A region in the cytosolic domain of the epidermal growth factor receptor antithetically regulates the stimulatory and inhibitory guanine nucleotide-binding regulatory proteins of adenylyl cyclase

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ABSTRACT Epidermal growth factor (EGF) stimulates adenylyl cyclase in the heart via activation of the stimulatory GTP-binding protein G_s . Therefore, employing peptides corresponding to regions in the cytosolic domain of the EGF receptor, we have investigated the ability of sequences within the EGF receptor to activate G_s . A 13-aa peptide (EGFR-13) corresponding to the juxtamembrane region in the cytosolic domain of the EGF receptor stimulated GTP binding and GTPase activity of G_s . This peptide did not stimulate GTP binding to G_i but increased the GTPase activity of this protein. Additionally, phosphorylation of the protein kinase C site (threonine residue) within EGFR-13 decreased the ability of the peptide to stimulate G_s and increase GTPase activity of G_i . Further, in functional assays of G_s employing S49 cyc⁻ cell membranes, EGFR-13 increased the ability of G_s to stimulate adenylyl cyclase; phospho-EGFR-13 and a 14-aa peptide corresponding to a sequence in the cytosolic domain of the EGF receptor did not alter the functional activity of G_s . Hence, the juxtamembrane region of the EGF receptor can activate G_s and, by stimulating GTPase activity of G_i , inactivates this latter G protein. Phosphorylation of the threonine residue within this region attenuates the activity of the peptide as a modulator of G-protein function.

Epidermal growth factor (EGF) activates a number of cellular processes ranging from modulation of DNA synthesis and cell growth to regulating metabolic events such as gluconeogenesis, glycolysis, and transport of ions and metabolites across the plasma membrane (1–4). In the rat heart, EGF increases cAMP accumulation and augments contractility and beating rate (5). The EGF-mediated increase in cAMP accumulation in the heart is the result of stimulation of cardiac adenylyl cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] (6) via a process involving G_{sa} , the α subunit of the stimulatory GTP-binding regulatory protein of adenylyl cyclase, G_s (7). The protein-tyrosine kinase activity of the EGF receptor (EGFR) is important for EGF-elicited stimulation of cardiac adenylyl cyclase (8).

To further determine the mechanism(s) involved in EGF-mediated stimulation of adenylyl cyclase and to incorporate the involvement of G_s and EGFR protein-tyrosine kinase activity, we have formulated a hypothesis that upon binding of EGF to its receptor, activation of protein-tyrosine kinase, and resultant alteration in receptor conformation, a region of the EGFR is exposed to interact with and activate G_s . While activation of the EGFR tyrosine kinase, autophosphorylation, and resultant alteration in conformation are known to occur (e.g., refs. 1 and 9), the possibility that a region of the receptor can interact with and activate G_s has hitherto not been examined. The latter possibility is based

upon the findings of others with receptors which couple to G proteins. For instance, Okamoto *et al.* (10) have shown that a 15-aa peptide corresponding to the β_2 -adrenergic receptor can activate G_s . Similarly, a 14-aa peptide corresponding to a region in the receptor for insulin-like growth factor II has also been shown to stimulate the inhibitory GTP-binding regulatory protein of adenylyl cyclase, G_i (11). From their studies with regions of receptors that activate G proteins, Okamoto *et al.* (10, 11) have described three criteria for a region of the receptor to be a candidate for G-protein activator: the presence of (i) two basic residues in the amino terminus, (ii) the sequence BBXB or BBXXB (B, basic residue; X, any residue) in the carboxyl terminus, and (iii) a region (or peptide) length of 14–18 aa. Within the cytosolic domain of the rat EGFR[§] we have identified one region resembling the peptide sequence in the β_2 -adrenergic receptor which activates G_s (10). This 13-aa region, Arg⁶⁴⁶–Arg⁶⁵⁸ (sequence, RRREIVRKRTLRR; EGFR-13) immediately follows the transmembrane region (Ile⁶²³–Met⁶⁴⁵) and meets all of the criteria for a G_s activator described by Okamoto *et al.* (10). This 13-aa region is also conserved in the human EGFR (12). Another region, His⁶⁸⁰–Lys⁶⁹³ (HL-RILKETEFKKIK; EGFR-14), fulfills only two (length and carboxyl-terminus requirement) of the three criteria mentioned above. Therefore, employing peptides corresponding to these sequences in the EGFR, we have determined whether or not region(s) in the cytosolic domain of the EGFR can interact with and activate G_s . Moreover, we have also investigated the interactions of these regions with G_i .

