Molecular mechanisms for the growth factor action of gastrin

ANDREA TODISCO,† YOSHIKI TAKEUCHI,† ANDREJ URUMOV,† JUNKO YAMADA,† VINZENZ M. STEPAN,† AND TADATAKA YAMADA†,‡

Departments of †Internal Medicine and ‡Physiology, University of Michigan Medical Center, Ann Arbor, Michigan 48109

Todisco, Andrea, Yoshiaki Takeuchi, Andrej Urumov, Junko Yamada, Vinzenz M. Stepan, and Tadataka Yamada. Molecular mechanisms for the growth factor action of gastrin. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G891–G898, 1997.—We have previously observed that gastrin has a cholecystokinin B (CCK-B) receptor-mediated growth-promoting effect on the AR42J rat pancreatic acinar cell line and that this effect is paralleled by induction of expression of the early response gene c-fos. We undertook these experiments to elucidate the mechanism for induction of c-fos and the linkage of this action to the trophic effects of gastrin. Gastrin (0.1–10 nM) dose dependently induced luciferase activity in AR42J cells transfected with a construct consisting of a luciferase reporter gene coupled to the serum response element (SRE) of the c-fos promoter. This effect was blocked by the specific CCK-B receptor antagonist D2 but not by the specific CCK-A receptor antagonist L-364,718 or by pertussis toxin, indicating that gastrin targets the SRE via specific CCK-B receptors through a mechanism independent of Gi. Inhibition of protein kinase C (PKC) either by prolonged (24 h) exposure of the cells to the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (100 nM) or by incubation with the selective inhibitor GF-109203X (3.5 µM) resulted in an 80% reduction in luciferase activity. Similar results were observed in the presence of the specific extracellular signal-regulated kinase (ERK) kinase (MEK) inhibitor PD-98059 (50 µM). We measured ERK2 activity in AR42J cells via in-gel kinase assays and observed that gastrin (1 pM–100 nM) induced ERK2 enzyme activity in a dose-dependent manner. Addition of GF-109203X and PD-98059, either alone or in combination, produced, respectively, partial and total inhibition of gastrin-induced ERK2 activity. Gastrin induction of ERK2 activity also resulted in a threefold increase in the transcriptional activity of Elk-1, a factor known to bind to the c-fos SRE and to be phosphorylated and activated by ERK2. PD-98059 blocked the growth-promoting effect of gastrin on the AR42J cells, demonstrating that this effect depends on activation of MEK. Our data lead us to conclude that the trophic actions of gastrin are mediated by ERK2-induced c-fos gene expression via PKC-dependent and -independent pathways.

cholecytokinin-B receptor; cellular proliferation; early response genes; protein kinases; transcriptional regulation; c-fos; mitogen-activated protein kinase; extracellular signal-regulated protein kinases

Although characterized as a stimulant of gastric acid secretion (11), the peptide hormone gastrin also exerts growth-promoting effects on normal and malignant gastrointestinal tissues (16, 28, 29). Gastrin is an important growth factor for the fetal pancreas (4, 34), and in the stomach it is a potent stimulant for the growth of the gastric mucosa (33). In addition, recent reports have indicated that gastrin induces the growth of colon carcinomas both in vivo and in vitro, thus underscoring the importance of gastrin as a growth factor for gastrointestinal neoplasms (23, 28).

The process of cellular proliferation is under the control of a complex cascade of phosphorylation reactions that is triggered by the interaction of growth factors with their specific cellular receptors (14). One of the best-characterized pathways linked to the control of cellular growth is known to involve the activation of the serine-threonine protein kinase Raf through its interaction with the small GTP binding protein Ras (14). Raf is responsible for the phosphorylation and activation of a dual-specificity protein kinase known as mitogen-activated protein kinase (MAPK) or extracellular signal-regulated protein kinase (ERK) kinase (MEK), which in turn phosphorylates both serine and tyrosine residues in a family of serine-threonine protein kinases known as MAPKs or ERKs (14). The ERKs phosphorylate numerous cellular proteins, including transcription factors such as p62TCF or Elk-1, that play an important role in the transcriptional regulation of the promoter of the early response gene c-fos through the serum response element (SRE) (14, 18). Thus c-fos appears to be an important central node for the propagation of mitogenic signals from second messengers to the nucleus and for the activation or repression of the next set of genes in the biological programs initiated by the extracellular signals (14, 20).

