Epidermal growth factor regulates adenylate cyclase activity via G_s and G_{i1-2} proteins in pancreatic acinar membranes

Danuta STRYJEK-KAMINSKA, Albrecht PIIPER and Stefan ZEUZEM*

II. Medical Department, University of Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt/Main, Federal Republic of Germany

In the present study, Western-blot and radioreceptor analyses have revealed the presence of the epidermal growth factor (EGF) receptor in pancreatic acinar membranes. Isolated pancreatic acinar membranes, which allow access of functional antibodies to individual components of the signal transduction cascade, were used to examine EGF-induced regulation of adenylate cyclase activity. Forskolin, vasoactive intestinal peptide (VIP) and to a smaller extent EGF increased cAMP production in pancreatic acinar membranes. Preincubation of the membranes with anti- G_{sz} antibody abolished EGF- and VIP-induced cAMP production, but had no effect on forskolin-induced cAMP accumulation. In the presence of either VIP or forskolin, EGF inhibited the VIP- and forskolin-induced cAMP production with an IC_{50} of 5 nM. Anti- G_{zi1-2} protein antibody, but not anti- G_{zi3} antibody, increased basal cAMP production, indicating that G_i proteins exert an inhibitory influence on basal adenylate cyclase

activity. Anti- G_{zi1-2} antibody, but not anti- G_{zi3} antibody, abolished the inhibitory effect of EGF on the forskolin- and VIP-induced cAMP accumulation. A peptide corresponding to the juxtamembrane region in the cytosolic domain of the rat EGF receptor increased cAMP production in pancreatic acinar membranes in an anti- G_{sz} antibody-sensitive fashion, whereas the EGF receptor peptide did not mimic the inhibitory effect of the native EGF receptor. The tyrosine kinase inhibitors genistein and pp60^{v-src} (137–157) inhibited both the stimulatory and the inhibitory effects of EGF on cAMP production. Thus the data of the present study show that EGF regulates adenylate cyclase via activation of G_s and G_i proteins by a tyrosine phosphorylation-dependent mechanism in pancreatic acinar membranes. This leads to stimulation of basal and inhibition of forskolin- and VIP-induced adenylate cyclase activity respectively.

INTRODUCTION

Epidermal growth factor (EGF) is a 53-amino-acid polypeptide with potent mitogenic effects on many cell types [1]. In addition, EGF exerts biological effects apparently unrelated to its mitogenicity, such as inhibition of gastric acid secretion and vasodilatation [2,3]. EGF binds to a receptor with a cytoplasmic protein tyrosine kinase domain. Activation of the EGF receptor initiates a complex series of events including autophosphorylation of the receptor and tyrosine phosphorylation of various substrates [1,4].

G-proteins are not yet generally recognized as important mediators in growth factor signalling. In hepatocytes the EGF receptor interacts with pertussis toxin-sensitive G-proteins and activates phospholipase C via pertussis toxin-sensitive G-proteins [5–8]. Similarly, in renal epithelial cells EGF increases turnover of inositol phospholipids by a pertussis toxin-sensitive mechanism [9]. In parietal cells of the stomach, EGF inhibits histamine-induced cAMP production via a pertussis toxin-sensitive mechanism [10], suggesting that EGF-induced activation of G_i -type G-proteins is coupled to inhibition of adenylate cyclase in these cells. In the rat heart and parotid, EGF increases cAMP production apparently via activation of G_s proteins [11,12]. Taken together, these results suggest that G_s - and G_i -type G-proteins might be involved in EGF receptor signalling in a cell specific fashion.

Incubation of pancreatic acini with EGF results in altered ADP-ribosylation of α -subunits of the G_s and G_{i1} , G_{i2} and G_{i3} proteins [13], suggesting EGF-induced activation of these G-

proteins. In the present study antisera raised against sequences in the C-terminal part of different G-protein α -subunits that abolish interaction of G_α with receptors were used to characterize the role of G-protein isoforms in EGF-induced regulation of adenylate cyclase in isolated pancreatic acinar membranes.

