Involvement of Phosphatidylinositol 3-Kinase and Mitogen-activated Protein Kinases in Glycine-extended Gastrin-induced Dissociation and Migration of Gastric Epithelial Cells*

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The various molecular forms of gastrin can act as promoters of proliferation and differentiation in different regions of the gastrointestinal tract. We report a novel stimulatory effect of glycine-extended gastrin17 on cell/cell dissociation and cell migration in a non-tumorigenic mouse gastric epithelial cell line (IMGE-5). In contrast, both amidated and glycine-extended gastrin17 stimulated proliferation of IMGE-5 cells via distinct receptors. Glycine-extended gastrin17-induced dissociation preceded migration and was blocked by selective inhibitors of phosphatidylinositol 3-kinase (PI3-kinase) but did not require mitogen-activated protein (MAP) kinase activation. Furthermore, glycine-extended gastrin17 induced a PI3-kinase-mediated tyrosine phosphorylation of the adherens junction protein β-catenin, partial dissociation of the complex between β-catenin and the transmembrane protein E-cadherin, and delocalization of β-catenin into the cytoplasm. Long lasting activation of MAP kinases by glycine-extended gastrin17 was specifically required for the migratory response, in contrast to the involvement of a rapid and transient MAP kinase activation in the proliferative response to both amidated and glycine-extended gastrin17. Therefore, the time course of MAP kinase activation appears to be a critical determinant of the biological effects mediated by this pathway. Together with the involvement of PI3-kinase in the dissociation of adherens junctions, long term activation of MAP kinases seems responsible for the selectivity of this novel effect of G17-Gly on the adhesion and migration of gastric epithelial cells.

Gastrin is an important hormone for the development and function of the gastrointestinal tract (for review see Ref. 1). Amidated gastrin (G17-NH2) has been shown to activate various intracellular transduction pathways depending on the cell type. In the rat pancreatic cell line AR4-2J, gastrin-induced cell proliferation is thought to be mediated by activation of MAP kinases, leading to subsequent expression of immediate early genes like c-fos and c-jun (3, 10), whereas gastrin-promoted cell growth in the rat pituitary adenoma cell line GH3 is supported by a Ca2+-dependent mechanism (11). Furthermore, in a rat intestinal epithelial cell line (IEC-6) (12) and in Chinese hamster ovary cells expressing transfected gastrin/CCK-B receptors (13), gastrin stimulates c-Src-like tyrosine kinases upstream of phosphatidylinositol 3-kinase (PI3-kinase) and MAP kinase.

To date, G17-Gly-induced transduction pathways have been studied mostly in tumor cell lines. In AR4-2J cells (3), as well as in the human colon cancer cell lines HT29 and LoVo (14), G17-Gly stimulates c-Jun amino-terminal kinase activation independently of the MAP kinase pathway. Binding studies strongly suggest that G17-Gly effects are mediated by a novel receptor that is insensitive to G17-NH2 and classical gastrin/CCK-B receptor antagonists (5, 6, 14), although a different receptor binding with a similar affinity to G17-Gly and G17-NH2 was also identified on Swiss 3T3 fibroblasts (15). More studies are necessary in order to determine the signal transduction pathways activated by G17-Gly on other tumoral and non-tumoral cell lines and to correlate these processes with the biological roles of the peptide.

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1 The abbreviations used are: G17-NH2, amidated gastrin 17; CCK, cholecystokinin; MAP, mitogen-activated protein; PI3-kinase, phosphatidylinositol 3-kinase; FCS, fetal calf serum; PBS, phosphate-buffered saline; BrdUrd, bromodeoxyuridine; DMEM, Dulbecco’s modified Eagle’s medium; DTT, dithiothreitol; PCR, polymerase chain reaction; ANOVA, analysis of variance; G17-Gly, glycine-extended gastrin 17; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.
For this work, we used a recently established gastric epithelial cell line (IMGE-5) (16) to compare the effects of amidated and glycine-extended gastrins on proliferation as well as cell adhesion and migration. The phenotype of these non-tumorigenic cells can be modulated in vitro by shifting them from a permissive temperature (33 °C) in the presence of γ-interferon to a non-permissive temperature (39 °C), allowing their differentiation toward an epithelial phenotype (16). The gastric origin of IMGE-5 cells together with their differentiated phenotype make them particularly suitable to study the cellular effects of gastrin-related peptides.

In this paper, we report for the first time that, while both molecular forms of gastrin stimulate proliferation of IMGE-5 cells, dissociation and migration of these cells in a wound healing assay was induced only by G17-Gly. G17-Gly also induced tyrosine phosphorylation of the adherens junction protein β-catenin and dissociation of the complex between β-catenin and the transmembrane protein E-cadherin, followed by the partial disappearance of β-catenin from the cell membrane. We also show that a differential time course in the activation of MAP kinases by the two gastrin derivatives, as well as the involvement of PI3-kinase in the effect of G17-Gly on β-catenin, seem responsible for the selectivity of the effects of G17-Gly on the adhesion and migration of IMGE-5 cells.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Cell Culture**—G17-NH2 was from Research Plus (Baltimore, MD), and G17-Gly was from Auspep (Melbourne, Australia). The CCK-A receptor antagonist L365,260 (18) were gifts from Dr. V. J. Lotti (Merck). The LY 294002 was from Sigma. E-cadherin, a receptor antagonist L365,260 (18) were gifts from Dr. V. J. Lotti (Merck).