Recent reports have indicated that gastrin can induce the ERKs and that this effect is paralleled by its ability to induce the early response gene c-fos (26, 30, 31). However, little is known about the signal-transduction pathways that target the ERKs and c-fos in response to gastrin and whether this effect is responsible for stimulation of cellular growth. Thus we undertook these studies to elucidate the mechanisms for induction of c-fos and the linkage of this action to the trophic effects of gastrin. Our data indicate that gastrin, binding to specific cholecystokinin (CCK)-B receptors on the AR42J cells, induces a cascade of phosphorylation reactions that target and activate the ERKs via both protein kinase C (PKC)-dependent and -independent pathways. The ERKs, in turn, appear to induce c-fos gene expression through phosphorylation and transcriptional activation of Elk-1. The net result of this cascade of events is stimulation of cellular proliferation.

MATERIAL AND METHODS

Plasmids. 5×Gal-Luc (13) was a gift from M. Karin (San Diego, CA); Gal4-ElkC (18) was a gift from R. Treisman (London, UK). SRE-Luc (35) was obtained from J. Pessin.
(Iowa City, IA), and pCMV-βGal was a gift from M. Uhler (Ann Arbor, MI).

Cell culture, transient transfection, and luciferase assays. For our experiments, we used the rat exocrine pancreatic cell line AR42J, which is known to express receptors for both gastrin (G17) and glycine-extended progastrin-processing intermediates (G-Gly) (27). The cells (obtained from American Type Culture Collection, Rockville, MD) were grown at 37°C in 35-mm dishes in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in 5% CO2-95% O2. Subconfluent AR42J cells were transfected with 5 µg of the luciferase reporter plasmid and, where indicated, with 0.5 µg of the expression vectors. Transfections were carried out using lipofectamine (GIBCO-BRL, Grand Island, NY), as previously described (31). The day after transfection, the media were removed, and the cells were fed with serum-free media (DMEM) for 24 h, then incubated for 5 h with or without human gastrin heptadecapeptide (Bachem, Torrance, CA) (0.1–10 nM) or 12-O-tetradecanoylphorbol 13-acetate (TPA, Sigma, St. Louis, MO; 100 nM). In some experiments, bisindolylmaleimide I (GF-109203X, 3.5 µM; Calbiochem, La Jolla, CA), PD-98059 (50 µM; a gift from Dr. Alan Saltiel, Park Davis Pharmaceutical Research, Ann Arbor, MI), L-364,718 (1 nM; a gift from Merck Sharp & Dohme, Rahway, NJ), or D2 (10 nM; a gift from Dr. Yoshi Goto, Daiichi Pharmaceuticals, Tokyo, Japan) was added 30 min before the addition of gastrin. In additional studies, the cells were also preincubated for either 16 h in 200 ng/ml pertussis toxin (Sigma) or for 24 h with TPA (100 nM) prior to the addition of gastrin. Bisindolylmaleimide I, TPA, L-364,718, and PD-98059 were dissolved in dimethyl sulfoxide (DMSO, Sigma). All other test substances were dissolved in water.

Control experiments were performed by incubating the cells in either incubation buffer or vehicle without the test substances. The end of the incubation period, the cells were washed and lysed, and luciferase assays were performed as previously described (31). Luciferase activity was expressed as relative light units and normalized for β-galactosidase activity. β-Galactosidase activity was measured by the luminometric light emitted from 1.0 µl of each sample incubated in 100 µl of Lumi-Gal S30 (Lumigen, Southfield, MI) and used to correct for luciferase assay data for transfection efficiency.

