Reconstitution of Epidermal Growth Factor Receptor Transmodulation by Platelet-derived Growth Factor in Chinese Hamster Ovary Cells*

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Platelet-derived growth factor (PDGF) causes an acute decrease in the high affinity binding of epidermal growth factor (EGF) to cell surface receptors and an increase in the phosphorylation state of the EGF receptor at threonine654. The hypothesis that PDGF action to regulate the EGF receptor is mediated by the activation of protein kinase C and the subsequent phosphorylation of EGF receptor threonine654 was tested. The human receptors for PDGF and EGF were expressed in Chinese hamster ovary cells that lack expression of endogenous receptors for these growth factors. The heterologous regulation of the EGF receptor by PDGF was reconstituted in cells expressing [Thr654]EGF receptors or [Ala654]EGF receptors. PDGF action was also observed in phorbol ester down-regulated cells that lack detectable protein kinase C activity. Together these data indicate that neither protein kinase C nor the phosphorylation of EGF receptor threonine654 is required for the regulation of the apparent affinity of the EGF receptor by PDGF.

The maximal growth of fibroblasts is sustained by the coordinated effects of several factors. Treatment of murine 3T3 fibroblasts with platelet-derived growth factor (PDGF) causes the cells to become "competent" for DNA replication and cell division. Only a brief treatment with PDGF is required because PDGF can be removed after 30 min without the loss of PDGF action on cell growth (Pledger et al. 1977). This effect of PDGF to make 3T3 cells competent for progression through the cell cycle distinguishes PDGF from other growth factors (such as epidermal growth factor (EGF) and insulin-like growth factors) that are required to be present for prolonged periods to support cell growth (for review, see Stiles 1983). The molecular mechanism by which the effects of growth factors are coordinated is not understood. One level of interaction between EGF and PDGF is mediated by the cell surface receptors for these growth factors. Treatment of cells with PDGF causes rapid alterations in the apparent affinity and tyrosine protein kinase activity of the EGF receptor. This process has been termed "transmodulation" (for review, see Schlessinger 1988). The relationship between the acute effects of PDGF to regulate the properties of the EGF receptor and the action of PDGF to increase EGF-stimulated growth is an important question that has not been resolved.

PDGF causes a decrease in the high affinity binding of EGF to cell surface receptors and a decrease in the tyrosine protein kinase activity of the EGF receptor (Wran et al., 1980; Wharton et al., 1982; Heldin et al., 1982; Shupnick et al., 1982; Collins et al., 1983; Bowen-Pope et al., 1983; Olashaw et al., 1986; Davis and Czech, 1985a, 1987). These actions of PDGF are similar to those of tumor-promoting phorbol esters. It has been shown that phorbol ester causes the phosphorylation of the EGF receptor at threonine654 by protein kinase C (Iwashita and Fox, 1984; Coch et al., 1984; Hunter et al., 1984; Davis and Czech, 1985b; Downward et al., 1985). Phosphorylation of the EGF receptor at threonine654 in vitro has been reported to cause a decrease in the receptor tyrosine protein kinase activity and a decrease in the apparent affinity of the receptor for EGF (Coch et al., 1984; Friedmann et al., 1984; Fearn and King, 1985; Downward et al., 1985). Together these data suggest that the mechanism of phorbol ester action to cause EGF receptor transmodulation is the phosphorylation of the EGF receptor at threonine654. Lin et al. (1986) have reported evidence to support this conclusion using site-directed mutagenesis of the EGF receptor at threonine654 (but see Livneh et al. (1988) and Davis (1988)).

Although the information gained from the study of the effects of phorbol ester on the EGF receptor has been useful for investigating the mechanism of action of protein kinase C, the relevance of these data to the effects of PDGF is not clear. This is because PDGF causes an increase in EGF-stimulated growth (Pledger et al., 1977). In contrast, phorbol ester causes a decrease in the EGF-stimulated growth of human fibroblasts (Decker, 1984) and NIH-3T3 cells (Livneh et al., 1988). In view of the disparate actions of phorbol ester and PDGF on EGF-stimulated growth it is apparent that conclusions derived from experiments using phorbol ester may not be relevant to PDGF action. Thus, further studies are required to investigate the physiological effects of PDGF, rather than the pharmacology of phorbol ester action.