**Detection of G-CCK-B Receptors by Reverse Transcriptase-PCR**—Molecular forms of gastrin stimulate proliferation of IMGE-5 cells, differentiation toward an epithelial phenotype (16). The gastric origin and the transmembrane protein E-cadherin, followed by the partial disappearance of β-catenin from the cell membrane.

**Detection of G-CCK-B Receptors by Reverse Transcriptase-PCR**—Total RNA was prepared from confluent and non-confluent cells cultured in DMEM with 1% FCS and 100 μg/ml of 1 unit/ml of penicillin and streptomycin. The RNA was isolated using TriReagent (Promega, Madison, WI), and 2 μl of template cDNA. The standard PCR procedure involved denaturation of samples at 94 °C, annealing at 65 °C, and elongation of DNA strands at 72 °C. The samples were run on a 1.8% agarose gel containing ethidium bromide and photographed under UV light.

**Binding Assay for 125I-Gly-Gly—**G17-Gly was iodinated using the IODO-GEN method (22) and purified by HPLC. 2.7 × 106 IMGE-5 cells were seeded in 6-well plates and grown at 33 °C until 70% confluent. They were then shifted to 39 °C for 24 h and incubated for 90 min at 39 °C in 200 μM Tris-HCl (pH 7.2) containing 10 μM KCl, 2 mM MgCl2, 1 mM DTT, 1 mM benzamidine, 0.1% bovine serum albumin, and 10 μM cold orthovanadate (1 mM DTT (WLB buffer), using 3 μg of anti-β-catenin antibody for 2 h at 4 °C, followed by 100 μl of 20% protein A-Sepharose CL-4B (Amersham Pharmacia Biotech) overnight. Samples were washed three times in WLB buffer, and spun for 10 s at 10,000 × g.

**Western Blotting**—Cells were grown in 100-mm Petri dishes under permissive conditions until they reached 90% confluency. They were then transferred to non-permissive conditions and serum-starved for 24 h, stimulated with the indicated concentrations of G17-NH2 or G17-Gly for various times with or without 15 μM preincubation with 10 μM LY 294002 or 50 μM PD 98059, and lysed using the standard procedure described previously (23). In the case of β-catenin/E-cadherin association studies, 100 μg of protein lysate per sample was immunoprecipitated in Tris/NaCl (pH 7.5) containing 1% Nonidet P-40. Membranes were processed for non-permissive conditions. Morphology and migration of IMGE-5 cells were assessed using a wound healing model on a near-confluent. They were then grown in 12-well plates under permissive conditions until they reached 90% confluency. They were then shifted to 39 °C for 24 h, stimulated with the indicated concentrations of G17-NH2 or G17-Gly in the presence of 1 μM MD200 digital camera and DC viewer software (1280 × 1024 pixels/image).

**Detection of G-CCK-B Receptors by Reverse Transcriptase-PCR**—Total RNA was prepared from confluent and non-confluent cells cultured in DMEM with 1% FCS and 100 μg/ml of 1 unit/ml of penicillin and streptomycin. The RNA was isolated using TriReagent (Promega, Madison, WI), and 2 μl of template cDNA.

**RESULTS**

**Dual Biological Effect of G17-Gly but Not G17-NH2 on IMGE-5 Cells**—The proliferative effects of G17-NH2 and G17-Gly on IMGE-5 cells under non-permissive conditions were investigated. Both forms of the peptide were found to stimulate BrdUrd incorporation into IMGE-5 cell nuclei in a dose-dependent manner and with a similar amplitude (Fig. 1A). G17-Gly was found to be significantly more potent than G17-NH2 as a stimulant of IMGE-5 proliferation (EC50 22.8 ± 1.5 μM for G17-Gly and 84.3 ± 2.9 μM for G17-NH2).

