) and expressed as the percentage of maximal binding. 125I-EGF binding studies were also studied at 4°C for 2, 4, and 8 h of incubation.

125I-TGF-α binding. Selective binding studies were repeated with 125I-TGF-α label. The binding conditions were the same as those for 125I-EGF binding studies.

Immunostaining of junctional tyrosine phosphorylation. Monolayers were either treated with MAb528 or with apical EGF before paraformaldehyde (3.7%) fixation. Cells were coincubated with primary antibodies specific for ZO-1 (rabbit polyclonal, 1:1,000; Zymed) and phosphotyrosine (monoclonal, 1:50; Upstate Biotechnology) and then immunofluorescently stained using corresponding FITC- (1:200, Jackson ImmunoResearch Laboratories, West Grove, PA) and tetramethylrhodamine isothiocyanate-conjugated secondary antibodies (1:50, Vector Laboratories, Burlingame, CA). Confocal microscopy (MRC1000, Bio-Rad Laboratories, Hercules, CA) was used to better visualize the apical tyrosine phosphorylation. Photomicrographs were generated by superimposing three adjacent horizontal (Z) optical sections of 0.5- to 1.0-μm thickness centered on the section displaying the most intense signal for the apical junctional marker ZO-1.

Electrophysiological measurement of TER. Monolayer TER was monitored using an EVM with chopstick electrodes. This technique allows repeated measurements of monolayer TER in a sterile condition for a prolonged period of time. Comparative studies using Ussing chambers as described previously (4) were selectively performed to ensure that similar results were obtained with both methods.

Mannitol flux. Apical-to-basolateral mannitol flux was determined by adding [3H]mannitol to the apical medium as described previously (4). Aliquots from the basolateral medium at the indicated time intervals were sampled to determine the radioactive activity.

Electrophysiological measurement of TER. Monolayer TER was monitored using an EVM with chopstick electrodes. This technique allows repeated measurements of monolayer TER in a sterile condition for a prolonged period of time. Comparative studies using Ussing chambers as described previously (4) were selectively performed to ensure that similar results were obtained with both methods.

Fig. 2. Inhibition of EGF receptor (EGFR) activation by the ipsilateral immunomoblocking with monoclonal antibody MAb528. Monolayers were treated apically (A) and basolaterally (B) with either vehicle, 1 nM EGF alone, and 50 nM EGF alone, or with 20 nM (1:25 dilution) MAb528 (added 30 min before EGF). TER was monitored with an EVM at indicated times after EGF treatment. Data are means ± SE of 3 replicate wells and are representative of experiments from 4 separate cell preparations.
Diego, CA), transferred to nitrocellulose membranes (Bio-Rad Laboratories), and probed with primary antibodies to antiphosphotyrosine (PY20, Transduction Laboratories) to detect EGFR or with antibody to β-catenin (Transduction Laboratories). Immunoreactive bands were visualized by enhanced chemiluminescence (Amersham International) with horseradish peroxidase-conjugated secondary antibodies (Bio-Rad Laboratories).

**Data analysis.** Kinetic binding data were analyzed using GraphPad Prism nonlinear regression software programs (San Diego, CA). Results are expressed as means ± SE. Statistical significance of differences between mean values was assessed using the Student’s paired t-test with \( P < 0.05 \) (Statistix, NH Analytical, Roseville, MN).

**RESULTS**

**EGF-mediated regulation of TER and paracellular permeability.** Canine oxyntic cells were cultured on Transwell filters until confluent. When the TER was >1,000 \( \Omega \cdot \text{cm}^2 \), monolayers were switched to serum-free medium for at least 6 h before growth factor studies were performed. Monolayers were exposed to 5 nM EGF at either the apical or basolateral surface. Both treatments resulted in detectable increases in TER within 2 min after adding EGF (Fig. 1A) and a maximal 150–200% increase in TER within 30 min. Basolateral addition consistently evoked greater increases in TER than apical addition. However, the response to basolateral EGF was transient; TER returned to baseline within 2–3 h with basolateral treatment, whereas the effects of apical EGF were relatively stable over an experimental period of several hours.