MATERIALS AND METHODS

Materials

Pertussis toxin, NAD⁺, human recombinant EGF, vasoactive intestinal peptide (VIP), 3-isobutyl-1-methylxanthine, forskolin, soybean trypsin inhibitor, collagenase type III and donkey anti-sheep IgG were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). Affinity-purified horseradish peroxidase-conjugated goat anti-rabbit IgG was from Bio-Rad Laboratories (Munich, Germany). Anti-EGF receptor antibody was from UBI (Lake Placid, NY, U.S.A.). The cAMP assay kit, a polyclonal antibody raised against the α -subunit of G_i (QL) and ¹²⁵I-labelled EGF (0.8–1.1 Ci/ μ mol) were from DuPont–New England Nuclear (Bad Homburg, Germany). Affinity-purified polyclonal G-protein antibodies raised against G_{sz} (385–394), G_{zi1-2} (345–354) and G_{zi3} (345–354) were purchased from Calbiochem (Frankfurt, Germany). EGF receptor substrate comprising pp60^{v-src} (137–157) was obtained from Biomol (Hamburg, Germany). The enhanced chemiluminescence (ECL) system was from Amersham Buchler (Braunschweig, Germany). The peptide corresponding to Arg⁶⁴⁶–Arg⁶⁵⁸ of the rat EGF receptor (Arg–Arg–Arg–Glu–Ile–Val–Arg–Lys–Arg–Thr–Leu–Arg–Arg) [14] was synthesized with

Abbreviations used: ECL, enhanced chemiluminescence; EGF, epidermal growth factor; G_{sz} , α -subunit of adenylate cyclase-stimulating G protein (G_s protein); G_{zi1} , G_{zi2} , G_{zi3} , isoforms of α -subunits of adenylate cyclase-inhibiting G-proteins (G_i proteins); $G_{\alpha q/11}$, α -subunit of cholera and pertussis toxin-insensitive G-proteins ($G_{q/11}$ proteins); VIP, vasoactive intestinal peptide.

* To whom correspondence should be addressed.

fluoren-9-ylmethoxycarbonyl chemistry and was purified by C_{18} reversed-phase HPLC. Purity exceeded 95% (Neosystem, Strasbourg, France).

Preparation of pancreatic acinar membranes

Pancreatic acini were isolated from rat pancreas by collagenase digestion as described [15–17]. Isolated pancreatic acini were homogenized in six volumes of ice-cold homogenization buffer containing 25 mM Hepes, pH 7.0, 0.3 M sucrose, 0.5 mM $MgCl_2$, 1 mM benzamide, 0.5 mM PMSF, 0.01% aprotinin, 0.01% leupeptin and 0.01% trypsin inhibitor by 20 strokes in a glass/Teflon Potter–Elvehjem homogenizer. The homogenate was centrifuged at 8000 g for 15 min, 4 °C, and the membranes in the supernatant were pelleted by centrifugation at 50000 g for 25 min, 4 °C.

Receptor binding assay

Receptor binding studies were performed in isolated pancreatic acinar membranes. Membranes (0.4 mg/ml protein) were suspended in a binding medium containing 150 mM NaCl, 5 mM $MgCl_2$, 20 mM Tris/HCl, pH 7.4, 10 μ g/ml bacitracin, 0.5 mg/ml BSA, 30000 c.p.m. ^{125}I -labelled EGF (30 pM), and increasing amounts of unlabelled EGF in a final volume of 500 μ l. After incubation for 2 h at room temperature, membranes were separated from the incubation medium by centrifugation. Membrane-associated radioactivity was measured after the pellets had been washed twice with ice-cold saline solution. Non-saturable binding of radioligand was determined as the amount of radioactivity associated with the pellet in the presence of excess unlabelled ligand and was less than 30% of total binding.

