The receptor for insulin-like growth factor 1 (IGF-1) mediates multiple cellular responses, including stimulation of both proliferative and anti-apoptotic pathways. We have examined the role of cross talk between the IGF-1 receptor (IGF-1R) and the epidermal growth factor receptor (EGFR) in mediating responses to IGF-1. In COS-7 cells, IGF-1 stimulation causes tyrosine phosphorylation of the IGF-1R β subunit, the EGFR, insulin receptor substrate-1 (IRS-1), and the Shc adapter protein. Shc immunoprecipitates performed after IGF-1 stimulation contain coprecipitated EGFR, suggesting that IGF-1R activation induces the assembly of EGFR/Shc complexes. Tyrophostin AG1478, an inhibitor of the EGFR kinase, markedly attenuates IGF-1-stimulated phosphorylation of EGFR, Shc, and ERK1/2 but has no effect on phosphorylation of IGF-1R, IRS-1, and protein kinase B (Akt). Cross talk between IGF-1 and EGF receptors is mediated through an autocrine mechanism involving matrix metalloprotease-dependent release of heparin-binding EGF (HB-EGF), because IGF-1-mediated ERK activation is inhibited both by [Glu52]Diphtheria toxin, a specific inhibitor of HB-EGF, and the metalloprotease inhibitor 1,10-phenanthroline. These data demonstrate that IGF-1-stimulated IRS-1/PI3K/Akt pathway and the EGFR/Shc/ERK1/2 pathway occurs by distinct mechanisms and suggest that IGF-1-mediated “transactivation” of EGFR accounts for the majority of IGF-1-stimulated Shc phosphorylation and subsequent activation of the ERK cascade.

The insulin receptor family is comprised of three members, the insulin receptor, the insulin-like growth factor-1 receptor (IGF-1R), and the insulin receptor-related receptor, an orphan whose endogenous ligand is unknown. The three receptors share a common topology, each composed of two entirely extracellular α subunits containing the ligand-binding domain, and two β subunits that contain a single transmembrane domain and an intracellular domain possessing intrinsic ligand-stimulated tyrosine kinase activity. Like classical receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR), stimulation of insulin receptors leads both to receptor auto-phosphorylation and to the recruitment and activation of signaling proteins that contain specific phosphotyrosine-binding SH2 homology 2 (SH2) domains.

In the case of EGFR, phosphorylation of tyrosine residues within the intracellular domain provides docking sites for SH2 domain proteins, including the Ras guanine nucleotide exchange factor complex Grb2-mSos, Ras-GTPase-activating protein, phospholipase Cγ, the p85-p110α phosphatidylinositol 3-kinase (PI3K) complex, and the nonreceptor tyrosine kinases c-Src and c-Fyn, as well as for adapter proteins such as Shc and Gab1. Thus the phosphorylated EGFR serves as a scaffold for the assembly, at the plasma membrane, of a multienzyme-signaling complex that mediates the intracellular response to EGFR (1). Unlike the EGFR receptor, autophosphorylated insulin family receptors do not signal via the direct recruitment of these signaling proteins. Instead, insulin and IGF-1 induce phosphorylation of separate adapter proteins such as the insulin receptor substrate (IRS) proteins and Shc. These adapter proteins, in turn, function as intermediates between the receptor and SH2 domain-containing signaling proteins (2).

Substantial data support the hypothesis that the IRS and Shc adapter proteins play distinct roles in mediating insulin/ IGF-1 responses. Tyrosine phosphorylation of IRS-1, for example, mediates recruitment of the p85-p110α PI3K complex, leading to both protein kinase B (Akt)-dependent suppression of the BAD/Bcl-X apoptotic pathway (3, 4) and to signals required for insulin-induced translocation of GLUT4 (5, 6). Although both IRS-1 and Shc can bind Grb2-mSos, the mitogenic response to insulin or IGF-1 often correlates with Shc, but not IRS-1, phosphorylation (7, 8). Furthermore, IRS proteins are not required for phosphorylation of Shc or for activation of Ras and the extracellular signal-regulated kinase (ERK)1/2 mitogen-activated protein (MAP) kinase cascade in many cell types, indicating that the IRS-1 and Shc pathways are truly independent.