Mannitol flux experiments were performed on a parallel set of cell cultures to further characterize EGF-dependent changes in paracellular permeability. Apical EGF (20 nM) decreased \([\text{H}]\)mannitol flux across monolayers by ~50% compared with untreated controls, with this effect sustained for a 7-h period (Fig. 1B). Basolateral EGF produced a similar, statistically significant decrease in mannitol flux over the first 3 h after treatment (Fig. 1B). However, between 3 and 7 h after basolateral EGF treatment, mannitol flux increased and was not significantly different from control \( (P > 0.5; n = 4) \) (Fig. 1B).

![Fig. 3. Specific binding of \(^{125}\text{I}-\text{EGF}\) and inhibition of EGF binding by immunoblocking with MAb528. Binding studies were performed as described in METHODS. \(^{125}\text{I}-\text{EGF}\) was added either basolaterally (A) or apically (B) in the presence of ipsilateral (●, ○) or contralateral (□, ○) MAb528. Binding was performed at 37°C for 45 min with various dilutions of antibody. Data represent means ± SE from 3 experiments using separate cell preparations. Relative binding is reported as % maximally bound \(^{125}\text{I}-\text{EGF}\). C: \(^{125}\text{I}-\text{EGF}\) binding to apical and basolateral EGFR. \(^{125}\text{I}-\text{EGF}\) was added to either apical or basolateral solution in the presence of increasing concentrations of ipsilateral cold EGF. Data are means ± SE from 4 separate preparations and are expressed as the % maximal binding. Basolateral (D) and apical binding (E) of \(^{125}\text{I}-\text{EGF}\) and \(^{125}\text{I}-\text{Labeled transforming growth factor-α (TGF-α)}\) to monolayers was studied using 20 nM of tracer. Cells were incubated in the absence (0) or presence of unlabeled EGF (10⁻⁷ M, E-7) or TGF-α (10⁻⁷ M, T-7) in the same side of monolayer where labeled ligand was added.\]
We suspected that the return of mannitol flux rates toward control level 3 h after treatment with basolateral EGF reflected the transient nature of EGF-induced increase in TER with basolateral treatment (Fig. 1A). This hypothesis was tested by adding [3H]mannitol 2 h after addition of EGF, a time at which TER with basolaterally treated monolayers had returned to near baseline. Under these conditions, basolaterally treated monolayers displayed control rates of mannitol flux, whereas apically treated monolayers displayed the decreased flux rates (Fig. 1C) similar to those observed when [3H]mannitol was added 30 min after EGF treatment (Fig. 1B).

The relationship between 1/TER and mannitol flux is shown in a scatter graph (Fig. 1D). The inverse linear relationship \( r^2 = 0.85 \) between TER measured 30 min after addition of EGF and mannitol flux rates during the 2 h after [3H]mannitol addition suggests that EGF-dependent changes in TER are predominantly caused by changes in paracellular permeability.

Inhibition of EGF effects by immunoblocking EGFR with monoclonal antibody MAb528. We also investigated the effects of blocking EGF-induced increases in TER by anti-receptor antibodies. In previous studies with canine epithelial monolayers plated in plastic culture dishes, we (18) found that the immunoblocking monoclonal antibody against the human EGFR (MAb528) totally inhibited TGF-\( \alpha \)-stimulated thymidine incorporation and migration but did not reduce the effects of cytokines unrelated to EGF. Figure 2 illustrates that ipsilateral (same side) addition of MAb528 (1:25 dilution) suppressed increases in TER induced by low doses (1 nM) of either apical or basolateral EGF. These immunoblocking effects of MAb528 were surmounted by higher doses (50 nM) of EGF (Fig. 2). MAb528 at a 1:25 dilution appeared somewhat more effective in blocking basolateral than apical receptor-mediated increases in TER. Monoclonal antibody against somatostatin CURE S6 (7, 35) at a similar dose did not alter TER effects of either apical or basolateral EGF treatments. Thus apical and basolateral receptors immunologically related to the human EGFR mediate ligand-dependent increases in TER.