Immunoblot analysis

After separation by SDS/PAGE, the membrane proteins were electrotransferred to nitrocellulose sheets (0.2 μ m). The blots were blocked for 30 min at room temperature in blocking buffer [2% (w/v) BSA, 20 mM Tris/HCl, pH 7.4, 140 mM NaCl, 0.1% Tween-20] and subsequently incubated with G-protein α -specific antibodies (1:1000 dilution in blocking buffer) for 2 h at room temperature. Antigen–antibody complexes were revealed with the appropriate horseradish peroxidase-conjugated antibodies and an ECL system.

cAMP production in isolated pancreatic acinar membranes

Pancreatic acinar membranes (100 μ l; 20 mg/ml protein) suspended in homogenization buffer were incubated with or without antisera (1:10 to 1:100) for 2 h at 4 °C. Then the acini were diluted in 1.9 ml of a medium containing 145 mM NaCl, 20 mM Hepes/Tris, pH 7.0, 10 μ M GTP, 1 mM ATP, 1 mM 3-isobutyl-1-methylxanthine, 10 mM creatine phosphate and 50 units/ml creatine kinase and incubated with agonists at 37 °C. At the beginning and after 1, 3 and 5 min of the incubation, 280 μ l aliquots were removed and mixed with 120 μ l of ice-cold trichloroacetic acid [20% (w/v)]. The cAMP content of the samples was determined by using a radioimmunoassay and was performed in accordance with the protocol provided by the manufacturer [15]. All experiments were repeated at least three times with membranes from different preparations. The data presented are means $\pm$ S.E.M. Statistical significance was calculated with Student's t -test for paired values.

RESULTS

Presence of the EGF receptor in rat pancreatic acinar membranes

Immunological analysis of pancreatic acinar membranes revealed the presence of the 170 kDa EGF receptor (results not shown). When isolated pancreatic acinar membranes were incubated with ^{125}I -labelled EGF (30 pM) and increasing amounts of unlabelled EGF, half-maximal displacement of the radioligand occurred at an EGF concentration of 1.0 ± 0.9 nM. This supports previous data showing that ^{125}I -labelled EGF binds to intact pancreatic acini with a K_d of 4.9 nM [18].

Effect of EGF on cAMP production in isolated pancreatic acinar membranes

We have recently shown that EGF causes cAMP accumulation in intact pancreatic acini [15]. As illustrated in Figure 1(A), 90 nM EGF also stimulated cAMP production in pancreatic acinar membranes, but was only 40% as effective as 10 nM VIP. Half-maximal stimulation of cAMP accumulation was observed at 7 nM EGF (Figure 1B).

Figure 1 Effect of EGF and VIP on cAMP accumulation in pancreatic acinar membranes

(A) Pancreatic acini were incubated with vehicle (water) (○), 90 nM EGF (●) or 10 nM VIP (△) for the indicated times. At the beginning of the incubation and after 1, 3 and 5 min, 280 μ l aliquots were removed and the cAMP content of the samples was determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. for six experiments. (B) Dose–response curve for EGF-induced cAMP accumulation after 5 min of incubation. The data shown are means $\pm$ S.E.M. for three different experiments. Asterisks indicate a significant increase of EGF- and VIP-induced cAMP accumulation over basal levels: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 1 Effect of anti- G_{zs} antibody on EGF-, VIP-, forskolin- and EGF receptor peptide-induced cAMP accumulation

Pancreatic acinar membranes were preincubated with normal rabbit serum (control) or anti- G_{zs} antibody at the indicated dilutions for 2 h at 4 °C. Then membranes were incubated with 90 nM EGF, 10 nM VIP, 0.1 mM forskolin, 10 μ M EGF receptor (EGFR) peptide or vehicle. At the beginning of the incubation and after 5 min, 280 μ l aliquots were removed and the cAMP content of the samples was determined as described in the Materials and methods section. The results are means $\pm$ S.E.M. for four different experiments. Asterisks indicate significant inhibition of EGF-, VIP-, forskolin- and EGF receptor peptide-induced cAMP accumulation by anti- G_{zs} antibody respectively (* P < 0.05, *** P < 0.001); results without asterisks are not significant; n.d., not determined.