We then investigated whether either form of gastrin was able to trigger changes in morphology and motility of IMGE-5 cells under non-permissive conditions.
performed in triplicate. H9262 presence of 100 starved for 24 h at 39 °C and stimulated for 18 h with the indicated concentrations of G17-Gly (closed circles) or G17-NH2 (open circles) in the presence of 100 μM BrdUrd. After fixation for 3 min in ice-cold methanol, BrdUrd incorporation was assessed by immunofluorescence using an anti-BrdUrd antibody. Data represent percent of total cells incorporating BrdUrd and are the means ± S.E. of four separate experiments, each performed in triplicate. B, cells were grown until subconfluent, serum-starved for 24 h at 39 °C, wounded linearly with a pipette tip, and then grown in the presence of 0.1% heat-inactivated FCS, without further treatment (a) or with 100 pM (b), 500 pM (c), 1 nM (d), 5 nM (e), 10 nM (f), or 50 nM (g) G17-Gly. Microphotographs of a similar randomly chosen field for each one of the wounded monolayers were taken when the wound was created (0 h) as well as 16 and 24 h after wounding the cells. C, histogram representing the change in wound size (in μm) over time in untreated IMGE-5 cells (control) or cells treated with 100 pM to 100 nM G17-Gly. For each sample, measurements were performed on 5 distinct fields along the wound at t = 0 (black bars), 16 (light gray), and 24 h (dark gray). Statistical significance was assessed by one-way analysis of variance (*, p < 0.05, and **, p < 0.01 compared with untreated cells, n = five experiments).

Different roles for G17-NH2 and G17-Gly in the proliferation and migration of gastric epithelial cells. A, cells were serum-starved for 24 h at 39 °C and stimulated for 18 h with the indicated concentrations of G17-Gly (closed circles) or G17-NH2 (open circles) in the presence of 100 μM BrdUrd. After fixation for 3 min in ice-cold methanol, BrdUrd incorporation was assessed by immunofluorescence using an anti-BrdUrd antibody. Data represent percent of total cells incorporating BrdUrd and are the means ± S.E. of four separate experiments, each performed in triplicate. B, cells were grown until subconfluent, serum-starved for 24 h at 39 °C, wounded linearly with a pipette tip, and then grown in the presence of 0.1% heat-inactivated FCS, without further treatment (a) or with 100 pM (b), 500 pM (c), 1 nM (d), 5 nM (e), 10 nM (f), or 50 nM (g) G17-Gly. Microphotographs of a similar randomly chosen field for each one of the wounded monolayers were taken when the wound was created (0 h) as well as 16 and 24 h after wounding the cells. C, histogram representing the change in wound size (in μm) over time in untreated IMGE-5 cells (control) or cells treated with 100 pM to 100 nM G17-Gly. For each sample, measurements were performed on 5 distinct fields along the wound at t = 0 (black bars), 16 (light gray), and 24 h (dark gray). Statistical significance was assessed by one-way analysis of variance (*, p < 0.05, and **, p < 0.01 compared with untreated cells, n = five experiments).

In untreated samples, the cells at the edge of the wound did not seem to dissociate from one another at any stage, and the β-catenin staining remained similar to that detected before or upon wounding for up to 24 h (Fig. 2, A1–A8). On the contrary, cells treated with G17-Gly significantly dissociated from each other at the front edge of the wound as early as 8 h after treatment (shown here for 10 nM G17-Gly, Fig. 2B6), and dissociation correlated with a strong shift of β-catenin localization from the membrane, mainly to the nucleus of these cells (Fig. 2B2). Interestingly, once the treated cells started to migrate into the wound, only about 30% consistently maintained a strong nuclear staining for β-catenin, whereas staining in the others was evenly decreased (Fig. 2B3). In all cases, upon closure of the wound, cells lost their spindle-like morphology and re-established contact with their neighbors, and β-catenin returned to a mostly membrane-bound localization (Fig. 2B4), around 24–26 h after treatment with G17-Gly concentrations above 5 nM.

Different Receptors Are Responsible for Effects of G17-NH2 and G17-Gly on IMGE-5 Cells—The only receptor selective for G17-NH2 cloned to date is the gastrin/CKC-B receptor, expression of which in IMGE-5 cells was assessed by reverse transcriptase-PCR. IMGE-5 cells expressed gastrin/CKC-B receptor mRNA only under non-permissive conditions (Fig. 3A).

The presence of a selective receptor for G17-Gly was investigated by binding of 125I-G17-Gly to adherent IMGE-5 cells. Although specific binding was detected under permissive conditions (data not shown), it was greatly increased under non-permissive conditions. Binding was reduced in a dose-dependent manner by concomitant incubation of cells with increasing

**Fig. 1.** Different roles for G17-NH2 and G17-Gly in the proliferation and migration of gastric epithelial cells. A, cells were serum-starved for 24 h at 39 °C and stimulated for 18 h with the indicated concentrations of G17-Gly (closed circles) or G17-NH2 (open circles) in the presence of 100 μM BrdUrd. After fixation for 3 min in ice-cold methanol, BrdUrd incorporation was assessed by immunofluorescence using an anti-BrdUrd antibody. Data represent percent of total cells incorporating BrdUrd and are the means ± S.E. of four separate experiments, each performed in triplicate. B, cells were grown until subconfluent, serum-starved for 24 h at 39 °C, wounded linearly with a pipette tip, and then grown in the presence of 0.1% heat-inactivated FCS, without further treatment (a) or with 100 pM (b), 500 pM (c), 1 nM (d), 5 nM (e), 10 nM (f), or 50 nM (g) G17-Gly. Microphotographs of a similar randomly chosen field for each one of the wounded monolayers were taken when the wound was created (0 h) as well as 16 and 24 h after wounding the cells. C, histogram representing the change in wound size (in μm) over time in untreated IMGE-5 cells (control) or cells treated with 100 pM to 100 nM G17-Gly. For each sample, measurements were performed on 5 distinct fields along the wound at t = 0 (black bars), 16 (light gray), and 24 h (dark gray). Statistical significance was assessed by one-way analysis of variance (*, p < 0.05, and **, p < 0.01 compared with untreated cells, n = five experiments).
concentrations of unlabeled G17-Gly but not with G17-NH₂ or with the G/CCK-B receptor antagonist L365,260 (Fig. 3B).