Binding of \(^{125}\text{I}-\text{EGF} \) to apical and basolateral sites. We (5) previously reported that both \(^{125}\text{I}-\text{EGF} \) and \(^{125}\text{I}-\text{TGF-} \alpha \) bound to gastric epithelial monolayers in plastic culture plates. We now find that \(^{125}\text{I}-\text{EGF} \) specifically bound to high-affinity binding sites on both apical and basolateral surfaces of monolayers grown on Transwell inserts. The binding to both surfaces was blocked by ipsilateral, but not contralateral, addition of MAb528 (Fig. 3, A and B). At the end of the 45-min binding experiment, only 0.26 ± 0.04% (mean ± SE; \( n = 4 \)) of \(^{125}\text{I} \) radioactivity was detected in the contralateral bathing medium, indicating that apical and basolateral membrane domains were completely separate and that EGF was not translocated to the contralateral surface under these conditions. We found...
that basolateral EGFR specifically bound ~25-fold more 125I-EGF than apical receptors (10,000 ± 4,300 vs. 390 ± 120 counts·min⁻¹·filter⁻¹; mean ± SE; n = 3). This low proportion of apical to total EGFR is similar to reports (14, 15) for other cell types.

Competition experiments with unlabeled EGF suggested that both basolateral and apical receptors had similar affinity for EGF (dissociation constant = 70 ± 34 and 150 ± 47 pM, respectively; Fig. 3C; mean ± SE; n = 4). Apical EGFR binding displayed a higher proportion of nonspecific binding than basolateral EGFR, a finding reflecting the much lower total apical binding. In addition, MAb528 appeared to be less effective in displacing EGF from apical than basolateral binding sites (compare Fig. 3, A and B); however, proportionate displacement is comparable if nonspecific binding is subtracted.

125I-TGF-α binding was also studied. TGF-α binding to apical and basolateral surfaces reflected the same relative magnitude as 125I-EGF binding (Fig. 3, D and E). With the 125I-TGF-α tracer used, the absolute level of apical binding was somewhat lower.

125I-EGF binding was also studied at 4°C for 2-, 4-, and 8-h incubation periods. Maximal and specific binding was comparable to that reported at 37°C for 45 min (n = 3, data not shown).

Apical EGF induces apical and junctional tyrosine phosphorylation. We next examined protein tyrosine phosphorylation in response to apical EGF treatment (Fig. 4). Cells exposed to apical EGF for 20 min were coabeled with antibodies against phosphotyrosine and antibodies against the tight junction protein ZO-1 to mark the apical/basolateral membrane interface. Immunofluorescent confocal microscopy revealed marked increases in apical and perijunctional tyrosine phosphorylation in response to apical EGF (Fig. 4B) compared with control cells (Fig. 4A). Enhanced tyrosine phosphorylation was detected in most but not all cultured cells, suggesting that within this mixed cell model, not all cell types respond equally to apical EGF treatment. Nevertheless, these results provide further evidence that apical EGFR are functional and stimulate tyrosine kinase activity on ligand binding. In these experiments, control cells (Fig. 4A) were treated with MAb528 to reduce background signals due to endogenous secretion of TGF-α by parietal cells (5). However, we also detected increased tyrosine phosphorylation in EGF-treated cells compared with cells not exposed to MAb528 (data not shown, n = 3). Although we focused our studies on the apical region, as marked by ZO-1, phosphorylation at deeper sites within cells was also evident. We did not detect changes in ZO-1 intensity among control, EGFR-blocked, or apically EGF-treated monolayers.

We further compared the ability of apical and basolateral receptors to mediate EGF-dependent tyrosine phosphorylation of two specific target proteins, the EGFR itself (autophosphorylation) and β-catenin, a component of the adherens junction. Immunoprecipitation and Western blot experiments showed that both apical and basolateral treatment with EGF stimulated phosphorylation of a 180-kDa band as early as 1–2 min after ligand addition (Fig. 5A). However, basolateral treatment resulted in markedly greater EGFR auto-phosphorylation (8-fold increase) compared with apical treatment (2-fold increase; Fig. 5B). This is consistent with the relative amounts of EGFR present on each surface (see Fig. 3). We confirmed that this band in Fig. 5A was EGFR by immunoprecipitating with the MAb528 and probing Western blots for tyrosine phosphorylation (n = 2, data not shown).