	cAMP accumulation (pmol/min per mg of protein)			
	Control	Anti- G_{zs} dilution		
		1:100	1:50	1:10
Basal	0.7 $\pm$ 0.2	n.d.	n.d.	1.1 $\pm$ 0.2
EGF	2.5 $\pm$ 0.4	2.2 $\pm$ 0.5	2.0 $\pm$ 0.6	1.6 $\pm$ 0.5*
VIP	3.4 $\pm$ 0.5	2.7 $\pm$ 0.5	2.3 $\pm$ 0.5*	2.0 $\pm$ 0.4***
Forskolin	4.6 $\pm$ 0.7	n.d.	n.d.	4.3 $\pm$ 0.8
EGFR-peptide (Arg ⁶⁴⁶ -Arg ⁶⁵⁸)	1.8 $\pm$ 0.2	n.d.	n.d.	0.8 $\pm$ 0.2*

To investigate whether EGF-induced cAMP production depends on tyrosine phosphorylation, EGF-induced cAMP accumulation was measured in the presence of the protein kinase inhibitor pp60^{v-src} (137–157). The EGF receptor substrate pp60^{v-src} (137–157) (20 μ M) decreased EGF-induced cAMP production by 80 % (n = 3, P < 0.05). The EGF receptor substrate had no effect on basal cAMP production or that induced by 10 nM VIP or 0.1 mM forskolin, confirming the specificity of the effect of EGF receptor substrate on adenylate cyclase activity. Similar data were obtained with the tyrosine kinase inhibitor genistein (80 μ M) (results not shown).

Anti- G_{zs} antibody inhibits EGF- and VIP-induced cAMP accumulation

To investigate the mechanism of EGF-induced cAMP production, pancreatic acinar membranes were preincubated with normal rabbit serum or antibodies raised against the C-terminus of the α -subunit of G_s -protein. The C-terminus of G_{zs} proteins is involved in binding the G-protein to the receptor [19]. Western blot analysis showed that this antibody recognizes the low- and high-molecular-mass forms of G_{zs} subunits in the membranes (results not shown). As illustrated in Table 1, preincubation of the membranes with anti- G_{zs} antibody led to a dose-dependent inhibition of EGF-induced cAMP production. At an antibody dilution of 1:10, anti- G_{zs} antibody inhibited EGF (90 nM)-induced cAMP accumulation by 55 $\pm$ 18 % (n = 4). Similarly, anti- G_{zs} antibody inhibited VIP (10 nM)-induced cAMP production (Table 1). Anti- G_{zs} antibody had no effect on basal cAMP accumulation or on cAMP production in response to 0.1 mM forskolin, which directly activates adenylate cyclase. These results indicate that the effect of anti- G_{zs} antibody is specific. A synthetic peptide of the juxtamembrane region in the cytosolic domain of the EGF receptor that has recently been shown to stimulate isolated G_s proteins [14] mimicked the stimulatory effect of EGF on cAMP accumulation, i.e. it increased cAMP production in an anti- G_{zs} antibody-sensitive fashion (Table 1). In contrast, the EGF receptor peptide had no effect on forskolin-induced cAMP production, indicating that

Figure 2 Effect of EGF on VIP- and forskolin-induced cAMP production

Pancreatic acinar membranes were incubated for 5 min with 10 nM VIP or 0.1 mM forskolin in the presence and absence of EGF. The amount of cAMP was determined as described in the Materials and methods section. Results are means $\pm$ S.E.M. for four different experiments. Significance levels: * P < 0.05, *** P < 0.001. Symbols: ■, forskolin; ▲, VIP.

Table 2 Effect of the protein tyrosine kinase inhibitors genistein (80 μ M) and pp60^{v-src} (20 μ M) on EGF (90 nM)-induced inhibition of cAMP accumulation induced by 10 nM VIP or 0.1 mM forskolin

The amount of cAMP generated was determined as described in the Materials and methods section. Results are means $\pm$ S.E.M. for three different experiments. Asterisks indicate a significant effect of the respective tyrosine kinase inhibitor: * P < 0.05; results without asterisks are not significant.

	cAMP accumulation (pmol/min per mg of protein)		
	Control	pp60 ^{v-src}	Genistein
VIP	3.4 $\pm$ 0.9	3.1 $\pm$ 0.4	4.0 $\pm$ 0.7
VIP + EGF	1.9 $\pm$ 0.4	3.0 $\pm$ 0.7*	3.2 $\pm$ 0.6*
Forskolin	4.4 $\pm$ 0.9	4.6 $\pm$ 1.2	4.4 $\pm$ 0.7
Forskolin + EGF	2.2 $\pm$ 0.7	4.3 $\pm$ 0.9*	4.4 $\pm$ 1.0*

this peptide does not mimic the inhibitory effect of EGF on forskolin-induced cAMP accumulation (results not shown).