It is increasingly apparent that EGFR serves as a point of convergence for mitogenic signals arising from diverse stimuli. EGFR transactivation can follow activation of G protein-coupled receptors, including the lysophosphatidic acid, α- and β-adrenergic, muscarinic cholinergic, angiotensin, thrombin, and bradykinin receptors (9–11), and cytokine receptors, including the growth hormone and prolactin receptors (12); integrin engagement (13); and even physical stimuli such as membrane depolarization (14, 15) and cell stress produced by ultraviolet and ionizing radiation (16, 17). The molecular mechanisms that account for cross talk between the EGFR and these diverse inputs remain poorly understood, although recent data indicate that autocrine release of soluble EGFR ligands, such as Diphtheria toxoid, mediates EGFR activation and subsequent signaling.
as heparin-binding EGF (HB-EGF), can account for EGFR transactivation in at least some systems (18).

Here, we report that cross talk between the IGF-1R and EGFR, occurring via an autocrine mechanism involving matrix metalloprotease-dependent release of HB-EGF, accounts for the majority of IGF-1-stimulated Shc phosphorylation and activation of the ERK cascade in COS-7 cells. The finding, that activation of the ERK pathway in response to IGF-1 is mediated via an autocrine mechanism in at least some cell types, suggests that pharmacologic approaches to dissociate the proliferative effects of insulin family receptors from their anti-apoptotic and metabolic effects is feasible.

**EXPERIMENTAL PROCEDURES**

**Materials**—The EGFR-specific tyrphostin AG1478, the platelet-derived growth factor receptor-specific tyrphostin AG1295, the PI3K inhibitors wortmannin and LY294002, IGF-1, and EGF were from Calbiochem. The EGFR-specific tyrphostin AG1478, the platelet-derived growth factor receptor-specific tyrphostin AG1295, the PI3K inhibitors wortmannin and LY294002, IGF-1, and EGF were from Calbiochem. The HB-EGF inhibitor [Glu52]Diphtheria toxin (CRM197), the metalloprotease inhibitor 1,10-phenanthroline, and phorbol 12-myristate 13-acetate (PMA) were from Sigma. Eukaryotic expression plasmids for the expression of cDNAs encoding IGF-1 receptor, human EGFR, and IRS-1 were generously provided by J. M. Olefsky, G. Gill, and M. F. White, respectively.

**Cell Culture and Transient Transfection**—COS-7 cells were from the American Type Culture Collection and were maintained in Dulbecco's modified eagle medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum and 50 μg/ml gentamicin. HEK-293 cells stably expressing the human IGF-1 receptor (HEK-IGF-1R) were provided by F.-T. Lin (19) and were maintained in minimum essential medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum and 50 μg/ml gentamicin. Transient transfection of COS-7 and HEK-IGF-1R cells was performed using LipofectAMINE (Life Technologies, Inc). as described previously (20). Monolayers of transfected cells were incubated in serum-free growth medium supplemented with 10 μM HEPES, pH 7.4, 0.1% bovine serum albumin, and gentamicin for 16–20 h prior to stimulation.

**Immunoprecipitation and Immunoblotting**—Agonist treatments were performed at 37 °C in serum-free medium following preincubation without inhibitors as described in the figure legends. After stimulation, cell monolayers were placed on ice, washed with ice-cold Dulbecco's phosphate-buffered saline, and lysed in 1 ml of solubilization buffer. For immunoprecipitation studies, radioimmune precipitation buffer (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 0.25% w/v sodium deoxycholate, 0.1% v/v Nonidet P-40, 1 mM NaF, 1 mM sodium pyrophosphate, 100 μM NaVO₃, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml apronitin) was employed. Solubilized lysates were briefly sonicated and centrifuged, and diluted to a protein concentration of 1 mg/ml. Where appropriate, a 50-μl aliquot of clarified whole-cell lysate was mixed with an equal volume of 2× Laemmli sample buffer and resolved by SDS-polyacrylamide gel electrophoresis (PAGE) for confirmation of plasmid expression or determination of ERK1/2 and Akt phosphorylation by protein immunoblotting.