The purpose of the studies reported here was to rigorously test the hypothesis that the mechanism of regulation of the EGF receptor by PDGF is the phosphorylation of the EGF receptor at threonine654. To test this hypothesis it was necessary to develop an experimental model that could be used to examine the mechanism of PDGF action. The strategy that we employed was to use CHO cells that lack expression of the receptors for PDGF and EGF and to introduce the human receptor cDNAs by transfection. The result of this approach is that cell lines can be obtained expressing EGF and PDGF receptors with defined structures. We report that the heterologous regulation of the EGF receptor caused by PDGF was reconstituted in the transfected CHO cells. Furthermore, we report that neither protein kinase C nor the phosphorylation of the EGF receptor at threonine654 is required for PDGF
action. We conclude that other mechanisms must account for the regulation of the EGF receptor by PDGF.

**EXPERIMENTAL PROCEDURES**

**Materials**—[^H]Thymidine was from ICN Radiochemicals [γ-^32P]ATP, Na[^32P]I, and ^125^I-PDGF were obtained from Amersham Corp. EGF was purified (Savage and Cohen, 1972; Matrignani et al., 1982) and iodinated to a specific activity of 70–90 Ci/g as described (Pessin et al., 1983). Restriction enzymes were from Boehringer Mannheim. Porcine PDGF was obtained from Bioprocessing Ltd. Amethopterin was from Sigma.

The [Thr^23]EGF receptor and [Ala^654]EGF receptor cDNAs have been described previously (Davis, 1988) and were cloned as 4-kilobase XbaI-HindIII fragments into the expression vector pX (obtained from Dr. G. Johnson, Jewish National Center, Denver, CO) which contains the murine dihydrofolate reductase gene as a selectable marker and allows the expression of the EGF receptor cDNA using the SV40 early promoter and polyadenylation signals. The plasmids obtained were designated pXHER and pXHER(Ala^654).

**Tissue Culture**—CHO cells expressing the human PDGF receptor (B-type) cDNA using a pZipNeoSV(X) vector (Cepko et al., 1984) were obtained from Drs. L. Claesson-Welsh and C.-H. Heldin (Univ. of Uppsala, Uppsala, Sweden). The cells were maintained in Ham’s F-12 medium supplemented with 10% fetal bovine serum and 0.25 mg/ml G418 (Geneticin, Gibco). The cells were transfected with the plasmids pXHER and pXHER(Ala^654) using the calcium phosphate technique. After 3 days, the cells were passaged and selected using minimum Eagle’s medium—a supplemented with 10% dialyzed fetal bovine serum, 0.5 mM amethopterin, and 0.25 mg/ml G418. Stable colonies were isolated using cloning rings and screened for the expression of EGF receptors by measuring the cell surface binding of ^125^I-EGF at 4 °C.

**Analysis of **[^125]I-**EGF and**[^125]I-**PDGF Binding**—CHO cells were seeded in 16-mm wells and grown to a density of 5 × 10^4^ cells/well. The cells were then incubated for 24 h in medium supplemented with 0.1% calf serum. The monolayers were washed with 120 mM NaCl, 6 mM KCl, 1.2 mM CaCl_2_, 1 mM MgCl_2_, 25 mM HEPES (pH 7.4), 50 mM bovine serum albumin and incubated for 30 min at 37 °C in the same medium. The cells were treated with and without phorbol ester or PDGF at 37 °C and then rapidly cooled to 0 °C. The binding of ^125^I-EGF and ^125^I-PDGF to cell surface receptors was measured by incubation of the cells at 0 °C for 3 h as described (Davis and Czech, 1987). Nonspecific binding was estimated in incubations containing a 500-fold excess of unlabeled ligand.