In contrast, despite the wide differences in EGFR numbers on apical and basolateral surfaces, β-catenin was comparably tyrosine phosphorylated in response to apical and basolateral addition of EGF (Fig. 6, A and C). Neither basolateral nor apical treatment of EGF altered the total amount of β-catenin (Fig. 6, B and D).

![Fig. 5. EGF-mediated tyrosine phosphorylation of EGFR. All immunoprecipitations were performed on an equal number of cells. Monolayers were transferred to serum-free media for overnight and then apically or basolaterally treated with 20 nM EGF for the indicated time periods. Cells were washed and lysed as described in METHODS. A: anti-phosphotyrosine blot of EGFR at various time points after addition of apical or basolateral EGF (20 nM). Soluble lysates were immunoprecipitated with anti-phosphotyrosine antibodies bound to protein A/G-agarose beads (4G10), and the membrane was blotted with another antiphosphotyrosine antibody PY20. This 180-kDa band was further verified using MAb528 for the western blot. The results shown are representative of 3 independent preparations. B: quantification of apical and basolateral stimulated EGFR tyrosine phosphorylation. The autoradiograms shown in A were analyzed using the NIH Image program, and the intensity of the specific bands was expressed in densitometry units (%basal). Values are means ± SE from 3 separate preparations. *P < 0.05, **P < 0.01 vs. basal.]
DISCUSSION

Our data demonstrate that exogenous EGF at the physiological concentrations added to the apical or basolateral surface of canine gastric monolayers in primary culture causes a rapid increase in TER and a corresponding fall in paracellular permeability, as evidenced by reduced mannitol flux. The tight linear relationship between 1/TER and mannitol flux supports the conclusion that changes in paracellular permeability underlie changes in TER under these conditions and that EGF-induced change in TER reflects direct effects on paracellular permeability.

The response to apical EGF was stable for hours. In contrast, the response to basolateral EGF was transient, returning to baseline after ~2 h. It is unclear why the effects of EGF on TER and mannitol flux are more stable with apical compared with basolateral treatment. It is possible that EGF is more rapidly internalized and degraded at basolateral EGFR or that downregulation of EGFR is more prominent at basolateral receptors. Regardless, the temporal correlation, evident by sustained apical and transient basolateral EGFR effects on both TER and mannitol flux, further supports the conclusion that increases in TER reflect decreased paracellular permeability.

Our data provide several lines of evidence supporting the existence of EGFR on the apical surface of these monolayers that are closely related to classical EGFR. 125I-EGF specifically bound to sites on the apical surface. However, these apical sites were sparse compared with basolateral receptors. Immunoblockade of EGFR by the anti-human EGFR antibody MAb528 displaced binding from both apical and basolateral sites. In addition, MAb528 blocked EGF-induced increases in TER at both apical and basolateral receptors. Anti-EGFR treatment inhibited the effects of low concentrations of EGF on TER, and this inhibition could be surmounted by higher concentrations of EGF. Apparent differences in MAb528 blockade of apical binding reflected the high proportion of nonspecific binding to the lower concentration of apical receptors. Taken together, these findings indicate that apical EGFR are present on these monolayers and that they are closely related to basolateral EGFR. Although mechanisms were not elucidated, studies (19) using polarized kidney epithelial cells with overexpressed apical EGFR demonstrated differences in EGFR downregulation and endocytosis between basolateral and apical EGFR. Studies of internalization would be difficult on our monolayers because of the low concentrations of apical EGFR.

Controls indicated that the apical and basolateral compartments of these culture inserts were functionally separated. Ipsilateral but not contralateral addition of MAb528 inhibited EGF binding to basolateral and apical receptors, respectively. Furthermore, the 125I-EGF tracer did not significantly cross the monolayer in these culture inserts.

It is well established that protein tyrosine phosphorylation mediates activation by EGFR, a process initiated by autotyrosine phosphorylation of EGFR itself (23). Tyrosine phosphorylation of EGFR substrates involved in the mitogenic pathway (SHC, ERK1, and ERK2) was detected in response to both apical and basolateral EGFR activation in fibroblast and polar...