Immunoprecipitations and in-gel ERK2 assay. Immunoprecipitations and in-gel ERK2 assays were performed according to previously described techniques (25, 31). Subconfluent AR42J cells were cultured for 24 h in serum-free DMEM, then incubated for 5 min with or without gastrin (1 pM–100 nM) or TPA (100 nM). In some experiments, GF-109203X (3.5 µM), bisindolylmaleimide V (3.5 µM), or PD-98059 (0.1 mM) were added during the last hour of the treatment period. Cells were washed four times with lysis buffer. The samples were then added to the tubes, and the solutions were mixed for one additional hour. After centrifugation, the pellets were washed four times with lysis buffer. The samples were resuspended in 60 µl of electrophoresis buffer (for 10 µl: 1 ml glycerol, 0.5 ml 2-mercaptoethanol, 3 ml 10% sodium dodecyl sulfate (SDS), 1.25 ml 1 M tris(hydroxymethyl)methyamine (Tris) buffer, 2 ml 0.1% bromophenol blue, and 0.6 g urea), boiled for 5 min, and applied to a 10% SDS-polyacrylamide gel containing 0.5 mg/ml myelin basic protein (Sigma). After electrophoresis, the gel was washed with two changes of 20% 2-propanol in 50 mM Tris (pH 8.0) for 1 h and then with two changes of 50 mM Tris (pH 8.0) containing 5 mM 2-mercaptoethanol for 1 h. The enzyme was denatured by incubating the gel with two changes of 6 M guanidine hydrochloride for 1 h and then rehydrated with five changes of 50 mM Tris (pH 8.0) containing 0.04% Tween 20 and 5 mM 2-mercaptoethanol for 1 h. The kinase reaction was performed in conditions inhibitory to cyclic nucleotide-dependent protein kinase and Ca2+-dependent protein kinases by incubating the gel at 25°C for 1 h with 40 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (pH 8.0) containing 0.5 mM EGTA, 10 mM MgCl2, 2 µM protein kinase inhibitor peptide, 40 µM ATP, and 2.5 µCi/ml of [γ-32P]ATP (6000 Ci/mmol). After incubation, the gel was washed with a 5% (wt/vol) trichloroacetic acid solution containing 1% (wt/vol) sodium pyrophosphate, dried, and subjected to autoradiography.

Proliferation studies. These studies were conducted according to previously described techniques (27). Briefly, the AR42J cells were grown in 35-mm dishes in DMEM supplemented with 10% FBS in 5% CO2 at 37°C. Subconfluent AR42J cells were cultured for 24 h in serum-free DMEM containing 0.2 mM unlabeled thymidine. After being washed with serum-free medium, the cells were treated with gastrin (1 nM) or G-Gly (1 nM) for 18 h. In some experiments, PD-98059 (50 µM) was added 30 min prior to the addition of gastrin and G-Gly. Control experiments were performed by incubating the cells in either incubation buffer or vehicle without the test substances. DNA synthesis was estimated by measuring [3H]thymidine incorporation into the trichloroacetic acid-precipitable material according to the method of Seva et al. (27). [3H]Thymidine (0.1 µCi/ml, 10 Ci/mmol) was added during the last hour of the treatment period. Cells were washed with serum-free media to remove unincorporated [3H]thymidine, and the DNA was precipitated with 5% trichloroacetic acid at 4°C for 15 min. Precipitates were washed twice with 95% ethanol, dissolved in 1 ml of 0.1 N NaOH, and analyzed in a liquid scintillation counter (Beckman Instruments, Palo Alto, CA).

Data analysis. Data are presented as means ± SE, where n is equal to the number of separate transfections performed with the AR42J cells. Statistical analysis was performed using Student’s t-test. P < 0.05 was considered significant.