METHODS

Purification of G_{sa} and Bovine Brain $\beta\gamma$ Subunits. The BL21(DE3) strain of *Escherichia coli* transformed with plasmid pQE-60, containing cDNA encoding the 45-kDa form of bovine G_{sa} , was obtained from Alfred Gilman (University of Texas Southwestern Medical Center). Expression of the recombinant G_{sa} from its cDNA subcloned into pQE-60 was induced by the addition of isopropyl β -D-thiogalactopyranoside and the protein was purified essentially as described (13). Bovine brain $\beta\gamma$ subunits were purified to homogeneity (14, 15). Heterotrimeric G_s was reconstituted by mixing G_{sa} and $\beta\gamma$ subunits ($G_{sa}/\beta\gamma$ ratio, 1:5) and incubating for 1 hr at 4°C (see ref. 16).

Binding Studies Using Guanosine 5'-[γ -³⁵S]thio]Triphosphate (GTP[γ -³⁵S]). GTP[γ -³⁵S] binding to G_s was monitored by a slight modification of the method of Northup *et al.* (17). The reconstituted G_s protein was incubated with or without

Abbreviations: EGF, epidermal growth factor; EGFR, EGF receptor; GTP[γ -S], guanosine 5'-[γ -thio]triphosphate.

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§Rat EGFR sequence was provided by H. Shelton Earp, University of North Carolina. As compared with the human EGFR, the rat EGFR has one additional amino acid in the extracellular domain.

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the peptides of interest for 5 min before initiation of GTP[γ - 35 S] binding in 100 μ l of 50 mM Hepes, pH 8.0/120 μ M MgCl₂/100 μ M EDTA/1 mM dithiothreitol. GTP[γ - 35 S] binding was initiated by addition of 100 nM GTP[γ - 35 S]. The final concentration of G_s was 10 nM as assessed by maximal binding in the presence of 1 μ M GTP[γ - 35 S] and 25 mM MgCl₂. The binding reactions were terminated by addition of 2 ml of ice-cold 25 mM Tris-HCl, pH 7.4/10 mM NaCl/25 mM MgCl₂. The bound GTP[γ - 35 S] was separated from unbound nucleotide by rapid filtration through Schleicher & Schuell BA85 nitrocellulose filters. Nonspecific binding was monitored under identical conditions but in the presence of excess (100 μ M) unlabeled GTP[γ -S]. None of the peptides employed bound any GTP[γ - 35 S].

GTPase Activity Measurements. The method described by Brandt *et al.* (18) and as modified by Okamoto *et al.* (10) was followed. Essentially, the reconstituted G_s protein was incubated with or without the peptides of interest at 25°C in 25 mM Hepes-NaOH, pH 8.0/110 μ M EDTA/200 μ M MgSO₄/1 mM dithiothreitol. At various times after initiation of incubation with 100 nM GTP[γ - 32 P], aliquots were withdrawn and transferred into tubes containing ice-cold 5% (wt/vol) Norit A in 50 mM NaH₂PO₄ (pH 8.0). After centrifugation of the mixture, the supernatant was decanted for determination of [32 P]phosphate content by scintillation counting. The rates of GTPase activity were linear under all experimental conditions. The peptides by themselves did not demonstrate any GTPase activity.