Fig. 6. EGF-mediated tyrosine phosphorylation of β-catenin. A: experiments were performed as described in Fig. 5 except that the membranes were blotted with antibody to β-catenin. B: soluble lysates were immunoprecipitated with antibody to β-catenin, and the nitrocellulose membrane was blotted with antibody to β-catenin. The results shown here are representative of 3 independent preparations. Quantification of apical and basolateral stimulated tyrosine phosphorylation of β-catenin and total β-catenin is shown in C and D, respectively. Data are means ± SE from 3 preparations. *P < 0.05, **P < 0.01 vs. basal.
ized epithelial cells (9, 19, 22). These data suggest that in some experimental models for the mitogenic response to EGF apical EGFR may activate similar pathways to basolateral EGFR.

Although tyrosine phosphorylation has been proposed to play a role in tight junction regulation, supporting evidence remains limited. Selective protein kinase and tyrosine phosphatase inhibitors have been observed to alter protein phosphorylation, junction morphology, and paracellular permeability (8, 11, 33), but the specificity of these effects is controversial.

We used immunohistochemistry to study the effects of EGF on tyrosine-phosphorylated proteins (32). Confocal microscopy of monolayers grown on filter inserts revealed a typical apical junctional pattern with antibodies to ZO-1. The addition of EGF did not obviously alter the intensity or distribution of ZO-1 staining. In control monolayers or monolayers treated with MAb528, anti-phosphotyrosine antibodies detected proteins scattered throughout the cell. However, after treatment with apical or basolateral EGF, confocal microscopy indicated early, marked enhancement of apical and perijunctional tyrosine phosphoprotein immunoreactivity. Induction of tyrosine phosphorylation by apical EGFR activation provided additional evidence for the biological activity of these apical receptors.

We also used immunoprecipitation and Western blotting to study tyrosine phosphorylation of EGFR and β-catenin (16) in response to apical and basolateral EGFR activation. Autophosphorylation of the 180-kDa EGFR was found with both apical and basolateral EGF treatment. However, basolateral EGFR activation produced a roughly comparable degree of phosphorylation of β-catenin. These findings showing that apical EGFR activation induces tyrosine phosphorylation of EGFR and β-catenin provide further support for the potential physiological relevance of apical EGFR in gastric cells. These findings also suggest that apical EGFR may phosphorylate certain junctional proteins, such as β-catenin, more efficiently than basolateral receptors.

The mechanisms coupling EGFR activation to regulation of paracellular permeability remain to be unraveled. EGF-dependent tyrosine phosphorylation of β-catenin has previously been observed (10, 30) in a variety of transformed or immortalized cell lines. However, in these models (10, 30), phosphorylation correlated with decreased cell adhesion or increased permeability. Our findings suggest that EGF induces tyrosine phosphorylation of β-catenin simultaneously with a decrease in paracellular permeability. We can only speculate that apical EGFR exert physiological regulation of paracellular permeability and that tyrosine phosphorylation of β-catenin plays a role in these actions of EGF. Our primary cell culture monolayers provide a model for dissecting the relevance and mechanisms of growth factor regulation of paracellular permeability via effects at the apical junctional complex. This model appears to be physiologically relevant by virtue of the presence of an apical barrier to acid that mimics this critical in vivo function of the gastric mucosa and responsiveness to regulation by growth factors.

The physiological importance of the regulation of paracellular permeability and apical EGFR remains to be determined. Our data indicate that apical EGFR on canine gastric monolayers specifically bind ligands, exhibit tyrosine kinase activity, and mediate a decrease in paracellular permeability. Other experiments in our laboratory have shown that EGF-dependent decreases in paracellular permeability correlate with increased resistance to apical acidification (unpublished observations). We postulate that apical EGFR exert physiologically relevant actions in gastric mucosa, regulating the paracellular pathway, and thereby decreasing the permeability to acid and contributing to the remarkable ability of gastric mucosal glandular cells to withstand the acidic environment of the gastric lumen.

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