RESULTS

We first investigated the effect of increasing concentrations of gastrin on SRE-luciferase activity. For these experiments, we cotransfected the AR42J cells with luciferase reporter plasmids containing the c-fos SRE upstream of the thymidine kinase (TK) gene minimal promoter and the luciferase reporter gene together with the pCMV-βGal expression vector. As shown in Fig. 1, gastrin dose dependently stimulated SRE-luciferase activity (1.76 ± 0.08-fold induction over control, n = 3; 3.24 ± 0.4-fold induction over control, n = 3; and 3.46 ± 0.87-fold induction over control, n =
3, in the presence of 0.1, 1, and 10 nM gastrin, respectively). Gastrin interacts with at least two different cellular receptor subtypes, designated CCK-A and CCK-B, respectively (5, 15). Thus we investigated which receptor subtype mediates the stimulatory action of gastrin on SRE-luciferase activity. As depicted in Fig. 2, 10 nM gastrin induced SRE transcriptional activity, and this effect was blocked by the highly selective CCK-B receptor antagonist D2 (3.73 ± 0.75-fold induction over control, \( n = 3 \), in the presence of gastrin vs. 1.36 ± 0.14-fold induction over control, \( n = 3 \), in the presence of gastrin in combination with 10 nM D2). In contrast, no inhibition was observed in the presence of the selective CCK-A receptor antagonist L-364,718 (4.03 ± 0.57-fold induction over control, \( n = 3 \), in the presence of gastrin vs. 4.23 ± 0.49-fold induction over control, \( n = 3 \), in the presence of gastrin in combination with 10 nM L-364,718), suggesting that the CCK-B receptor mediates gastrin induction of c-fos gene transcription. Treatment of the cells with either the D2 compound or L-364,718 alone did not have any significant effect on SRE transcriptional activity (1.11 ± 0.13-fold induction over control, \( n = 3 \), and 1.23 ± 0.18-fold induction over control, \( n = 3 \), in the presence of D2 and L-364,718, respectively). As previously reported, cells transfected with a plasmid containing the TK gene minimal promoter but devoid of the SRE exhibited no induction in response to treatment with gastrin (31).

To characterize the signaling pathways that target the c-fos SRE in response to stimulation with gastrin, we performed studies in which we preincubated the AR42J cells with 200 ng/ml pertussis toxin, an agent known to ADP ribosylate and thereby inactivate some GTP binding proteins such as \( G_i \). Pertussis toxin pretreatment failed to inhibit gastrin stimulation of SRE-luciferase activity (4 ± 0.6-fold induction over control, \( n = 3 \), in the presence of gastrin vs. 4.23 ± 0.67-fold induction over control, \( n = 3 \), in the presence of gastrin in combination with pertussis toxin) (Fig. 3), indicating that this effect of gastrin is mediated by a mechanism independent of \( G_i \). Treatment of the cells with pertussis toxin alone exhibited a 1.14 ± 0.15-fold induction over control (\( n = 3 \)).

One of the best-characterized signal-transduction pathways activated by gastrin on interaction with the CCK-B receptor involves PKC stimulation (5). Accordingly, we performed studies to examine whether PKC mediated gastrin induction of SRE-luciferase activity. As depicted in Fig. 4A, the selective PKC inhibitor GF-109203X (3.5 µM) significantly inhibited, although not completely, gastrin-stimulated SRE luciferase activity (2.78 ± 0.19-fold induction over control, \( n = 3 \), in the presence of gastrin vs. 1.37 ± 0.21-fold induction over control, \( n = 3 \), in the presence of gastrin in combination with GF-109203X). Identical results were obtained when PKC was inhibited by prolonged (24 h) incubation of the cells with 100 nM TPA (data not shown). TPA
(100 nM) stimulated SRE transcriptional activity, and this effect was blocked by 3.5 µM GF-109203X (2.78 ± 0.07-fold induction over control, n = 4, in the presence of TPA vs. 0.79 ± 0.045-fold induction over control, n = 4, in the presence of TPA in combination with GF-109203X) (Fig. 4B), demonstrating that GF-109203X is able to inhibit PKC completely in AR42J cells. Identical results were obtained when the cells were preincubated for 24 h with 100 nM TPA (data not shown). No induction of SRE transcriptional activity was detected in the presence of either GF-109203X alone (0.97 ± 0.1-fold induction over control, n = 3) (Fig. 4A) or vehicle (0.1% DMSO) (data not shown). Thus gastrin specifically targets the c-fos SRE via both PKC-dependent and -independent pathways.