**Protein tyrosine phosphorylation** was detected by a 1:1000 dilution of horseradish peroxidase (HRP)-conjugated anti-phosphotyrosine monoclonal antibody (PY20; Transduction Laboratories). Immunoblotting of EGFR was performed using sheep anti-human EGFR polyclonal IgG (Upstate Biotechnology). Immunoprecipitation of IRS-1 was performed using 2.5 μg/ml rabbit anti-IRS-1 polyclonal IgG (Upstate Biotechnology). Immunoprecipitation of Shc was performed using 2.5 μg of rabbit anti-Shc polyclonal IgG (Transduction Laboratories). Immune complexes were collected using 50 μg of clarified whole-cell lysate and 0.5% v/v Nonidet P-40.

**ERK1/2 and Akt Phosphorylation**—For determination of ERK1/2 and Akt phosphorylation, approximately 15 μg of whole cell lysate protein/lane was resolved by SDS-PAGE. ERK1/2 phosphorylation was detected by protein immunoblotting using a 1:1000 dilution of rabbit polyclonal phospho-specific MAP kinase IgG (New England Biolabs) with HRP-conjugated goat anti-rabbit IgG (Jackson Immunoresearch Laboratories) as secondary antibody. Akt phosphorylation was detected by protein immunoblotting using a 1:1000 dilution of rabbit polyclonal anti-phospho-Akt IgG (New England Biolabs) with HRP-conjugated goat anti-rabbit IgG (Jackson Immunoresearch Laboratories) as secondary antibody.

**Quantitation of ERK1/2 and Akt phosphorylation** was performed by scanning laser densitometry. After quantitation of ERK1/2 phosphorylation, PVDF membranes were stripped of immunoglobulin and reprobed using rabbit polyclonal anti-ERK1/2 IgG (Santa Cruz Biotechnology) or rabbit polyclonal anti-Akt IgG to confirm equal protein loading.
IGF-1 Receptor Stimulation Induces EGFR Transactivation and EGFR-dependent Tyrosine Phosphorylation of the Shc Adapter Protein—

To assay for IGF-1-stimulated cross talk between IGF-1R and EGFR, we initially compared the ability of IGF-1 and EGF to induce tyrosine phosphorylation of IGF-1R, EGFR, IRS-1, and Shc in COS-7 cells. Because COS-7 cells express relatively low levels of endogenous IRS proteins, assays of IGF-1-stimulated phosphorylation of IRS-1 were performed in COS-7 cells that were transiently transfected with cDNA encoding IRS-1. As shown in Fig. 1A, treatment of COS-7 cells with IGF-1, but not with EGF, rapidly increased tyrosine autophosphorylation of the endogenous IGF-1R β subunit. Similarly, tyrosine phosphorylation of IRS-1, a direct substrate of the IGF-1R kinase (21), was stimulated by IGF-1, but not by EGF. In contrast, both IGF-1 and EGF induced tyrosine phosphorylation of endogenous EGFR and Shc in COS-7 cells. Maximal IGF-1-stimulated EGFR and Shc phosphorylation occurred within 5 min (data not shown) and was less robust than the response to EGF.

As shown in Fig. 1B, Shc immunoprecipitates from IGF-1- or EGF-stimulated cells lysed in Nonidet P-40 solubilization buffer contained coprecipitated tyrosine phosphoproteins of approximately 60, 130, and 170 kDa (Fig. 1B, left panel). Reprobing these immunoblots with anti-EGFR IgG revealed the presence of coprecipitated EGFR (right panel). Thus, IGF-1 stimulation induced both EGFR phosphorylation and binding of EGFR to tyrosine-phosphorylated Shc.