**Analysis of **[^3]HThymidine Incorporation by **CHO Cells**—CHO cells were seeded in 16-mm wells and grown to a density of 5 × 10^4^ cells/well. The cells were then incubated for 48 h in medium supplemented with 0.1% calf serum. Growth factors were added to the medium up to 20% serum, 0.5 mM amethopterin, and 0.25 mg/ml G418. Stable colonies were isolated using cloning rings and screened for the expression of EGF receptors by measuring the cell surface binding of ^125^I-EGF at 4 °C.

**Expression of the Human PDGF and EGF Receptors in CHO Cells**—The aim of this study was to examine the role of EGF receptor threonine[^634] in the heterologous regulation of the EGF receptor by PDGF. The strategy that we employed was to express [Thr^23]EGF receptors and [Ala^654]EGF receptors in cultured cells and to examine the regulation of these receptors by PDGF. CHO cells were used because these cells express no endogenous EGF receptors (Livneh et al., 1986; Davis, 1988). However, CHO cells also do not express endogenous PDGF receptors. Therefore, CHO cells transfected with the human PDGF receptor (B-type) cDNA were used to investigate PDGF action. Subsequent transfection of these cells with the human EGF receptor cDNA allowed the isolation of CHO cells expressing both the human EGF receptor and the human PDGF receptor. The cells obtained were characterized by measuring the binding of ^125^I-PDGF and ^125^I-EGF to cell surface receptors at 4 °C. The cells were seeded in 16-mm wells and grown to a density of 5 × 10^4^ cells/well. The specific binding of 0.5 nM ^125^I-PDGF to cells expressing [Thr^23]EGF receptors and [Ala^654]EGF receptors was measured to be 12,400 ± 1,100 cpm/well and 10,600 ± 870 cpm/well, respectively (mean ± S. D., n = 3). The specific binding of 0.5 nM ^125^I-EGF to cells expressing [Thr^23]EGF receptors and [Ala^654]EGF receptors was measured to be 4,080 ± 340 cpm/well and 3,690 ± 290 cpm/well, respectively (mean ± S. D., n = 3). (Nonspecific binding of ^125^I-PDGF and ^125^I-EGF was observed to be less than 10 and 5% of the total binding, respectively.)

**Heterologous Regulation of the EGF Receptor by PDGF**—CHO cells expressing human EGF and PDGF receptors were examined to investigate whether the heterologous regulation of the EGF receptor was reconstituted in the CHO cells. Fig. 1 shows that treatment of CHO cells with 4β-phorbol 12β-myristate 13α-acetate (PMA) or PDGF at 37 °C caused a decrease in the binding of ^125^I-EGF to cell surface receptors. Maximal effects were observed when the cells were incubated with 1 nM PDGF. The inhibition of ^125^I-EGF binding was observed to be rapid, but the effect of PDGF treatment was transient. Maximal inhibition of ^125^I-EGF binding was observed after 15 min of PDGF treatment. The measured ^125^I-EGF binding returned close to control levels after 60 min of PDGF treatment (Fig. 2). Analysis of the ^125^I-EGF binding isotherm indicated that PDGF action was mediated by a
promoting phorbol ester, PMA. One marked difference be-
tween transfected with the human PDGF receptor cDNA (Table I). The
tase-deficient CHO cells (Livneh

The regulation of the high affinity binding of 125I-EGF to cell surface receptors
(Fig. 3, Table I).

Previously the heterologous regulation of the EGF receptor
by PDGF has been examined in human and murine fibroblasts
(Wrann et al., 1980; Wharton et al., 1982; Heldin et al., 1982;
Shupnick et al., 1982; Collins et al., 1983; Bowen-Pope et al.,
1983; Olashaw et al., 1986; Davis and Czech, 1985a, 1987).
Comparison of the time course, effective concentration of
PDGF, and the analysis of the 125I-EGF binding isotherm
indicates that the observed effects of PDGF on CHO cells
(Figs. 1-3) are similar to those reported for human and murine
fibroblasts. We conclude that the expression of the cDNAs
allows the reconstitution of the heterologous regulation
of the EGF receptor.