G/CCK-B and CCK-A receptors are currently the best characterized receptors for gastrin-related peptides. By using reliable selective antagonists, the involvement of these subtypes in the proliferative effects of G17-NH₂ and G17-Gly on IMGE-5 cells was investigated. Interestingly, the stimulation induced by G17-NH₂ was dose-dependently reversed by the selective G/CCK-B antagonist L365,260 (Fig. 3C), whereas L364,718, a selective CCK-A receptor antagonist (17), had a very weak effect, consistent with its interaction at high concentrations (Fig. 3B). Neither antagonist had any effect on G17-Gly-induced proliferation, at any of the concentrations tested (Fig. 3C).

**Phosphatidylinositol 3-Kinase and MAP Kinase Pathway Are Involved in the Biological Effects Induced by G17-Gly**—The role of PI3-kinase in the mediation of the proliferative signal triggered by G17-Gly and G17-NH₂ was investigated. When IMGE-5 cells were preincubated with either of two PI3-kinase inhibitors (10 nM wortmannin or 10 μM LY 294002), the proliferative effect of G17-Gly was almost abolished, whereas neither inhibitor significantly affected the stimulation induced by G17-NH₂ (Fig. 4A). In contrast, the stimulatory effects of both peptides were significantly decreased by the MEKi2 inhibitor PD 98059 (50 μM).

Furthermore, preincubation with 10 μM LY 294002 (Fig. 4Be) prevented the stimulation of IMGE-5 cell migration induced by 10–100 nM G17-Gly (Fig. 4Bd for effect of 100 nM), indicating that PI3-kinase is directly involved in this effect. Interestingly, although pretreatment with the MEK inhibitor PD 98059 abolished the motility response to G17-Gly, numerous cells were still able to dissociate from their neighbors at the edges of the wound (Fig. 4Bf).

**Role of Phosphatidylinositol 3-Kinase and MAP Kinases in the Tyrosine Phosphorylation and Membrane Delocalization of β-Catenin**—When confluent IMGE-5 cells were incubated for periods ranging from 30 min to 24 h with 100 pst to 100 nM G17-Gly under non-permissive conditions, a marked decrease was detected in the amount of β-catenin located at the plasma membrane from 1 h after treatment (shown for 100 nM), whereas cytoplasmic staining for the protein was greatly
Increased (Fig. 5Ab). The enhanced cytoplasmic localization of β-catenin persisted for at least 4 h, but the protein returned to the plasma membrane within 12 h after G17-Gly treatment (Fig. 5Ab). This shift of β-catenin from the membrane to the cytoplasm was largely prevented by preincubating the cells with the PI3-kinase inhibitor LY 294002 (Fig. 5Ad) or with the tyrosine kinase inhibitor tyrphostin 25 (Fig. 5Ac), whereas the MEK1/2 inhibitor PD 98059 was found to have no effect on the G17-Gly-induced delocalization of β-catenin (Fig. 5Ac). Furthermore, G17-NH2 did not display any effect on the membrane localization of β-catenin during 24 h of treatment (Fig. 5Af).

The effect of similar doses of G17-Gly on β-catenin in IMGE-5 cells was then studied by Western blotting. G17-Gly induced a rapid increase in the tyrosine phosphorylation of β-catenin (Fig. 5B) and a partial dissociation of β-catenin from its adherens junction partner E-cadherin (Fig. 5B). Increased tyrosine phosphorylation of β-catenin, as well as its dissociation from E-cadherin, was significant after 15 min and maximal 30 min after G17-Gly treatment. The dissociation between β-catenin and E-cadherin returned to control values 4 h after treatment, while at that time the tyrosine phosphorylation levels of β-catenin were still slightly higher than those found in control cells (Fig. 5Bc), perhaps because β-catenin can partially reassociate with E-cadherin before being completely dephosphorylated. Alternatively, a slight difference in sensitivity may exist between the detection of phosphorylated β-catenin and of the amount of E-cadherin co-immunoprecipitated with β-catenin. Tyrosine phosphorylation of β-catenin returned to control levels within 12 h after addition of G17-Gly (Fig. 5Bf). Thus, the time course of G17-Gly-induced changes in β-catenin phosphorylation and association with E-cadherin correlates well with the partial cytoplasmic relocalization of β-catenin detected by immunocytochemistry.