Several distinct receptor types, including G protein-coupled receptors and cytokine receptors, have been shown to induce EGF-independent activation of the intrinsic EGFR kinase activity (9–11), a process termed transactivation. For many G protein-coupled receptors, among them the M2 muscarinic, thrombin, bombesin, endothelin, and lysophosphatidic acid receptors (22, 23), inhibiting EGFR function prevents G protein-coupled receptor-induced Shc phosphorylation and activation of the ERK1/2 pathway.

To determine whether transactivated EGFR contributes to IGF-1-induced tyrosine phosphorylation of adapter proteins, we assayed the effect of the EGFR-specific inhibitor tyrphostin AG1478 on IGF-1-stimulated tyrosine phosphorylation of IRS-1 and Shc. Fig. 2A demonstrates the specificity of tyrphostin AG1478 for the EGFR. HEK-293 cells overexpressing both IGF-1R and EGFR were preincubated with varying concentrations of AG1478 before determination of agonist-stimulated receptor autophosphorylation. As shown, AG1478 concentrations of greater than 50 nM completely inhibited EGFR phosphorylation, with no detectable effect on IGF-1R. Fig. 2B depicts the effect of 100 nM AG1478 on IGF-1 and EGF-stimulated tyrosine phosphorylation of the IGF-1R β subunit, IRS-1, EGFR, and Shc in COS-7 cells. As shown, IGF-1-mediated phosphorylation of the IGF-1R β subunit and its substrate...
IRS-1 were unaffected by AG1478. In contrast, IGF-1-stimulated EGFR and Shc phosphorylation were both markedly attenuated in the presence of AG1478, suggesting that IGF-1-induced EGFR transactivation accounts for the majority of the Shc phosphorylation in response to IGF-1. These data indicate the existence of two distinct mechanisms of IGF-1 receptor-mediated tyrosine phosphorylation. Phosphorylation of direct IGF-1 receptor substrates, such as IRS-1, is independent of functional EGFR, whereas phosphorylation of Shc occurs predominantly as a result of cross talk between IGF-1 and EGF receptors.

Functional Dissociation of the IRS-1/PI3K/Akt and the Transactivated EGFR/Shc/ERK1/2 Cascades in IGF-1-stimulated Cells—In many cell types, IGF-1-stimulated mitogenesis correlates with tyrosine phosphorylation of Shc, whereas the generation of anti-apoptotic signals, such as Akt-dependent suppression of the BAX/Bcl-2 anti-apoptotic pathway (5, 4), reflects P38-dependent signaling downstream of IRS proteins (24). Our data suggest that, in COS-7 cells, IGF-1-induced Shc phosphorylation, but not IRS-1 phosphorylation, is mediated via transactivated EGFR. Thus, we hypothesized that Shc-dependent mitogenic signals, such as activation of the ERK1/2 MAP kinase cascade, would be EGFR-dependent, whereas IRS-1-dependent anti-apoptotic signals, such as activation of Akt, would be EGFR independent. To ascertain the role of transactivated EGFR in IGF-1 signaling, we determined whether inhibiting the EGFR affected the ability of IGF-1 to activate the ERK1/2 and Akt pathways. These data are presented in Fig. 3 (A and B). Fig. 3A compares the effects of two tyrophostins, the EGFR-specific tyrophostin AG1478 and the platelet-derived growth factor receptor-specific tyrophostin AG1295, on IGF-1- and EGF-stimulated ERK1/2 and Akt phosphorylation in COS-7 cells. As expected, EGF-stimulated phosphorylation of both ERK1/2 and Akt was sensitive to AG1478, but not to AG1295. EGFR phosphorylation was strongly attenuated by AG1478 and was AG1295-insensitive. Significantly, IGF-1-stimulated Akt phosphorylation was insensitive to both AG1478 and AG1295, consistent with the failure of AG1478 to block IGF-1-stimulated IRS-1 phosphorylation.