Effect of EGF on forskolin- and VIP-induced cAMP production

Because EGF inhibits VIP- and forskolin-induced cAMP production in intact pancreatic acini [15], we examined the effect of EGF on forskolin- and VIP-induced cAMP production in isolated pancreatic acinar membranes. As shown in Figure 2, EGF dose-dependently inhibited the cAMP production induced by 10 nM VIP or 0.1 mM forskolin. The EC₅₀ of EGF-induced inhibition of the VIP- and forskolin-induced cAMP accumulation was 5 nM EGF (n = 3). These results confirm the results from intact pancreatic acini that EGF inhibits VIP- and forskolin-induced activation of adenylate cyclase [15].

To investigate whether EGF-induced inhibition of VIP- and forskolin-induced cAMP production depends on tyrosine phosphorylation, cAMP accumulation induced by 10 nM VIP or 0.1 mM forskolin was measured in the presence of protein tyrosine kinase inhibitors. As shown in Table 2, the specific tyrosine kinase inhibitor pp60^{v-src} (137–157) (20 μ M) diminished the inhibitory effect of 90 nM EGF on the cAMP production induced by 10 nM VIP or 0.1 mM forskolin by 70 % and 86 %

Table 3 Effect of anti- $G_{\alpha i}$ antibodies on the inhibitory effect of EGF on VIP- and forskolin-induced cAMP production.

Pancreatic acinar membranes were preincubated with vehicle (normal rabbit serum, diluted 1:10), anti- $G_{\alpha 1-2}$ or anti- $G_{\alpha 3}$ at a dilution of 1:10 for 2 h. Then membranes were incubated with 90 nM EGF, 10 nM VIP, 0.1 mM forskolin, EGF + VIP or EGF + forskolin for 5 min. The cAMP content was determined in 280 μ l aliquots as described in the Materials and methods section. Results are means $\pm$ S.E.M. for four different experiments with different membrane preparations. Asterisks indicate a significant effect of the respective tyrosine kinase inhibitor: * $P < 0.05$; results without asterisks are not significant.

	cAMP accumulation (pmol/min per mg of protein)		
	Control	Anti- $G_{\alpha 1-2}$	Anti- $G_{\alpha 3}$
Basal	0.8 $\pm$ 0.2	1.5 $\pm$ 0.3*	0.7 $\pm$ 0.4
EGF	1.6 $\pm$ 0.4	2.1 $\pm$ 0.5	1.1 $\pm$ 0.6
VIP	3.0 $\pm$ 0.5	4.0 $\pm$ 0.9*	3.4 $\pm$ 0.5
VIP + EGF	1.4 $\pm$ 0.5	3.6 $\pm$ 0.7*	1.3 $\pm$ 0.6
Forskolin	4.5 $\pm$ 0.7	4.6 $\pm$ 0.8	5.1 $\pm$ 0.8
Forskolin + EGF	1.3 $\pm$ 1.0	4.2 $\pm$ 0.9*	1.5 $\pm$ 0.6

respectively ($n = 3$, $P < 0.05$). The effect was observed during the complete observation period, but was most pronounced 1 min after the start of the experiment. These results indicate that tyrosine kinase activity is required for the inhibitory effect of EGF on VIP- or forskolin-induced activation of adenylate cyclase. Similar data were obtained with the less specific tyrosine kinase inhibitor genistein (80 μ M) (Table 2).