Adenylyl Cyclase Assays with S49 cyc⁻ Cell Membranes. The functional activity of G_s was measured by the ability of G_s to activate adenylyl cyclase activity in mouse lymphoma S49 cyc⁻ membranes. S49 cyc⁻ cell membranes were a gift from Ravi Iyengar (Mt. Sinai Medical Center) and were isolated as described (19). Reconstituted G_s was incubated in the presence or absence of peptides and GTP[γ -S] (100 nM) for 1 hr as described above for GTP[γ -S] binding. Thereafter, 0.1 pmol (10 μ l) of G_s was added to 10 μ g of S49 cyc⁻ cell membrane protein in the presence of 1 nmol of guanosine 5'-[β -thio]diphosphate. Adenylyl cyclase reactions (in a final volume of 100 μ l) were initiated by the addition of 80 μ l of reaction mixture described previously (6, 7) to the cyc⁻ membranes/G_s complex, except that the Mg²⁺ concentration was 1 mM. Assays were conducted for 15 min in triplicate. The peptides alone (no G_s) did not alter adenylyl cyclase activity.

Materials. The peptides EGFR-13, EGFR-14, β III-2, and Rev-EGFR-13 were synthesized by the solid-phase system on an Applied Biosystems (model 430) synthesizer. Peptides were recovered from the resin by HF cleavage and extraction with 5% acetic acid. They were desalted, and purified by HPLC on a Vydac 218TP54 column with a linear (10–60% gradient of acetonitrile). The threonine-phosphorylated analog of peptide EGFR-13 (phospho-EGFR-13) was custom synthesized by SynPep (Dublin, CA). All synthetic peptides were <95% pure as assessed by HPLC analysis. Purified recombinant G_{ia2} expressed in *Spodoptera frugiperda* Sf9 cells was obtained from James C. Garrison (University of Virginia, Charlottesville).

RESULTS AND DISCUSSION

To investigate the hypothesis that a region of the EGFR can modulate G_s activity, experiments were performed to determine whether peptides corresponding to the two aforementioned regions of the EGFR could alter GTP[γ - 35 S] binding to G_s. None of the peptides altered GTP[γ - 35 S] binding to the pure G_s monomer (data not shown). However, GTP[γ - 35 S] binding was altered when the G_s was reconstituted with purified bovine brain $\beta\gamma$ subunits to form the G_s heterotrimer. Since most consistent effects of the peptides were observed in the presence of G_s and $\beta\gamma$ subunits at a molar ratio of $\geq 1:3$, in our studies, G_s was reconstituted from G_s and $\beta\gamma$ subunits

at a molar ratio of 1:5. Initially, the ability of the 13-aa peptide EGFR-13 (sequence, RRREIVRKRTLRR) to increase GTP[γ - 35 S] binding to G_s was monitored as a function of time. Controls were performed either in the absence of peptides or with EGFR-14 (sequence, HLRILKETEFKKIK), which meets only two (length and carboxyl-terminal sequence) of the three criteria for a G_s activator. The peptide EGFR-13 (1 μ M) increased the rate and extent of GTP[γ - 35 S] binding to G_s (Fig. 1A). On the other hand, an equivalent concentration (1 μ M) of EGFR-14 only slightly increased ($\approx 20\%$) the extent of binding of GTP[γ - 35 S] to G_s (Fig. 1A). In control incubations performed with peptides alone, neither of the peptides by themselves bound any GTP[γ - 35 S] (data not shown). Since equilibrium binding of GTP[γ - 35 S] to G_s was achieved by 60 min either in the presence or the absence of peptides (Fig. 1A), in subsequent studies this time point was employed to monitor this parameter. The peptide EGFR-13 increased GTP[γ - 35 S] binding to G_s in a concentration-dependent manner, with half-maximal and maximal activation of G_s being observed at 700 nM and 10 μ M EGFR-13, respectively (Fig. 1B). In contrast, EGFR-14 at concentrations up to 10 μ M did not stimulate GTP[γ - 35 S] binding to G_s, but very high concentrations (100 μ M) of EGFR-14 increased GTP[γ -S] binding to the same extent as EGFR-13 (Fig. 1B). Similarly, peptide 3 (sequence, CIASTTTFGG), with a sequence irrelevant to any region of the EGFR, did not alter GTP[γ - 35 S] binding to G_s at concentrations up to 100 μ M. Since EGFR-13 and EGFR-14 are similar in length and also contain the carboxyl-terminal motif (BBXXBB or BBXB; B, basic residue; X, any amino acid) required of a G_s activator (10), the data in Fig. 1B suggest that the basic residues in the amino terminus of EGFR-13 are important in determining the efficacy of this peptide as a G_s activator.