The binding data were obtained from cells treated without (control)
and with 0.5 nM PDGF or 10 nM PMA for 30 min at
37°C. The specific binding of 200 pm 125I-EGF to cell surface receptors
was measured at 4°C. The data are presented as the mean ± S. D. of
triple determinations and are normalized (100%) to the specific
binding observed to control cells (2154 cpm/well and 1963 cpm/well
for cells expressing [Thr654]EGF receptors and [Ala654]EGF receptors,
respectively). Similar results were obtained in three separate exper-
iments.

The data obtained are presented by

The data presented in Fig. 3 were used to investigate the equilib-
rium binding properties of EGF receptors expressed in CHO cells.
The binding constants were estimated using the computer program LIGAND (Munson and Rodbard, 1980) and are presented as the mean
± S. E. The data were fitted to two models: a model that assumes  a
single class of EGF binding sites at the cell surface and a model that
assumes two classes of binding sites. In each case, the binding
constants from the model that provides the better (statistically sig-
nificant, F test) fit of the data are presented.

| Receptor | Sites/cell | nM | 
|-----------|------------|-----| 
| Thr654EGF  | 107 ± 21  | 7.6 ± 3.1 | 
| PDGF      | 129 ± 14 | 9.5 ± 2.5 | 
| Ala654EGF  | 151 ± 18 | 6.6 ± 2.3 | 
| Control    | 62 ± 8   | 0.66 ± 0.3 | 
| PMA        | 21 ± 5   | 0.32 ± 0.2 | 
| PDGF      | 23 ± 4   | 0.38 ± 0.2 | 
| Site 1     | 62 ± 8   | 0.66 ± 0.3 | 
| Site 2     | 21 ± 5   | 0.32 ± 0.2 | 

In previous studies, the affinity of the human EGF receptor
expressed in CHO-K1 cells (Davis, 1988) and in dihydrofolate reduc-
tase-deficient CHO cells (Livneh et al., 1986) was observed to be
greater than the affinity of the EGF receptor expressed in CHO cells
transfected with the human PDGF receptor cDNA (Table I). The
reason for the difference in EGF receptor affinity between these CHO
cell lines is not understood.
PDGF (Figs. 1 and 2). This difference between the actions of PMA and PDGF has been previously observed in experiments using murine and human fibroblasts (Wran et al., 1980; Wharton et al., 1982; Heldin et al., 1982; Shupnick et al., 1982; Collins et al., 1983; Bowen-Pope et al., 1983; Olashaw et al., 1986; Davis and Czech, 1985a, 1987).

Role of EGF Receptor Threonine$^{634}$—The role of EGF receptor threonine$^{634}$ was examined by investigation of the regulation of $^{125}$I-EGF binding by PDGF using CHO cells expressing a mutated human EGF receptor in which threonine$^{634}$ had been substituted with an alanine residue. It was observed that PDGF caused an inhibition of $^{125}$I-EGF binding to the [Ala$^{634}$]EGF receptor (Fig. 1). The transient nature of the inhibition of $^{125}$I-EGF binding to the [Thr$^{634}$]EGF receptor was also observed in experiments using cells expressing [Ala$^{634}$]EGF receptors (Fig. 2). The extent of inhibition of $^{125}$I-EGF binding to CHO cells expressing [Ala$^{634}$]EGF receptors was greater than that observed in experiments using cells expressing [Thr$^{634}$]EGF receptors (Figs. 1–3).

In further experiments, the regulation by PMA of the high affinity binding of $^{125}$I-EGF to CHO cells expressing [Ala$^{634}$] EGF receptors was examined. It was observed that there was no significant difference between the regulation of $^{125}$I-EGF binding caused by PMA when cells expressing [Thr$^{634}$]EGF receptors and [Ala$^{634}$]EGF receptors were compared (Figs. 1–3). These data are in contrast to the observations made by Lin et al. (1996) but are consistent with the data reported by Davis (1988) and Livneh et al. (1988).