Because activation of the c-fos SRE is known to require induction of the ERKs and of their upstream activator, MEK, we conducted experiments to examine the effect of the highly specific MEK inhibitor PD-98059, known to induce complete ERK inhibition at doses ranging between 50 and 100 µM (1). We observed, as shown in Fig. 5, that PD-98059 (50 µM) potently inhibited SRE-luciferase activity stimulated by gastrin (3.17 ± 0.64-fold induction over control, n = 4, in the presence of gastrin vs. 1.28 ± 0.26-fold induction over control, n = 4, in the presence of gastrin in combination with PD-98059), confirming the involvement of MEK in gastrin induction of the c-fos SRE. Treatment of the cells with PD-98059 alone had no effect on SRE luciferase activity (1.08 ± 0.22-fold induction over control, n = 4).

Because ERK2 activation is required for induction of the SRE in response to growth factor stimulation (18), we tested the effect of gastrin on ERK2 activity using in-gel kinase assays. Gastrin induced ERK2 in a dose-dependent fashion with a maximal effect detected between 10^{-6} and 10^{-7} M (Fig. 6). We then examined whether inhibition of PKC resulted in inhibition of gastrin-stimulated ERK2 activity. As shown in Fig. 7, addition of 3.5 µM GF-109203X produced partial inhibition of gastrin-stimulated ERK2 activity, whereas no
Effect was observed in the presence of the inactive PKC inhibitor bisindolylmaleimide V (3.5 µM). In contrast, GF-109203X completely inhibited the induction observed in the presence of 0.1 µM TPA, confirming the notion that gastrin targets ERK2 through pathways that depend only partially on activation of PKC. Identical results were obtained when the cells were preincubated for 24 h with 0.1 µM TPA prior to the addition of either gastrin or TPA (data not shown). To study the relative contribution of PKC and MEK to the stimulatory effect of gastrin on ERK2 activation, we performed studies in which we compared the inhibitory effects of GF-109203X and PD-98059 alone with those of both agents combined. Addition of GF-109203X (3.5 µM) and of a supramaximal dose of PD-98059 (100 µM), either alone or in combination, produced, respectively, partial and total inhibition of gastrin-induced ERK2 activity (Fig. 8), suggesting that both MEK and PKC are activated in response to gastrin stimulation.

ERK2 activation induces c-fos gene expression by phosphorylation of the transcription factor Elk-1, which binds to the c-fos SRE (18). To examine whether this pathway mediated the effects of gastrin, we used a yeast hybrid system involving cotransfection of the cells with a chimeric Gal4-ElkC expression vector and the 5×Gal-luciferase reporter plasmid. In this system, Gal4-ElkC transactivates and stimulates luciferase activity only if the carboxy terminus of Elk-1 is phosphorylated by ERK2. As depicted in Fig. 9, 10 nM gastrin induced a 3.22 ± 0.5-fold increase (n = 3) in 5×Gal-luciferase activity, demonstrating that gastrin induction of the c-fos SRE depends, at least in part, on ERK2 activation and Elk-1 phosphorylation.

We finally examined whether inhibition of MEK resulted in inhibition of cellular proliferation. For these experiments, the AR42J cells were incubated in the presence of gastrin, alone or in combination with PD-98059. Gastrin (1 nM) induced [3H]thymidine incorporation into AR42J cells by 1.64 ± 0.2-fold (n = 6) over unstimulated control cells, and this effect was inhibited in the presence of PD-98059 (1.11 ± 0.08-fold over control, n = 6), demonstrating that activation of MEK and ERK is required for the growth-promoting effect of gastrin (Fig. 10A). In contrast, as shown in Fig. 10B, PD-98059 failed to inhibit AR42J cell proliferation stimulated by 1 nM G-Gly (1.33 ± 0.03-fold induction over control, n = 5, in the presence of G-Gly vs. 1.34 ± 0.03-fold induction over control, n = 5, in the presence of G-Gly in combination with PD-98059), which, as previously reported, is known to activate different

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**Fig. 7.** Effect of the PKC inhibitor GF-109203X on G17- and TPA-stimulated extracellular signal regulated kinase-2 (ERK2) activity in AR42J cells. A: ERK2 in lysates from AR42J cells stimulated with 10 nM G17 and 0.1 µM TPA, alone or in combination with either GF-109203X (3.5 µM) or bisindolylmaleimide V (3.5 µM), was immunoprecipitated, and its activity was measured by in-gel kinase assays. B: linear transformation of the densitometric analysis of the autoradiograms. Data are representative of a single experiment.