As shown quantitatively in Fig. 3B, application of AG1478 dissociated IGF-1-stimulated ERK1/2 phosphorylation, which was AG1478-sensitive, from IGF-1-stimulated Akt phosphorylation, which was AG1478-insensitive. In contrast, activation of both the ERK1/2 and Akt pathways by EGF was sensitive to AG1478. This probably reflects the requirement for EGFR autophosphorylation to support SH2 domain-mediated recruitment of both the Shc-Grb2-mSos Ras activation complex and
the p85-p110α PI3K complex directly to the phosphorylated EGFR (25). Despite the clear ability of EGFR to mediate Akt phosphorylation in response to EGF, the failure of AG1478 to attenuate IGF-1-mediated Akt phosphorylation suggests that in the presence of an intact IGF-1/IRS-1/PI3K pathway the transactivated EGFR does not contribute significantly to IGF-1-stimulated Akt phosphorylation. Thus, although these data strongly suggest that IGF-1R/EGFR cross talk represents the predominant mechanism of IGF-1-stimulated activation of ERK1/2, they indicate that IGF-1-mediated activation of the IRS-1/PI3K/Akt pathway is independent of EGFR transactivation.

Reflecting the well established role of IRS protein-mediated recruitment of the p85-p110α PI3K complex in signaling by insulin family receptors, several cellular responses to insulin and IGF-1 are sensitive to inhibitors of PI3K. In addition to activating Akt, IRS-1-dependent PI3K recruitment has been implicated in insulin-stimulated translocation of the GLUT4 glucose transporter (5, 6) and in insulin- or IGF-1-induced membrane ruffling (26). In contrast, neither wortmannin nor a dominant negative mutant of the p85 subunit of PI3K affects insulin-stimulated Ras-GTP loading in Chinese hamster ovary cells stably overexpressing insulin receptors (6, 27). To determine whether IGF-1-mediated ERK1/2 activation requires IGF-1-stimulated PI3K activity, we measured IGF-1-stimulated Akt and ERK1/2 phosphorylation in the presence of the PI3K inhibitors wortmannin and LY294002. As shown in Fig. 3C, IGF-1-stimulated Akt phosphorylation was inhibited by wortmannin and LY294002 with IC50 concentrations of approximately 10 nM and 1 μM, respectively. IGF-1-stimulated ERK1/2 phosphorylation was also sensitive but with 3- to 5-fold higher IC50 concentrations of each inhibitor. In fact, 50–60% of the ERK1/2 signal persisted at concentrations of wortmannin (30 nM) and LY294002 (10 μM) sufficient to abolish IGF-1-stimulated Akt phosphorylation. Because Akt phosphorylation is a direct reflection of PI3K activation (28), these data suggest that IGF-1-stimulated ERK1/2 activation does not require IRS-1-mediated PI3K recruitment. Our results are also consistent with previous reports that wortmannin inhibits insulin activation of p70 S6 kinase (29) and glycogen synthase (27) via a mechanism that is independent of its effect on PI3K.

**Autocrine Release of HB-EGF Mediates IGF-1-stimulated ERK1/2 Activation—** Recent data suggest that transactivation of EGFR can occur via an autocrine/paracrine mechanism involving the release of soluble EGF-like ligands (11, 18). HB-EGF is a peptide mitogen of the EGF family that is released by proteolytic cleavage of a larger membrane-anchored precursor. Cell surface shedding of HB-EGF is mediated by matrix metalloproteases, whose regulation by extracellular stimuli is not completely understood (30).

Transmembrane HB-EGF is the receptor for Diptheria toxin. Thus, binding of Diptheria toxin to the extracellular HB-EGF domain potently and specifically inhibits its mitogenic activity (31). To determine whether HB-EGF shedding contributes to IGF-1-mediated activation of the ERK1/2 cascade, we assessed whether treatment with CRM197, a catalytically inactive [Glu52] mutant of Diptheria toxin, or the metalloprotease inhibitor 1,10-phenanthroline affected IGF-1-mediated ERK1/2 phosphorylation in COS-7 cells. As shown in Fig. 4A, preincubation with AG1478, CRM197, or 1,10-phenanthroline each markedly attenuated IGF-1-stimulated ERK1/2 phosphorylation. As expected, the response to EGF was sensitive only to AG1478, because direct application of the EGFR ligand circumvented the requirement for the paracrine release of endogenous HB-EGF. Phorbol ester-stimulated ERK1/2 phosphorylation, which is mediated via a tyrosine kinase and Ras-
Grb2 counts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF. The transactivated EGFR accounts for the majority of IGF-1-stimulated HB-EGF.