Both the G17-Gly-induced tyrosine phosphorylation of β-catenin (Fig. 5D) and its dissociation from E-cadherin (Fig. 5C) were abolished by preincubation with the PI3-kinase inhibitor LY 294002. In contrast, the apparent inability of the MEK1/2 inhibitor PD 98059 to block G17-Gly-induced delocalization of β-catenin and dissociation of IMGE-5 cells in the wound healing assay was further supported by a similar lack of effect of this inhibitor on the G17-Gly-induced stimulation of tyrosine phosphorylation of β-catenin (Fig. 5Dd) and of its dissociation from E-cadherin (Fig. 5Dd).

Finally, G17-NH2 showed no effect on the phosphorylation level of β-catenin (Fig. 5De) or on its association with E-cadherin (Fig. 5E).

Differential Activation of the MAP Kinase Pathway by G17-NH2 and G17-Gly Is Partly Responsible for the Difference in Biological Effects—The time course of p42/p44 MAP kinase activation by G17-NH2 and G17-Gly in IMGE-5 cells was then investigated. A very rapid but transient activation of p42/p44 phosphorylation was induced by G17-NH2 (Fig. 6A, left panel); activation was found to be maximal within 1 min of stimulation, and the phosphorylation level returned to control levels between 15 and 30 min later (Fig. 6A, graph). On the contrary, the profile of activation by G17-Gly was quite different (Fig. 6A, right panel); p42/p44 phosphorylation was also detected from 1 min after stimulation, but the intensity of phosphorylation was found to increase continuously until 30 min after stimulation, to remain high for up to 3 h, and to return to control levels 6 h after stimulation (Fig. 6A, graph).

In order to determine whether the differential MAP kinase activation could explain, at least in part, the different biological effects displayed by both molecular forms of gastrin, activation of the MAP kinase pathway was blocked at different stages of the simulation induced by either form of gastrin, and the consequences on their respective biological activities were investigated. When the early (15 min) activation of the MAP kinase pathway was blocked by preincubating the cells with 50 μM PD 98059 prior to G17-NH2 or G17-Gly stimulation, the increase in proliferation was abolished (Fig. 6B). On the contrary, when the MAP kinase pathway inhibitor was added simultaneously with each one of the peptides, the early activation of p42/p44 MAP kinases was still detected, and the proliferative effect of G17-NH2 or G17-Gly was no longer abolished (Fig. 6B). In contrast, the stimulation of migration by G17-Gly was always blocked whether the MAP kinase pathway was blocked 5, 15, or 30 min after addition of the peptide, whereas G17-Gly-induced cell dissociation was unaffected in these conditions (Fig. 6C).

G17-Gly Activates Akt/PKB Phosphorylation—Finally, we assessed whether G17-Gly and G17-NH2 were capable of regulating a PI3-kinase-dependent pathway related to apoptosis, through control of Akt/PKB phosphorylation. Although G17-NH2 had no effect (Fig. 7, upper panel), G17-Gly significantly increased Akt phosphorylation. Activation was detected about 15 min after addition of the peptide, was maximal after 30 min (Fig. 7, lower panel), and decreased regularly down to control values after 60 min. This activation was reversed by preincubation with the PI3-kinase inhibitor LY 294002.
DISCUSSION

The results presented in this paper demonstrate the existence of similarities but also major differences in the biological effects of G17-Gly and G17-NH2 on a non-tumorigenic gastric epithelial cell line (IMGE-5). On the one hand, both G17-NH2 and G17-Gly stimulated IMGE-5 proliferation, as has been described previously in several other models (1, 4–6). On the other hand, a major difference between the two gastrins is the novel effect of G17-Gly only on the dissociation and migration of gastric epithelial cells. Our results underline the existence of a biological role for G17-Gly that seems specific to this peptide and is not exhibited by G17-NH2. This finding contrasts with previous work on G17-Gly, which described biological effects also displayed by G17-NH2, such as cell proliferation (1, 5, 6) or...
stimulation of gastric acid secretion (24). However, it is in agreement with the recent description of a stimulatory effect of G₁₇-Gly on the invasiveness of the human colon cancer cell line LoVo (8). The enhancement of cell migration by G₁₇-Gly reported herein may be profoundly important in the previously reported roles of progastrin-derived peptides during the differentiation of the gastrointestinal tract, as well as during cancer development and metastasis (see Refs. 1 and 9 for review).

Several reports have recently implicated gastrins in the activation of proteins involved in epithelial cell/matrix adhesion and morphology, such as p130Cas and paxillin (9). Recent results showing the phosphorylation and activation of p125FAK by G₁₇-NH₂ via a Src-related pathway are also compatible with a role in cell motility (25), as the role of this non-receptor tyrosine kinase in cell migration is well documented (26). It is worth noting, however, that G₁₇-NH₂ was shown to have an inhibitory effect on the spontaneous motility of glioblastoma cell lines (27). The results reported in this paper provide, to our knowledge, the first demonstration of a direct stimulatory effect for a non-amidated progastrin-derived peptide on cell/cell adhesion between epithelial cells.