**Fig. 8.** Effect of the MEK inhibitor PD-98059 and of the PKC inhibitor GF-109203X on G17-stimulated ERK2 activity in AR42J cells. A: ERK2 in lysates from AR42J cells stimulated with 10 nM G17, alone or in combination with either 100 µM PD-98059 or 3.5 µM GF-109203X and in the presence of both 100 µM PD-98059 and 3.5 µM GF109203X combined, was immunoprecipitated, and its activity was measured by in-gel kinase assays. B: linear transformation of the densitometric analysis of the autoradiograms. Identical results were obtained in one other separate experiment.
means shown). Data are expressed as multiple-fold induction over control; reporter plasmid gave only background luciferase activity (data not shown). Data are expressed as multiple-fold induction over control; means ± S.E. *P < 0.05.

signal-transduction pathways (31). Treatment of the cells with PD-98059 alone exhibited no significant effect on AR42J cell proliferation.

DISCUSSION

Seven transmembrane receptors interact with heterotrimeric GTP binding proteins to transduce extracellular signals to downstream effector molecules (10, 21). A number of studies have recently indicated that these receptors can signal through pathways that were initially described in response to activation of receptor tyrosine kinases by growth factors (2, 7, 12, 17, 22, 24, 25). One of the best characterized of these pathways involves the activation of Ras, Raf, and of the dual-specificity protein kinase MEK, which in turn phosphorylates both serine and tyrosine residues in a family of serine-threonine protein kinases known as MAPKs or ERKs (14). The ERKs are proline-directed protein kinases that phosphorylate numerous cellular proteins, including downstream protein kinases such as 90-kDa S6 kinase (6, 9) and transcription factors like Elk-1 that regulate the activity of the promoter of the early response gene c-fos through the SRE (14, 18, 32). Thus the ERKs, via activation of early gene function and of downstream protein kinases, play a crucial role in the process of amplification, integration, and transmission of the extracellular signals from the cell surface to the nucleus.

The early response gene c-fos is a member of a family of genes that are turned on within minutes of cellular activation by growth factors, hormones, and neurotransmitters, and it appears to be essential for the activation or repression of the next set of genes in the biological programs initiated by the extracellular signals (14, 20). The promoter of c-fos has been the focus of extensive investigations, and numerous DNA regulatory elements have been mapped and characterized. One of these elements, the SRE, has been found to receive inputs from growth factor-activated signal-transduction pathways. The SRE is in fact regulated by numerous transcription factors that are constitutively bound to the DNA and are rapidly phosphorylated and activated in response to growth factor stimulation (14, 32). Elk-1 in particular is a member of a well-characterized family of transcription factors, which is known to interact with the serum response factor to form a ternary complex that plays an important role in the activation of c-fos transcription (14, 32). In response to growth factors, Elk-1 is rapidly phosphorylated and transcriptionally activated by protein kinases such as ERK2, resulting in stimulation of c-fos gene expression (32). In some systems, the activation of this pathway leads to induction of cellular growth and proliferation (19).