Effect of anti- $G_{\alpha i}$ antibodies on the inhibitory effect of EGF on VIP- and forskolin-induced cAMP production

Our previous results obtained in intact acini indicate that pertussis toxin-sensitive G-proteins mediate the inhibitory effect of EGF on VIP- and forskolin-induced activation of adenylate cyclase [15]. To test the possibility that EGF-induced inhibition of VIP- and forskolin-induced activation of adenylate cyclase occurs via G_i proteins, acinar cell membranes were preincubated for 2 h with different antibodies raised against the C-terminal part of the α -subunits of G_{i1} , G_{i2} and G_{i3} proteins. Western blot analyses confirmed specificity of these antisera (results not shown). Preincubation of the membranes with anti- $G_{\alpha 1-2}$ antibody abolished the inhibitory effect of 90 nM EGF on VIP- or forskolin-induced cAMP production (Table 3). EGF-mediated inhibition of VIP- or forskolin-induced cAMP production was not altered in membranes preincubated with either anti- $G_{\alpha 3}$ (Table 3) or anti- $G_{\alpha q}$ antibodies (results not shown). These results suggest that $G_{\alpha 1-2}$ antibody specifically inhibits the effect of EGF on VIP- and forskolin-stimulated cAMP accumulation.

Preincubation of pancreatic acinar membranes with anti- $G_{\alpha 1-2}$ antibody increased basal and VIP-induced cAMP production and tended to increase EGF-induced cAMP production. The latter effect, however, was not significant (Table 3). These data can be interpreted to mean that $G_{\alpha 1-2}$ proteins induce tonic inhibition of adenylate cyclase and that inhibition of G_i proteins leads to stimulation of adenylate cyclase.

DISCUSSION

Activation of the EGF receptor leads to tyrosine phosphorylation of several substrates [4,20]. Whereas EGF receptor signalling appears to be G-protein-independent in transformed cells over-expressing the EGF receptor, several lines of evidence suggest that EGF receptor signal transduction uses G-proteins in normal

cells. We have recently shown that in intact pancreatic acini EGF itself stimulates cAMP production but inhibits VIP- and forskolin-induced cAMP production by a pertussis toxin-sensitive mechanism [15]. In the present study we have extended these results to isolated pancreatic acinar membranes, which allow the use of functional antibodies to investigate the role of certain proteins in signal transduction. The data show that EGF stimulates adenylate cyclase via an anti- $G_{\alpha s}$ antibody-sensitive mechanism, whereas EGF inhibits VIP- and forskolin-induced cAMP accumulation via an anti- $G_{\alpha 1-2}$ -sensitive pathway. An anti- $G_{\alpha 3}$ antiserum did not influence EGF-induced regulation of cAMP production. These data strongly suggest that EGF activates adenylate cyclase via G_s proteins, whereas EGF inhibits activation of adenylate cyclase by VIP or forskolin via G_{i1-2} proteins. G_{i3} protein is apparently not involved in EGF-induced inhibition of adenylate cyclase, although a recent study by Pröfrock et al. [13] indicated that EGF is capable of activating all G_i isoforms.

There is a precedent for negative regulation of adenylate cyclase activity by EGF in hepatocytes and parietal cells of the stomach. In hepatocytes EGF inhibits glucagon-induced cAMP production [21]. Moreover, EGF inhibits the histamine-induced cAMP production in parietal cells of the stomach via pertussis toxin-sensitive G-proteins [10]. EGF-induced signal transduction in pancreatic acini differs from both parietal cells and hepatocytes with respect to its ability to stimulate adenylate cyclase. To our knowledge, pancreatic acini are the only cell type in which EGF exerts both stimulatory and inhibitory effects on adenylate cyclase and are thus a particularly interesting cell type for the study of EGF-mediated regulation of adenylate cyclase activity.

The mechanism by which growth factor receptors activate G-proteins is unknown. The growth factor receptors differ considerably from the 'classical' G-protein coupling receptors, which possess a seven-transmembrane-spanning α -helix structure. Although growth factor receptors lack the seven transmembrane α -helix motif, these receptors could interact with G-proteins direct. On the basis of experiments with oligopeptides corresponding to the insulin-like growth factor-2 receptor, Okamoto et al. [22,23] have postulated criteria for oligopeptides to activate G-proteins: (1) two basic amino acid residues in the N-terminus, (2) a BBXB or BBXXB (B, basic residue; X, any residue) motif at the C-terminus, and (3) a length of 14–18 amino acid residues. Recently, a peptide corresponding to the cytosolic juxtamembrane region of the rat EGF receptor (Arg⁶⁴⁶–Arg⁶⁵⁸) has been shown to stimulate isolated G_s proteins and to activate adenylate cyclase in S49 *cyc*[−] membranes after addition of G_s [14]. In the present study Arg⁶⁴⁶–Arg⁶⁵⁸ activated adenylate cyclase in an anti- $G_{\alpha s}$ antibody-sensitive fashion, whereas the peptide had no effect on forskolin-induced cAMP accumulation. This suggests that the juxtamembrane EGF receptor peptide mimicked only EGF receptor-induced stimulation of G_s but not that of G_i proteins. However, this peptide has not yet been demonstrated to inhibit binding of G_s proteins to the EGF receptor.