Role of Protein Kinase C—The hypothesis that PDGF-stimulated protein phosphorylation mediated by protein kinase C accounts for the heterologous regulation of the EGF receptor was examined. It has previously been documented that the incubation of cultured cells with phorbol ester causes the down-regulation of protein kinase C (Collins and Rozen- gurt, 1984). The down-regulation of protein kinase C in CHO cells was investigated using a synthetic peptide substrate for protein kinase C, Lys-Arg-Thr-Leu-Arg-Arg (Davis and Czech, 1987). It was observed that CHO cells incubated for 48 h with 500 nM PMA did not express detectable calcium and phospholipid-stimulated protein kinase activity (data not shown). PMA treatment of down-regulated cells did not cause an inhibition of $^{125}$I-EGF binding. However, PDGF caused a decrease in $^{125}$I-EGF binding to both the [Thr$^{634}$]EGF receptor and the [Ala$^{634}$]EGF receptor expressed in down-regulated CHO cells (data not shown). These data demonstrate that the functional desensitization of the protein kinase C signal transduction pathway does not alter the heterologous regulation of the EGF receptor by PDGF.

Regulation of $[^{3}H]$Thymidine Incorporation by EGF and PDGF—The interaction between EGF and PDGF was examined by investigation of the incorporation of $[^{3}H]$thymidine into DNA by CHO cells. In initial experiments the incorporation of thymidine by CHO cells expressing [Thr$^{634}$]EGF receptors was examined. Treatment of the CHO cells with PDGF caused a small increase in $[^{3}H]$thymidine incorporation. A significantly greater increase in $[^{3}H]$thymidine incorporation was observed when the cells were incubated with PDGF. Treatment of the cells with EGF and PDGF together resulted in a stimulation of $[^{3}H]$thymidine incorporation that was similar to that observed when the cells were incubated with 10% fetal calf serum (Fig. 4). Similar results were obtained in experiments using cells previously incubated for 48 h with 500 nM PMA to down-regulate protein kinase C (Fig. 4).

The incorporation of $[^{3}H]$thymidine by CHO cells expressing [Ala$^{634}$]EGF receptors was examined. In contrast to the small effect of EGF to increase $[^{3}H]$thymidine incorporation by CHO cells expressing [Thr$^{634}$]EGF receptors, EGF caused a marked increase in the $[^{3}H]$thymidine incorporation by cells expressing [Ala$^{634}$]EGF receptors. The increase in $[^{3}H]$thymidine incorporation observed was similar to that caused by PDGF (Fig. 4). This result suggests that mitogenic signal transduction by the [Ala$^{634}$]EGF receptor is more efficient than that by the [Thr$^{634}$]EGF receptor in CHO cells. Treatment of the CHO cells with PDGF together with EGF caused a larger stimulation of incorporation than that observed in the presence of 10% fetal calf serum (Fig. 4). Similar results were obtained in experiments using cells previously incubated for 48 h with 500 nM PMA to down-regulate protein kinase C (Fig. 4). These data indicate that PDGF augments the mitogenic effects of EGF mediated by the [Ala$^{634}$]EGF receptor in CHO cells.

**DISCUSSION**

**Functional Reconstitution of EGF and PDGF Receptors in CHO Cells**—We have investigated the process by which growth factors act coordinately to sustain the proliferation of cultured cells. In the present study, we have examined the interaction between PDGF and EGF. The strategy that we employed was to use CHO cells that lack expression of the receptors for PDGF and EGF and to introduce the human receptor cDNAs by transfection. The result of this approach was that cell lines could be obtained expressing EGF and PDGF receptors with defined structure. CHO cells expressing EGF and PDGF receptors responded to growth factor treat-
ment by increasing the incorporation of \(^{3}H\) thymidine into DNA. Treatment of the cells with EGF and PDGF together resulted in \(^{3}H\) thymidine incorporation at a level similar to that observed when the cells were incubated with 10% fetal calf serum (Fig. 4). These data indicate that the combined treatment of cells with PDGF and EGF resulted in potent mitogenic stimulation and that the interaction between the effects of PDGF and EGF on cell growth can be reconstituted in CHO cells. We conclude that CHO cells cotransfected with the human EGF and PDGF receptor cDNAs provide a suitable model system that can be exploited to gain an understanding of the regulation of cellular proliferation by growth factors.