Okamoto *et al.* (10) reported that the 15-aa peptide (β III-2; sequence, RRSSKFCLKEHKALK) corresponding to Arg²⁵⁹–Lys²⁷³ in the third cytosolic loop of the β_2 -adrenergic receptor activated G_s at concentrations of 100 nM. Since 10 μ M EGFR-13 maximally stimulated GTP[γ - 35 S] binding to G_s, we investigated the relative potencies of the two peptides. Under the conditions of our experiments, although β III-2 stimulated GTP[γ - 35 S] binding, EGFR-13 was ≈ 1 order of magnitude more efficacious as a G_s activator than β III-2 (Fig. 2). The

FIG. 1. Effect of peptides EGFR-13 and EGFR-14, corresponding to regions within the cytosolic domain of the EGFR, on GTP[γ -S] binding to G_s. Purified recombinant G_s (1 pmol) was reconstituted with 5 pmol of purified bovine brain $\beta\gamma$ subunits; final concentrations of G_s and $\beta\gamma$ were 10 nM and 50 nM, respectively. GTP[γ - 35 S] binding assays were performed in a final volume of 100 μ l of reaction mixture with or without the indicated peptides. (A) Time course of GTP[γ - 35 S] binding to reconstituted G_s in the presence of 1 μ M EGFR-13 (RRREIVRKRTLRR) (Δ) or EGFR-14 (HLRILKETEFKKIK) ($\circ$), corresponding to Arg⁶⁴⁶–Arg⁶⁵⁸ and His⁶⁸⁰–Lys⁶⁹³ of the rat EGFR, respectively. Controls ($\blacksquare$) in the absence of peptides are also shown. A representative experiment of three is presented. (B) Dose-response curve of GTP[γ - 35 S] binding to reconstituted G_s in the presence of various concentrations of EGFR-13 (Δ) or EGFR-14 ($\circ$). Effect of 100 μ M peptide 3 (CIASTTTFGG) ($\diamond$), which has no resemblance to any region of the EGFR, is also shown. Data are mean $\pm$ SD ($n = 3$ to 5).

FIG. 2. Comparison of the efficacies of EGFR-13 (Δ), the reverse sequence of EGFR-13 (rev-EGFR-13) ($\square$), and β III-2, corresponding to the G_s activating region (Arg²⁵⁹–Lys²⁷³) in the β_2 -adrenergic receptor (10) ($\blacktriangle$), on GTP[γ -³⁵S] binding to G_s . Experimental protocols were similar to those for Fig. 1B. A representative experiment of three in which each concentration was assayed in triplicates is shown (mean $\pm$ SD).

precise reason(s) for the difference in efficacy of β III-2 as a G_s activator in our studies as compared to those reported by Okamoto *et al.* (10) is not immediately obvious, since the experimental conditions employed were identical. One possible explanation is that Okamoto *et al.* (10) used purified bovine brain G_s in their experiments whereas we have employed a recombinant 45-kDa isoform of the G_{sa} and reconstituted it with bovine brain $\beta\gamma$ subunits. Nonetheless, the data in Fig. 2 demonstrate that, in our experiments, EGFR-13 is a more effective activator of reconstituted G_s when compared with peptide β III-2, which corresponds to a sequence in the β_2 -adrenergic receptor.