This effect was detected at G₁₇-Gly concentrations as low as 100 nM. Serum concentrations of G₁₇-Gly are generally thought to be around 20–50 pm in the fasting state. However, gastrin precursor production is significantly increased in various physiological conditions such as birth and weaning (28, 29), as well as during pathological processes such as gastric adenocarcinoma (30) and gastrinoma (31, 32). The migratory effect of G₁₇-Gly was maximal around 5 nM, as compared with 1 nM for gastrin receptor (31). If the migratory effect of G₁₇-Gly followed the same potency trend, it is possible that the apparent discrepancy might indicate that the G₁₇-Gly receptors could exist in various affinity states or that fractional occupancy only is necessary to activate the biological effects downstream of these receptors.

The signal transduction pathways activated by G₁₇-NH₂ in IMGE-5 cells have been reported previously in some, but not all, cell types. The results obtained in this study indicate that MAP kinase activation is essential for the proliferative effect of G₁₇-NH₂ on IMGE-5 cells, and Stepan et al. (10) recently showed that G₁₇-NH₂-stimulated proliferation through a pathway involving MAP kinases in AR42J cells. In contrast, in GH3 cells, G₁₇-NH₂ did not activate this pathway but induced proliferation in a Ca²⁺-dependent manner. In previous studies,
activation of G/0CK-B receptors by G17-NH2 has been shown to induce PI3-kinase activation in transfected Chinese hamster ovary cells (13), probably via the prior formation of a p60Src-p125FAK complex (25). Thus, there seems to be a certain degree of cell type specificity in the transduction pathways involved in the proliferative effects of G17-NH2.

Much less is known about the signal transduction pathways activated by G17-Gly. Reports by Todisco et al. (3) on pancreatic carcinoma cells and Stepan et al. (14) on colorectal carcinoma cells indicate that G17-Gly regulates the transcriptional activation of early genes, through the activation of enzymes such as c-Jun kinase. In these cell lines, the MAP kinase pathway is not involved in the proliferative effect of G17-Gly (3, 13). On the contrary, our results show that p42/p44 MAP kinase activation by G17-Gly as well as G17-NH2 was involved in their proliferative effect on IMGE-5 cells. However, PI3-kinase was involved selectively in the proliferative effect of G17-Gly only. Further studies on other cell lines will be necessary to assess whether there is also a cell type specificity in the transduction pathways triggered by G17-Gly, whether there are several subtypes of G17-Gly receptors, or whether the signal transduction coupled to these receptors is different in tumor cell lines.

The novel effect of G17-Gly on dissociation and migration of gastric epithelial cells involved both PI3-kinase and MAP kinase pathways. Activation of PI3-kinase was essential at least for cell dissociation, whereas the MAP kinase pathway seemed to be involved only in the motility response of IMGE-5 cells, without affecting their dissociation. This result is interesting, as the role of these two pathways in the dissociating effect of growth factors on other cell types is still unclear. PI3-kinase activation has been shown to participate in the ligand-induced migration of several cell types including renal epithelial cells (36) and vascular smooth muscle cells (37). However, the specific involvement of these pathways in the successive steps of a migratory response, i.e. cell dissociation and motility, has not been clearly defined. A report by Potempa and Ridley (38) showed that both PI3-kinase and MAP kinase activation (by Ras) seemed essential to hepatocyte growth factor-induced adherens junction disassembly in Madin-Darby canine kidney cells. However, in the same cell line, Royal et al. (36) reported that hepatocyte growth factor-induced motility required PI3-kinase activation, whereas pathways downstream from Grb2 (including MAP kinases) were involved in branching tubulogenesis. Recent results on HepG2 human hepatoma cells also showed that PI3-kinase was involved in cell dissociation, whereas inhibition of MEK blocked the motility response to growth factors (39). Our results also directly implicate the PI3-kinase pathway in the G17-Gly-induced tyrosine phosphorylation of β-catenin, as well as its dissociation from E-cadherin and delocalization from the adherens junctions. In contrast to HepG2 cells, the MAP kinase pathway does not appear to be involved in this G17-Gly-induced event in IMGE-5 cells.

We also found in this study that the time course of MAP kinase activation by G17-Gly and G17-NH2 was different, with the former triggering a rapid but short lived phosphorylation, whereas the latter induced a long term activation lasting for 3 h. Furthermore, we showed that the early activation of p42/44 MAP kinases is essential to the proliferative effect of G17-NH2 and G17-Gly, whereas a longer term activation seems necessary for the migratory response to G17-Gly. A previous study (39) suggested a correlation between the lack of effect of epidermal growth factors on HepG2 cell motility and its ability to induce only a short term increase in the phosphorylation of p42/p44 MAP kinases, whereas factors inducing a scattering of these cells, like hepatocyte growth factor, stimulated MAP kinases for a longer time. However, to our knowledge, this is the first direct demonstration that long term activation of MAP kinases is essential to the migratory response to an exogenous factor.