**DISCUSSION**

As depicted schematically in Fig. 5, our data suggest that cross talk between the IGF-1R and EGFR accounts for the functional dissociation of the IRS-1/P13K/Akt pathway from the Shc/Ras/ERK1/2 pathway in COS-7 cells. Because IRS proteins are direct substrates of the IGF-1R, signaling events occurring downstream of IRS phosphorylation, e.g. P13K recruitment and Akt phosphorylation, are independent of EGFR transactivation. In contrast, IGF-1R-mediated Shc phosphorylation and ERK1/2 activation predominantly reflect transactivation of EGFR in response to IGF-1. Transactivation of the EGF receptor is accomplished predominantly via the autocrine release of HB-EGF resulting from the IGF-1-stimulated activation of an as yet incompletely characterized matrix metalloprotease.

The extent to which EGFR transactivation contributes to mitogenic signaling by insulin family receptors is likely to vary between cell types. The Grb2 adapter protein that links mSos to tyrosine phosphoproteins can bind not only to phosphorylated EGFR and Shc but also to Tyr411 of IRS-1 (33). Thus, IRS-1 should be able to directly support Ras-dependent signaling, independent of IGF-1- or insulin receptor-mediated EGFR transactivation. Previous work has shown, however, that the relative contributions of Shc and IRS-1 to Ras-dependent ERK1/2 activation varies between cell types and is probably determined by the relative levels of expression of the receptors and the two adapter proteins (7, 8, 33, 34). In cells that express insulin receptor and IRS-1 at relatively low levels, activation of Ras and ERK1/2 is mediated via Shc phosphorylation (7, 8).

Each of the known endogenous ligands for EGFR, EGF, transforming growth factor α, HB-EGF, amphiregulin, betacellulin, and epiregulin (35) is synthesized as a transmembrane precursor, which undergoes regulated proteolysis to produce a soluble growth factor. Although initial studies on the mechanism of EGFR transactivation by G protein-coupled receptors failed to detect an autocrine mechanism (22, 36, 37), more recent data suggest that autocrine/paracrine release of soluble EGF-like ligands can account for EGFR transactivation by stimuli as diverse as phorbol esters (38, 39), ionizing radiation (40), and G protein-coupled receptors (41, 42). Our data indicate that IGF-1-induced EGFR transactivation may be mediated in a similar fashion.

Proteolysis of the HB-EGF precursor is mediated by members of the ADAM family of matrix metalloproteases (43), although the mechanisms of receptor-dependent metalloprotease regulation are poorly understood. Protein kinase C-dependent HB-EGF cleavage in response to phorbol esters reportedly involves the metalloprotease ADAM 9 (38), cell adhesion (44), and MAP kinase activity (41, 44). This cannot represent the sole mechanism for metalloprotease regulation, however, because G protein-coupled receptor-induced HB-EGF shedding is insensitive to inhibitors of protein kinase C (42). Ectodomain shedding apparently accounts for the sustained activation of MAP kinases observed following stimulation by some growth factors and is necessary for autocrine growth control of a variety of cell types (30) as well as for the regulation of cell migration (45).

Remarkable similarities exist between the mechanisms of ERK1/2 activation employed by the IGF-1R and many G protein-coupled receptors. As with most G-coupled receptors (46), IGF-1-stimulated ERK1/2 activation in some cell types is sensitive both to pertussis toxin and to expression of peptide inhibitors of Gαi subunits (47, 48). Although the precise role of Gαi subunits in IGF-1R signaling remains ill-defined, the finding that the IGF-1R, like many G protein-coupled receptors, activates the ERK1/2 cascade through EGFR cross talk suggests that both classes of receptor might employ a common mechanism of EGFR transactivation involving heterotrimeric G proteins.