Ligand-dependent association of the EGF receptor with pertussis toxin-sensitive G-proteins has been demonstrated in hepatocytes, because α -subunits of pertussis toxin-sensitive G-proteins co-immunoprecipitated with the EGF receptor in an agonist-dependent fashion [7,8]. In contrast, direct association of the EGF receptor and G_s proteins remains to be demonstrated.

The present study shows that both the stimulatory and the inhibitory effects of EGF on cAMP production are inhibited by the tyrosine kinase inhibitors genistein and pp60^{b-src} (137–157). Because pp60^{b-src} (137–157) is unlikely to interfere with GTPase activity, as recently reported for other tyrosine kinase

inhibitors [24], it is conceivable that EGF-induced activation of both G_s - and G_i -type G-proteins requires tyrosine kinase activity of the EGF receptor. However, EGF does not cause tyrosine phosphorylation of G_{as} or G_{ai} (results not shown), and evidence for EGF-induced tyrosine phosphorylation of G-protein α -subunits has not yet been reported.

EGF-induced activation of G-proteins also influences the phospholipase C pathway. This is best studied in hepatocytes in which pertussis toxin abolishes EGF-induced inositol phosphate production and anti- G_i protein antibodies inhibit EGF-induced mobilization of intracellular calcium [8]. In pancreatic acini, EGF does not cause a detectable increase in inositol 1,4,5-trisphosphate level, but inhibits cholecystokinin-stimulated inositol 1,4,5-trisphosphate production, release of intracellular calcium, and amylase secretion [25–27]. Because our recent results show that pretreatment of pancreatic acini with pertussis toxin abolishes EGF-induced inhibition of bombesin-stimulated inositol 1,4,5-trisphosphate production in pancreatic acini (D. Stryjek-Kaminska, A. Piiper, R. Klengel and S. Zeuzem, unpublished work), it seems that EGF inhibits both signalling cascades, i.e. adenylate cyclase and phosphoinositide-specific phospholipase C pathways, by activation of pertussis toxin-sensitive G-proteins.

The question of whether EGF is a physiological regulator of amylase secretion *in vivo* cannot be answered by the present study. EGF and the EGF receptor agonist transforming growth factor- α are produced in both pancreatic acinar and duct cells [28]. The inhibitory influence of EGF on amylase release in response to both adenylate cyclase-activating and phospholipase C-activating secretagogues suggests that EGF functions as a regulator of secretion *in vivo*.

In conclusion, the results of the present study show the presence of the 170 kDa EGF receptor in pancreatic acinar membranes and that EGF regulates adenylate cyclase activity via G_s and G_{i1-2} proteins by a tyrosine phosphorylation-dependent mechanism. This leads to stimulation of basal adenylate cyclase activity and inhibition of VIP- and forskolin-induced adenylate cyclase activity respectively. An obvious explanation for the inhibitory effect of tyrosine kinase inhibitors on both stimulatory and inhibitory effects of EGF on adenylate cyclase activity is that only the tyrosine-phosphorylated EGF receptor is capable of interacting with G-proteins.

This work was supported in part by the Deutsche Forschungsgemeinschaft (Ze 237/4-1).