Interestingly, the stimulation induced by G17-Gly triggered a delayed increase in the activation of Akt/PIK, which was not detected after stimulation with G17-NH2. This result contradicts recent data (40) showing the activation of an Akt-dependent anti-apoptotic pathway by the formation of E-cadherin-mediated cell/cell contacts in Madin-Darby canine kidney cells. However, the existence of a similar mechanism has been recently demonstrated by Taupin et al. (41), who showed that the migratory effect of intestinal trefoil factor on intestinal cell lines is coupled to anti-apoptotic signals. It is possible that a balancing mechanism would induce activation of an anti-apoptotic pathway when cells are induced to migrate by an exogenous physiological activator. Such a mechanism would be necessary when cells need to migrate in vivo during the epithelial/mesenchymal transition or during processes leading to mucosal restitution and ulcer repair.

Our data showed that IMGE-5 cells are sensitive to both amidated and glycine-extended forms of gastrin. To date the only cell line responding to both molecular forms of gastrin is the rat pancreatic carcinoma cell line AR42J (5). The ligand selectivity of the G17-Gly receptor identified on IMGE-5 cells was similar to that described previously (6) both on AR42J and on YAMC cells. Furthermore, the expression of receptors for both G17-Gly and G17-NH2 was correlated to the differentiation status of IMGE-5 cells. Although IMGE-5 cells do not express G/0CK-B receptors under permissive conditions, they still express binding sites for G17-Gly, albeit at a lower density. IMGE-5 could therefore represent a unique tool for the parallel and independent study of biological effects and signal transduction pathways associated with G17-Gly and G17-NH2 activation.

Finally, our results demonstrating an effect of G17-Gly on the migration of gastric epithelial cells could reflect a potential physiological role for this peptide during ontogeny, in gastroduodenal ulcer disease, or during the progression of carcinomas. Although the available results are still scarce, there seems to be a general tendency toward the expression of partially processed rather than mature forms of gastrin in the early stages of development (at a stage when migration of cells to form the gastric pits is maximal), as well as during carcinoma development (42–44). The role of these partially processed forms in colonic proliferation is well established in vivo (1, 9, 45). Furthermore, a correlation could exist between serum concentrations of total progastrin products and the presence of liver metastasis in colorectal cancer (46) as well as in patients affected by the rare Zollinger-Ellison syndrome (32). Furthermore, antibodies neutralizing both amidated and glycine-extended forms of gastrin have been shown to inhibit the spontaneous metastasis of a human colorectal tumor when injected into immunodeficient mice (47). Therefore, the potential role of gastrin-derived peptides on migration needs to be further investigated in other models, in order to assess to what extent the results presented in this paper extend beyond the gastric mucosa and represent a general regulatory mechanism in the gastrointestinal tract.

REFERENCES
1. Dockray, G. J. (1999) J. Physiol. (Lond.) 518, 315–324
2. Miyazaki, Y., Shinomura Y., Tatsui, S., Zushi, S., Higashimoto, Y., Kanayama, S., Hipshiyama, S., Taniguchi, N., and Matsuzawa, Y. (1999) Gastroenterology 116, 73–89
3. Todisco, A., Takeuchi, Y., Seva, C., Dickinson, C. J., and Yamada, T. (1995) J. Biol. Chem. 270, 28337–28341
4. Baldwin, G. S. (1995) J. Gastroenterol. Hepatol. 10, 215–222
5. Seva, C., Dickinson, C., and Yamada, T. (1994) Science 265, 410–412
6. Holland, F., Imidhal, A., Mantamadiotis, T., Cicicottost, G. D., Shulkes, A., and Baldwin, G. S. (1997) Gastroenterology 113, 1576–1588
7. Baldwin, G. S., Holland, F., Yang, Z., Karelina, Y., Paterson, A., Straus, R.,
Fourmy, D., Neumann, G., and Shulkes, A. (2001) J. Biol. Chem. 276, 7791–7796

Kermorgant, S., and Levy, T. (2001) Biochem. Biophys. Res. Commun. 285, 136–141

Rozengurt, E., and Walsh, J. H. (2001) Annu. Rev. Physiol. 63, 49–76

Stepan, V. M., Dickinson, C. J., Del Valle, J. D., Matsushima, M., and Todisco, A. (1999) Am. J. Physiol. 276, G1363–G1372

Stepan, V. M., Tatewaki, M., Matsushima, M., Dickinson, C. J., del Valle, J., and Todisco, A. (1999) Am. J. Physiol. 276, G415–G424

Singh, P., Narayan, S., and Adiga, R. B. (1995) J. Biol. Chem. 270, 20657–20663

Stepan, V. M., Sawada, M., Todisco, A., and Dickinson, C. J. (1999) Mol. Med. 5, 147–159