**Transmodulation of the EGF Receptor after PDGF Treatment**—The molecular mechanisms by which the actions of EGF and PDGF are coordinated are not understood. One level of interaction is mediated by the cell surface receptors for these growth factors. Treatment of fibroblasts with PDGF causes a rapid and transient decrease in the expression of high affinity \(^{125}I\)-EGF binding sites at the cell surface (Wrann et al., 1980; Wharton et al., 1982; Heldin et al., 1982; Shupnick et al., 1982; Collins et al., 1983; Bowen-Pope et al., 1983). The mechanism of PDGF action to inhibit the high affinity binding of EGF to cell surface receptors is not understood. The inhibition of EGF binding may be caused by either a decrease in the affinity of the EGF receptor or by the internalization of the high affinity binding sites (Wrann et al., 1980; Wharton et al., 1982; Heldin et al., 1982; Shupnick et al., 1982; Collins et al., 1983; Bowen-Pope et al., 1983). The action of PDGF to inhibit EGF binding was also observed in CHO cells expressing human PDGF and EGF receptors (Figs. 1–3). The mechanism of transmodulation of the EGF receptor caused by PDGF has been suggested to be mediated by the phosphorylation of the EGF receptor at threonine by protein kinase C as a result of phoshoatidyl breakdown and consequent stimulation of protein kinase C (for review, see Schlessinger, 1988)). The evidence for this mechanism is based on several published reports. 1) Phorbol ester and PDGF cause transmodulation of the EGF receptor (Wrann et al., 1980). 2) PDGF causes the phosphorylation of the EGF receptor at the C-kinase substrate site, threonine (Davis and Czech, 1985a, 1987). 3) Mutagenesis of EGF receptor threonine has been reported to block phorbol ester action on the EGF receptor (Lin et al., 1986) but see Davis (1988) and Livneh et al. (1988)). Direct evidence that the phosphorylation of EGF receptor threonine by protein kinase C accounts for the transmodulation of the EGF receptor caused by PDGF has not been reported. We therefore investigated the effect of substitution of EGF receptor threonine with an alanine residue on the transmodulation of the EGF receptor caused by PDGF. It was observed that PDGF inhibited the high affinity binding of EGF to [Ala\(^{654}\)]EGF receptors expressed in CHO cells (Figs. 1–3). We conclude that the mechanism of EGF receptor transmodulation caused by PDGF does not require the phosphorylation of the EGF receptor at threonine and that other mechanisms must account for PDGF action.

Although phorbol ester caused a similar inhibition of \(^{125}I\)-EGF binding to cells expressing [Thr\(^{654}\)]EGF receptors and [Ala\(^{654}\)]EGF receptors, it was observed that PDGF caused a greater inhibition of \(^{125}I\)-EGF binding to the [Ala\(^{654}\)]EGF receptor compared with the [Thr\(^{654}\)]EGF receptor (Figs. 1–3).

The reason for the greater response of the cells expressing [Ala\(^{654}\)]EGF receptors to regulation by PDGF is not understood. It is possible that the lack of phosphorylation of the mutant receptor at residue 654 is mechanistically related to the enhanced effects of PDGF. Alternatively, it is possible that the data obtained result from the effect of the substitution of threonine with an alanine residue on the conformation of the receptor independently of phosphorylation. Further studies are required to distinguish between these hypotheses.