To determine whether the amino- and carboxyl-terminal orientations of EGFR-13 are important for stimulation of GTP[γ -³⁵S] binding to G_s and also to investigate the requirement for the specific arrangement of the basic residues (i.e., BBXXBB vs. BBXXBBB; B, basic amino acid; X, any amino acid) in the carboxyl terminus of EGFR-13, experiments were performed with the reverse sequence of EGFR-13 (RRL-TRKRVIERRR). The reverse sequence (rev-EGFR-13) was as effective as EGFR-13 in stimulating GTP[γ -³⁵S] binding to G_s (Fig. 2). Hence, the orientation of the peptide is not important for stimulation of GTP[γ -³⁵S] binding to G_s . Moreover, the data in Fig. 2 demonstrate that there is not a very stringent requirement in the arrangement of the basic residues (i.e., BBXXBB vs. BBXXBBB) in the carboxyl terminus of the peptide for stimulation of G_s activity. One implication of the findings that EGFR-13 and the reverse sequence, rev-EGFR-13, are equally effective activators of G_s is that basic peptides by virtue of their positive charge would be able to activate GTP[γ -³⁵S] binding to G_s . To address this possibility, experiments with poly(L-lysine) and protamine were performed. Neither poly(L-lysine) nor protamine at concentrations up to 100 μ M altered GTP[γ -³⁵S] binding to G_s (data not shown). Thus, the ability of EGFR-13 and rev-EGFR-13 to activate GTP[γ -³⁵S] binding to G_s is not determined solely by the positive charge on the peptides, but some other structural features are also important.

As another monitor of alterations in G_s activity, the effect of the peptides corresponding to regions of the EGFR on GTPase activity of this protein was also monitored. EGFR-13 and rev-EGFR-13 were equally effective at increasing GTPase activity of G_s (Fig. 3). The peptide EGFR-14 stimulated GTPase activity only at very high concentrations. Moreover, as

FIG. 3. Stimulation of GTP hydrolysis by G_s in the presence of various concentrations of peptides EGFR-13 ($\blacktriangle$), EGFR-14 (Δ), rev-EGFR-13 ($\circ$), and β III-2 ($\square$). G_s reconstituted from 1 pmol of G_{sa} and 5 pmol of $\beta\gamma$ subunits was assayed in a final volume of 100 μ l for [γ -³²P]GTP (100 nM)-hydrolyzing activity over a 10-min period. Data are presented as mean $\pm$ SEM of three experiments.

described for the GTP[γ -³⁵S] binding experiments (Fig. 2), the peptide β III-2, corresponding to region in the third cytosolic loop of the β_2 -adrenergic receptor, was less effective in stimulating GTPase activity of G_s (Fig. 3). Thus, EGFR-13 is a potent stimulator of G_s activity (Figs. 1–3).