Singh, P., Owlia, A., Espeijo, R., and Dai, B. (1995) J. Biol. Chem. 270, 8429–8438

Holland, F., Blanc, E. M., Bali, J. P., Whitehead, R. H., Pelegrin, A., Baldwin, G. S., and Choquet, A. (2001) Am. J. Physiol. 280, G910–G921

Chang, R. S. L., and Lotti, V. J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 4923–4926

Lotti, V. J., and Chang, R. S. L. (1989) Eur. J. Pharmacol. 162, 273–280

Jat, P. S., Noble, M. D., Ataliotis, P., Tanaka, Y., Yannoutsos, N., Larsen, L., and Kinossi, D. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5096–5100

Whitehead, R. H., Van Eeden, P. E., Noble, M. D., Ataliotis, P., and Jat, P. S. (1995) Proc. Natl. Acad. Sci. U. S. A. 90, 587–591

Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159

Seet, L., Fabri, L., Nice, E. C., and Baldwin, G. S. (1987) Biomed. Chromatogr. 2, 159–163

Renne, S., Dhan, R., Waterfield, M. D., and Courtneidge, S. A. (1994) Biochem. J. 301, 703–711

Chen, D., Zhao, C.-M., Dockray, G. J., Varro, A., Van Hoek, A., Sinclair, N. P., Wang, T. C., and Koh, T. J. (2000) Gastroenterology 119, 756–765

Daulhac, L., Kowalski-Chauvel, A., Pradayrol, L., Vaysse, N., and Seva, C. (1999) FEBS Lett. 445, 251–255

Ilie, D., Damsky, C. H., and Yamamoto, T. (1997) J. Cell. Sci. 110, 401–407

De Hauser, C., Camby, I., Darro, F., Migette, I., Decaestecker, C., Verbeek, C., Danguy, A., Pasteels, J.-L., Brotschi, J., Salmon, I., Van Ham, P., and Kiss, R. (1998) J. Neurobiol. 37, 373–382

Torrance, C. J., and Rehfeld, J. F. (2000) J. Pediatr. Endocrinol. Metab. 13, 1563–1570

Hilsted, L., Bardram, L., and Rehfeld, J. F. (1988) Biochem. J. 255, 397–402

Henwood, M., Clarke, P. A., Smith, A. M., and Watson, S. A. (2001) Br. J. Surg. 88, 564–568

Azuma, T., Magami, Y., Habu, Y., Kawai, K., Taggart, R. T., and Walsh, J. H. (1990) J. Gastroenterol. Hepatol. 5, 525–529

Jais, P., Mignon, M., and Rehfeld, J. F. (1997) Int. J. Cancer 71, 308–309

Stepan, V. M., Krametter, D. F., Matsushima, M., Todisco, A., Delvalle, J., and Dickinson, C. J. (1999) Am. J. Physiol. 277, R572–R581

Kaise, M., Muraoa, A., Seva, C., Takeda, H., Dickinson, C. J., and Yamada, T. (1995) J. Biol. Chem. 270, 11155–11160

Iwase, K., Rvers, B. M., Hellmich, M. R., Guo, Y.-S., Higashide, S., Kim, H. J., and Townsend, C. M., Jr. (1997) Gastroenterology 113, 782–790

Royal, I., Fournier, T. M., and Park, M. (1997) J. Cell. Physiol. 173, 196–201

Duan, C., Bauchat, J. R., and Hsieh, T. (2000) Circ. Res. 86, 15–23

Potempa, S., and Ridley, A. J. (1998) Mol. Biol. Cell 9, 2185–2200

Sipeki, S., Bander, E., Buday, L., Farkas, G., Bacsy, E., Ways, D. K., and Farago, A. (1995) Cell. Signal. 11, 885–890

Peece, S., Chiariello, M., Murga, C., and Gutkind, J. S. (1999) J. Biol. Chem. 274, 19347–19351

Taupin, D. R., Kinosita, K., and Podolsky, D. K. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 799–804

Read, M. A., Chick, P., Hardy, K. J., and Shulkes, A. (1992) Endocrinology 130, 1688–1697

Read, M., and Shulkes, A. (1993) Mol. Cell. Endocrinol. 93, 31–38

Ciccotosto, G. D., and Shulkes, A. (1996) Regul. Pept. 62, 97–105

Wang, T. C., Koh, T. J., Varro, A., Cahill, R. J., Dangler, C. A., Fox, J. G., and Dockray, G. J. (1996) J. Clin. Invest. 98, 1918–1929

Kameyama, M., Fukuda, I., Imakoa, S., Nakamori, S., and Iwanaga, T. (1993) Dis. Colon Rectum 36, 497–500

Watson, S. A., Michaeli, D., Morris, T. M., Clarke, P., Varro, A., Griffin, N., Smith, A., Justin, T., and Hardecastle, J. D. (1999) Eur. J. Cancer 35, 1286–1291
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