The gastrointestinal hormone gastrin interacts with two separate cellular receptors known as CCK-A and CCK-B, respectively, which are members of the seven transmembrane receptor family (5, 15). This hormone has been found to be a potent growth factor for both normal and neoplastic gastrointestinal tissues (16, 23, 28, 29, 33). In our study, we have analyzed some of the signal-transduction pathways that are activated by gastrin via interaction with CCK-B receptors and that appear to be linked to stimulation of cellular proliferation. Gastrin is known to induce mobilization of intracellular Ca²⁺ and to activate PKC (5). In addition, we and others have reported that gastrin can induce the ERKs and stimulate c-fos gene expression via induction of SRE transcriptional activity (31). In the present study, we have confirmed, using highly specific CCK-B and CCK-A receptor antagonists, that the stimulatory effect of gastrin on c-fos gene transcription regulated by the SRE is mediated by the CCK-B receptor subtype. In addition, we have demonstrated that this effect of gastrin is independent of activation of the GTP binding protein G₁₇, since incubation of the cells with pertussis
toxin failed to affect gastrin induction of SRE transcriptional activity. Activation of the ERKs and c-fos by seven-transmembrane, G protein-coupled receptors has been reported to be both pertussis toxin sensitive and insensitive. Although the adrenergic \( \text{C}2 \alpha \) and the muscarinic M2 receptors, both known to couple to \( \text{G}_i \), are sensitive to treatment with pertussis toxin (8, 12), the \( \text{G}_q \)-coupled adrenergic \( \text{C}1 \beta \) and the muscarinic M1 receptors are not (12), demonstrating that both \( \text{G}_i \)-sensitive and -insensitive pathways can target the ERKs.

In our study, we reported that inhibition of either PKC or MEK with the use of highly selective and specific inhibitors led to only partial inhibition of ERK2 activation. In the presence of both agents combined, however, we observed that this inhibition was complete, suggesting that both PKC-dependent and -independent and MEK-dependent pathways appear to mediate gastrin action. Although PKC has been shown to induce \( \text{c-Raf} \) directly and to increase \( \text{Ras} \) activation (6, 9), it is clear that gastrin signals to ERK2 and c-fos through pathways that are only partially dependent on activation of PKC. Similar results were reported in the case of the thyrotropin-releasing hormone (22) and the angiotensin II receptors (24), which are known to target the ERKs through pathways that are, at least in part, pertussis toxin insensitive and PKC independent. These receptors can induce the activation of protein tyrosine kinases, which, in turn, phosphorylate the adapter molecule Shc and allow the formation of a complex with the adapter protein Grb2 and the mammalian guanine nucleotide exchange factor mSos that leads to the activation of \( \text{Ras} \) (22, 24). Furthermore, the observation that addition of supramaximal doses of the MEK inhibitor PD-98059 leads to incomplete inhibition of gastrin-stimulated ERK2 induction indicates that PKC does not totally lie upstream of MEK but that it can target ERK2 via novel, yet unidentified, signaling pathways.

In contrast to these findings, other investigators have examined the trophic effects of gastrin in a fibroblast cell line stably transfected with the human gastrin/CCK-B receptor and found that, in these cells, gastrin stimulates both MAPK and Raf-1 kinase via PKC-independent pathways (26), underscoring the complexity of the cellular activation events that are initiated by the interaction of gastrin with its specific cellular receptors.

We demonstrated that gastrin stimulation of the c-fos SRE is mediated by activation of ERK2 and induction of Elk-1 transcriptional activity. Thus gastrin can activate a cascade of intracellular phosphorylation reactions that involves PKC-dependent and -independent pathways that, in turn, induce c-fos through Elk-1 transcriptional activation. The physiological relevance of this chain of events was underscored by the finding that inhibition of the ERKs led to inhibition of cellular proliferation, thus demonstrating that this CCK-B receptor-linked signal-transduction pathway is essential for the transmission of mitogenic signals. The specificity of this effect was further confirmed by the observation that PD-98059 failed to inhibit AR42J cell proliferation stimulated by G-Gly. This processing intermediate of gastrin has been shown to induce AR42J cell proliferation via signal-transduction pathways that do not involve activation of the ERKs and induction of c-fos (31).

Our data suggest a working model for the growth-promoting effect of gastrin. Gastrin binding to specific CCK-B receptors on the AR42J cells induces a complex cascade of reactions that targets and activates ERK2 via both PKC-dependent and -independent pathways. ERK2, in turn, induces c-fos gene expression through phosphorylation and transcriptional activation of Elk-1. The net result of this cascade of events appears to be stimulation of cellular proliferation. These data underscore the complexity of cellular activation events initiated by ligand-receptor interaction on the AR42J cell membrane.

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Address for reprint requests: A. Todisco, 6520 MSRB I, Ann Arbor, MI 48109-0682.

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