To determine whether or not EGFR-13 selectively activated G_s , the ability of this peptide and EGFR-14 to alter GTP[γ -³⁵S] binding to, and GTPase activity of, purified recombinant $G_{i\alpha 2}$ reconstituted with brain $\beta\gamma$ subunits was investigated. Neither EGFR-13 (Fig. 4A) nor EGFR-14 (data not shown) significantly altered GTP[γ -³⁵S] binding to G_i . Interestingly, however, EGFR-13 increased GTPase activity of G_i to a similar extent as G_s (compare Fig. 4B and C), whereas EGFR-14 was ineffective at altering GTPase activity of G_i (Fig. 4C). To our knowledge, EGFR-13 is the first peptide or agent shown to increase GTPase activity of a G protein without altering binding of GTP. Moreover, the ability of EGFR-13 to increase GTPase activity without altering GTP[γ -³⁵S] binding to G_i indicates that the net effect of the peptide would be to inactivate G_i function. Thus, the ability of EGFR-13 to selectively activate G_s , coupled with inhibition of G_i activity, may amplify the actions of G_s on the effector, adenylyl cyclase. GTPase activity of α subunits of heterotrimeric G proteins such as $G_{q/11}$ and transducin are stimulated by effectors such as phospholipase C- β 1 (20) and cGMP phosphodiesterase (21), respectively. Moreover, a region within the α subunits of G proteins has been demonstrated to activate GTPase (22). However, to our knowledge, no receptor or its region has been shown to selectively increase the GTPase activity of a G protein. In this respect, EGFR-13 is the first example of a region within a receptor which increases GTPase activity of a G protein (e.g., G_i) without increasing GTP binding. Since the GTP binding and GTPase activating domains of heterotrimeric G protein α subunits are separate (22), it is conceivable that EGFR-13 interacts with both these domains in G_s and with only the GTPase-activating domain of G_i .

An interesting feature of the EGFR-13 sequence is that the threonine residue within this sequence (Thr⁶⁵⁵ in the rat EGFR) corresponds with Thr⁶⁵⁴ in the human EGFR and is the site of protein kinase C phosphorylation (23, 24); an extra amino acid residue in the ligand-binding domain of the rat EGF receptor is the reason for difference in the location of thr residues from the amino-terminus.[§] Protein kinase C activation results in the attenuation of EGFR kinase activity (25) and

FIG. 4. Modulation by phosphorylation of Thr⁶⁵⁵ in EGFR-13 on GTP[γ -³⁵S] binding, GTPase activities of G_s and G_i , and ability of G_s to stimulate adenylyl cyclase activity. (A) GTP[γ -³⁵S] binding to G_s (○, ●) and G_i (□, △) was assayed in the presence of various concentrations of EGFR-13 (○, □) and phospho-EGFR-13 (●, △). G_i was reconstituted from purified $G_{i\alpha 2}$ expressed in Sf9 cells and purified brain β - γ subunits. Data are presented as mean $\pm$ SD. *, $P < 0.001$; **, $P \leq 0.005$ as compared with identical concentration of EGFR-13. (B) Modulation of GTPase activity of G_s by EGFR-13 (stippled bars) and phospho-EGFR-13 (hatched bars). Experimental conditions were similar to those described under Fig. 3. Data are presented as mean $\pm$ SD. *, $P < 0.001$; **, $P \leq 0.005$ as compared with identical concentration of EGFR-13. (C) Modulation of GTPase activity of G_i by EGFR-13 (stippled bars) and phospho-EGFR-13 (hatched bars). Effect of EGFR-14 (10 μ M) on GTPase activity of G_i is also shown (bar with horizontal stripes). Data are presented as mean $\pm$ SD. *, $P < 0.001$; **, $P \leq 0.005$ as compared with identical concentration of EGFR-13. (D) Stimulation of adenylyl cyclase activity by G_s which had been preactivated in the presence or absence of EGFR-13 (stippled bar), phospho-EGFR-13 (hatched bar), and EGFR-14 (bars with horizontal stripes). Reconstituted G_s was incubated in the presence or absence of peptides and GTP[γ -S] (100 nM) for 1 hr. Thereafter, 0.1 pmol (10 μ l) of G_s was added to 10 μ g of S49 cyc⁻ cell membrane protein in the presence of 1 nmol of guanosine 5'-[β -thio]diphosphate. Adenylyl cyclase reactions (in 100 μ l) were conducted for 15 min. Data are presented as the mean $\pm$ SD of three determinations in two similar experiments. In controls with peptides alone (no G_s), adenylyl cyclase activity was not altered (data not shown).