REFERENCES

- 1 Carpenter, G. and Cohen, S. (1990) *J. Biol. Chem.* **265**, 7709–7712
- 2 Gregory, H. (1976) *Nature (London)* **257**, 325–327
- 3 Gan, B. C., McCannell, K. L. and Hollenberg, M. D. (1987) *J. Clin. Invest.* **80**, 199–206
- 4 Johnson, G. L. and Vaillancourt, R. R. (1994) *Curr. Opin. Cell Biol.* **6**, 230–238
- 5 Johnson, R. M., Connelly, P. A., Sisk, R. B., Pobiner, B. F., Hewlett, E. L. and Garrison, J. C. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2032–2036
- 6 Liang, M. and Garrison, J. C. (1991) *J. Biol. Chem.* **266**, 13342–13349
- 7 Yang, L., Baffy, G., Rhee, S. G., Manning, D., Hansen, C. A. and Williamson, J. R. (1991) *J. Biol. Chem.* **266**, 22451–22458
- 8 Yang, L., Camoratto, A. M., Baffy, G., Raj, S., Manning, D. R. and Williamson, J. R. (1993) *J. Biol. Chem.* **268**, 3789–3796
- 9 Teitelbaum, I., Strasheim, A. and Berl, T. (1990) *J. Clin. Invest.* **85**, 1044–1050
- 10 Lewis, J. J., Goldenring, J. R., Asher, V. A. and Modlin, I. M. (1990) *Am. J. Physiol.* **258**, G476–G483
- 11 Nair, B. G., Parikh, B., Milligan, G. and Patel, T. B. (1990) *J. Biol. Chem.* **265**, 21317–21322
- 12 Nakagawa, Y., Gammicha, J., Purushotam, K. R., Schneyer, C. A. and Humphreys-Beher, M. G. (1991) *Biochem. Pharmacol.* **42**, 2333–2340
- 13 Proffrock, A., Schnefel, S. and Schulz, I. (1991) *Biochem. Biophys. Res. Commun.* **175**, 380–385
- 14 Sun, H., Seyer, J. M. and Patel, T. B. (1995) *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2229–2233
- 15 Stryjek-Kaminska, D., Piiper, A. and Zeuzem, S. (1995) *Am. J. Physiol.* **269**, G676–G682
- 16 Zeuzem, S., Stryjek-Kaminska, D., Caspary, W. F., Stein, J. and Piiper, A. (1994) *Am. J. Physiol.* **267**, G350–G356
- 17 Piiper, A., Stryjek-Kaminska, D., Stein, J., Caspary, W. F. and Zeuzem, S. (1994) *Am. J. Physiol.* **266**, G363–G371
- 18 Korc, M., Matrisian, L. M., Planck, S. R. and Magun, B. E. (1983) *Biochem. Biophys. Res. Commun.* **111**, 1066–1073
- 19 Sullivan, K. A., Miller, R. T., Masters, S. B., Beiderman, B., Heideman, W. and Bourne, H. R. (1987) *Nature (London)* **330**, 758–760
- 20 Ullrich, A. and Schlessinger, J. (1990) *Cell* **61**, 203–212
- 21 Bosch, F., Bouscarel, B., Slaton, J., Blackmore, P. F. and Exton, J. H. (1986) *Biochem. J.* **239**, 523–530
- 22 Okamoto, T., Katada, T., Murayama, Y., Ui, M., Ogata, E. and Nishimoto, I. (1990) *Cell* **62**, 709–717
- 23 Okamoto, T. and Nishimoto, I. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8020–8023
- 24 Wolbring, G., Hollenberg, M. D. and Schnetkamp, P. P. M. (1994) *J. Biol. Chem.* **269**, 22470–22472
- 25 Stryjek-Kaminska, D., Piiper, A., Caspary, W. F. and Zeuzem, S. (1993) *Biochem. Biophys. Res. Commun.* **190**, 92–96
- 26 Stryjek-Kaminska, D., Piiper, A., Caspary, W. F. and Zeuzem, S. (1995) *Peptides* **16**, 123–128
- 27 Proffrock, A., Piiper, A., Eckhardt, L. and Schulz, I. (1991) *Biochem. Biophys. Res. Commun.* **180**, 900–906
- 28 Korc, M., Chandrasekar, B., Yamanaka, Y., Friess, H., Büchler, M. and Beger, H. G. (1992) *J. Clin. Invest.* **90**, 1352–1360