Clathrin-dependent endocytosis also plays an integral role in activation of the ERK pathway via both G protein-coupled receptors and receptor tyrosine kinases (49). EGF-stimulated ERK1/2 activation, but not receptor autophosphorylation or phospholipase Cγ activation, is impaired in HeLa cells expressing a dominant inhibitory dynamin mutant (50).
cal, physical, or recombinant protein inhibitors of receptor endocytosis impair ERK1/2 activation by G protein-coupled receptors in some, but not all, model systems (49). Similarly, IGF-1-stimulated Shc phosphorylation and ERK activation, but not IRS-1 phosphorylation, are inhibited by dansylcadaverine, a polyanine that blocks endocytosis by stabilizing clathrin cages (51). The common link to endocytosis in each of these systems may be the involvement of the EGFR. Recent data suggest that EGFR-mediated ERK1/2 activation requires intact endocytic machinery, whether the EGFR is activated by EGF or via cross talk with another receptor. ERK1/2 activation mediated by the α2A-adrenergic (52) and κ-opioid (53) receptors, which do not themselves undergo agonist-induced endocytosis, is blocked by inhibitors of clathrin-dependent endocytosis. In the case of the β2- and α2A-adrenergic receptors, endocytosis-dependent ERK1/2 activation correlates with G protein-coupled receptor-mediated EGFR transactivation and internalization of the transactivated EGFR (52).

Our data suggest that the dissociation of IRS protein-dependent signals from Shc-dependent signals mediated by the IGF-1R can be accounted for by IGF-1R-mediated transactivation of the EGFR. Furthermore, the finding that IGF-1R can mediate ERK1/2 activation via autocrine/paracrine release of HB-EGF suggests the intriguing prospect that it may be possible to selectively block the proliferative or hypertrophic effects of IGF-1 by targeting the extracellular components of the signaling pathway. Because IRS-1-dependent signals, such as PI3K-dependent anti-apoptotic signals and GLUT4 translocation, are independent of EGFR transactivation, such an approach would not be expected to adversely affect the other metabolic effects of IGF-1.

Acknowledgments—We thank R. J. Lefkowitz for helpful discussion and critical reading of the manuscript, and D. Addison and M. Holben for excellent secretarial assistance.

REFERENCES

1. Medema, R. H., and Bos, J. L. (1993) Crit. Rev. Oncog. 4, 615–661
2. White, M. F. (1997) Diabetologia 40, S2–S17
3. Franko, T. F., Kaplan, D. R., and Cantley, L. C. (1997) Cell 88, 435–437
4. Del Peso, L., Gonzalez-Garcia, M., Page, C., Herrera, R., and Nunez, G. (1997) Science 278, 673–679
5. Okada, T., Kawano, Y., Sakakibara, T., Hazeki, O., and Ui, M. (1994) J. Biochem. (Tokyo) 115, 2395–2400
6. Hara, K., Yonezawa, K., Ando, K., Kotani, K., Kojima, A., Waterfield, M. D., and Kasuga, M. (1995) J. Biol. Chem. 270, 697–702
7. Sakaue, H., Yonezawa, K., Harai, H., Tushima, T., Akanuma, Y., Kozasa, T., Fujita, T., Komuro, I., Kazaki, Y., and Kadowaki, T. (1995) Nature 376, 703–704
8. Myers, M. G., Jr., and White, M. F. (1995) Trends Endocrinol. Metab. 6, 209–215
9. Daub, H., Weiss, F. U., Wallasch, C., and Ulrich, A. (1996) Nature 379, 576–580
10. Hackel, P. O., Zwick, E., Prenzel, N., and Ullrich, A. (1999) J. Biol. Chem. 274, 4672–4680
11. Carpenter, G. (2000) Science's STKE. 15, pe1