downregulation of a number of biological actions of EGF (26–28), including stimulation of cardiac adenylyl cyclase activity (6). We found that the ability of the threonine-phosphorylated EGFR-13 (phospho-EGFR-13) to stimulate GTP[γ -³⁵S] binding to, and GTPase activity of, G_s was markedly attenuated (Fig. 4A and B). Likewise, although phospho-EGFR-13 did not alter GTP[γ -³⁵S] binding to G_i (Fig. 4A), its ability to increase GTPase activity of G_i was markedly less than that of dephospho-EGFR-13 (Fig. 4C). To test whether or not the peptides altered the functional activity of G_s , the ability of G_s which had been incubated with GTP[γ -S] in the presence or absence of the peptides to stimulate adenylyl cyclase activity in S49 cyc⁻ cell membranes, which do not contain G_s activity (29), was monitored. Consistent with its ability to stimulate GTP[γ -³⁵S] binding to G_s (Fig. 1), EGFR-13-activated G_s ,

stimulated adenylyl cyclase by a 2-fold greater extent than G_s which had not been exposed to peptides (Fig. 4D). Additionally, compared with the latter control, neither phospho-EGFR-13 nor EGFR-14 altered the ability of G_s to stimulate adenylyl cyclase in S49 cyc⁻ membranes (Fig. 4D). The data in Fig. 4 show that phosphorylation of Thr⁶⁵⁵ in the rat EGFR can decrease the ability of EGFR-13 to modulate G_s and G_i function. The finding that phosphorylation of the threonine residue in EGFR-13 attenuates the ability of EGFR-13 to stimulate G_s function (Fig. 4) is also consistent with the observation that protein kinase C activation inhibits the ability of EGF to stimulate cardiac adenylyl cyclase (6). Phosphorylation of Thr⁶⁵⁴ in the human EGFR (Thr⁶⁵⁵ in the rat receptor) has been demonstrated to decrease the number of cell surface receptors available for binding to EGF (30, 31) and

also attenuate the tyrosine kinase activity of the receptor (25). In addition to these mechanisms, our data show that phosphorylation of the threonine residue also attenuates its ability to modulate G-protein functions. Although there does not appear to be a stringent requirement for the arrangement of the basic amino acids in the carboxyl terminus, it is likely that the introduction of negative charges by phosphorylation of Thr⁶⁵⁵ in the carboxyl terminus of EGFR-13 alters the electrostatic charge interactions between EGFR-13 and G_s. However, the identity of the basic residue(s) among the various present on EGFR-13 which is critical for interactions with G_s remains unknown.

Other than the involvement of G_s, it has been shown that the intrinsic protein tyrosine kinase activity of the EGFR is also important in EGF-mediated stimulation of adenylyl cyclase (8). Since autophosphorylation of the EGFR alters the conformation of the intracellular domain from a compact dephosphorylated form to a more extended phosphorylated form (9), it appears that upon binding of EGF to its receptor, and autophosphorylation of the receptor, the resultant change in conformation allows the juxtamembrane region of the receptor to interact with G_s in a stimulatory manner and G_i in an inhibitory manner to subsequently activate adenylyl cyclase.

In conclusion, our studies have identified the juxtamembrane region in the cytosolic domain of the EGFR as an activator of G_s and inhibitor of G_i function. Moreover, the protein kinase C phosphorylation site modulates the interactions of this region with G proteins. The definition of the G protein-interacting sequence of the EGFR adds to the number of functionally important regions within the cytosolic domain of this receptor. Thus in addition to the well-characterized tyrosine kinase domain (32) and the importance of regions around the autophosphorylation sites in the carboxyl terminus for recognition of Src homology region 2-containing proteins (33), the juxtamembrane region of the receptor is important for interactions with G proteins coupled to adenylyl cyclase. Hence, the pleiotropic actions of EGF may be explained by the ability of the multifunctional cytosolic regions of the EGFR to activate a number of different types of signaling cascades.

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