It has been proposed that protein kinase C mediates the effects of both phorbol ester and PDGF (for review, see Schlessinger, 1988). Down-regulation of protein kinase C by the prolonged treatment of cells with a high concentration of phorbol ester causes the desensitization of the transmodulation of the EGF receptor by PMA. These data are consistent with the hypothesis that protein kinase C mediates the effect of PMA. In contrast, Olashaw et al. (1986) have reported that down-regulation of protein kinase C does not block the transmodulation of the EGF receptor in Balb/c 3T3 fibroblasts caused by PDGF. This result was confirmed in experiments using CHO cells expressing human PDGF and EGF receptors (data not shown). These data are inconsistent with a role for protein kinase C in the mechanism of PDGF action to transmodulate the EGF receptor. Investigation of the phosphorylation of the EGF receptor in down-regulated human fibroblasts demonstrated that PDGF caused the phosphorylation of EGF receptor threonine in the absence of detectable protein kinase C activity (Davis and Czech, 1987). However, the functional significance of this phosphorylation of the EGF receptor at threonine can be questioned because PDGF inhibits EGF binding to the [Ala\(^{654}\)]EGF receptor in down-regulated CHO cells (data not shown). Together these data indicate that neither protein kinase C nor the phosphorylation of EGF receptor threonine is required for the transmodulation of the EGF receptor caused by PDGF.

**Transmodulation of the EGF Receptor after Phorbol Ester Treatment**—Treatment of CHO cells with PMA caused an inhibition of the high affinity binding of EGF to [Thr\(^{654}\)]EGF receptors and [Ala\(^{654}\)]EGF receptors (Figs. 1–3). This effect of PMA is consistent with previous observations (Davis, 1988; Livneh et al., 1988). However, the effect of PMA to cause transmodulation of the [Ala\(^{654}\)]EGF receptor contrasts markedly with the data reported by Lin et al. (1986) which indicate that the [Ala\(^{654}\)]EGF receptor does not respond to PMA treatment. The reason for the conflict in the data concerning the transmodulation of the EGF receptor mutated at threonine is not understood but has been discussed previously in detail (Davis, 1988).

**Regulation of EGF-stimulated Growth**—Livneh et al. (1988) have reported that phorbol ester inhibits the EGF-stimulated growth of NIH-3T3 cells and that the expression of EGF receptors mutated at threonine in NIH-3T3 cells causes the release of this mitogenic block. These data suggest that the phosphorylation of the EGF receptor by protein kinase C results in functional desensitization of the EGF receptor (Whitley and Glaser, 1986) and are consistent with the reported correlation between the phosphorylation of threonine and the inhibition of the receptor tyrosine protein kinase activity (Cochet et al., 1984; Friedmann et al., 1984; Downward et al., 1985; Davis, 1988). Comparison of the EGF-stimulated incorporation of \(^{3}H\) thymidine by CHO cells indicates that signal transduction by the [Ala\(^{654}\)]EGF receptor is more potent than that by the [Thr\(^{654}\)]EGF receptor (Fig. 4). The effects of EGF mediated by the [Thr\(^{654}\)]EGF receptor were small relative to that caused by fetal calf serum (Fig. 4).
In contrast, CHO cells expressing [Ala<sup>654</sup>]EGF receptors were stimulated by EGF to an extent that was similar to that caused by fetal calf serum (Fig. 4). It is possible that the increased effect of EGF to stimulate [<sup>3</sup>H]thymidine incorporation by CHO cells expressing [Ala<sup>654</sup>]EGF receptors compared with [Thr<sup>654</sup>]EGF receptors may be the result of a defect caused by fetal calf serum (Fig. 4). The phosphorylation of the EGF receptor at threonine<sup>654</sup> by protein kinase C was greatly increased by CHO cells expressing [Ala<sup>654</sup>]EGF receptors compared with CHO cells expressing [Thr<sup>654</sup>]EGF receptors (Fig. 4). We conclude that the mechanism of action of PDGF on cellular proliferation does not require the phosphorylation of the EGF receptor at threonine<sup>654</sup> by protein kinase C.

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