Biochem. J. (1988) 256, 109–115 (Printed in Great Britain)

Transmodulation of the epidermal-growth-factor receptor in permeabilized 3T3 cells

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Binding of murine epidermal growth factor (EGF) to its high-affinity receptor can be modulated by a variety of structurally unrelated mitogens. The transmodulation, however, is temperature-dependent and has not been observed in isolated membranes. We report here the transmodulation of high-affinity EGF receptors by platelet-derived growth factors (PDGF) and tumour-promoting phorbol esters in 3T3 cells even when they are rendered incapable of fluid-phase endocytosis by treatment with phenylarsine oxide or by permeabilization with lysophosphatidylcholine. The relative affinity of the EGF receptors in the absence of modulating agents is not significantly altered by phenylarsine oxide treatment. Thus the difference in affinity between the two classes of EGF receptors seems to be unrelated to dynamic membrane changes or to differential rates of internalization. In permeabilized cells, non-hydrolysable GTP analogues transmodulate the high-affinity EGF receptor; however, the effects of these analogues are blocked by the protein kinase C inhibitor chlorpromazine. In contrast, transmodulation by PDGF is not blocked by chloropromazine. Thus the high-affinity EGF receptor can be transmodulated by both protein kinase C-dependent or -independent pathways, and the transmodulation processes do not require fluid-phase endocytosis.

INTRODUCTION

The epidermal-growth-factor (EGF) receptor exists on the surface of many cells in two different affinity states (Todaro & De Larco, 1976; Lin *et al.*, 1986). It has been suggested that the mitogenic response to EGF is mediated through the high-affinity EGF receptor. Prywes *et al.* (1986) have shown that mutations in the cytoplasmic domain of the EGF receptor can cause the loss of high-affinity binding sites. The mitogenic response to EGF is severely decreased in these mutants, even when the concentration of EGF used is close to the K_d of the low-affinity receptor. The physiological role of the high- and low-affinity receptors is still a matter of some debate, as EGF is required for 6–8 h to induce the mitogenic signal (Westermark & Heldin, 1985). During this length of time, the low-affinity sites might be capable of delivering a mitogenic signal to the cells.

The activation of the EGF receptor is usually accompanied by phosphorylation, internalization and degradation (Carpenter, 1984; Yarden & Schlessinger, 1985). The relationships between these three phenomena and mitogenesis are still unclear, and indeed it is difficult to separate these aspects of signal transduction. It is noteworthy that other mitogenic agents [e.g. platelet-derived growth factor (PDGF) and transforming growth factor-β (TGFβ)], which can synergize with EGF by increasing its mitogenic activity in the low-picomolar range, also induce the down-modulation of the high-affinity EGF receptor (Massague, 1985). It is apparent that in many situations this transmodulation of the high-affinity EGF receptor is associated with the activation of protein kinase C (Rozengurt, 1986). It is still not clear whether the transmodulation causes a change in surface display of the high-affinity EGF receptor, or whether the binding constant for the formation of the high-affinity EGF–EGF-receptor complex is altered.

There is some evidence that the high-affinity state of the EGF receptor can be retained on membrane preparations (King & Cuatrecasas, 1982), although the reported binding constants for the two states were rather low, suggesting that the receptor affinity was disturbed as a result of the membrane preparation procedure. There have been no reports of transmodulation of the high-affinity EGF receptor in membrane preparations. This may reflect the absence of the cytoplasmic proteins (or the proteins 'weakly' associated with the inner surface of the plasma membrane), which mediate the signal from one receptor to another. Previous studies have attempted to analyse the effect of PDGF on the EGF receptor in the absence of endocytosis (Collins *et al.*, 1983) by decreasing the temperature to 4 °C. In one study only low-affinity receptors ($K_d \sim 2$ nM) were detected by Scatchard analysis. It was reported that PDGF decreased the binding constant of the low-affinity EGF receptors with no change in the number of EGF receptors (Bowen-Pope *et al.*, 1983).

It has also been reported that the transmodulation of the EGF receptor by PDGF was inhibited at 4 °C (Collins *et al.*, 1983), and the possibility of selective internalization of the high-affinity state was not formally disproved. Their conclusion was that the high-affinity receptors were converted into the low-affinity state, but remained on the surface of the cell, and that this conversion could be due to a covalent modification by a temperature-sensitive enzyme. To clarify the mechanism of transmodulation of the EGF receptor by PDGF, we have investigated the relationship between endocytosis,

Abbreviations used: EGF, epidermal growth factor; PDGF, platelet-derived growth factor; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; GTP[S], guanosine 5'-[γ-thio]triphosphate; p[NH]ppG, guanosine 5'-[$\beta\gamma$-imido]triphosphate; DME, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline.

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the maintenance of the high-affinity state and transmodulation by using the endocytosis inhibitor phenylarsine oxide. Since endocytosis was not required for the transmodulation, and the high-affinity state was not affected by phenylarsine oxide, we investigated the transmodulation of EGF receptors on permeabilized cells (Nomura *et al.*, 1986). Permeabilized cells allow direct investigation of the intracellular biochemical pathway linking the PDGF and EGF receptors.

EXPERIMENTAL

Materials

Balb/c 3T3 cells were routinely grown in Dulbecco's modified Eagle's medium (DME) containing 10% (v/v) foetal-calf serum. Confluent monolayers were used in all experiments. Phenylarsine oxide, 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and L-α-lysophosphatidylcholine (lysolecithin), chlorpromazine and neomycin were purchased from Sigma (St. Louis, MO, U.S.A.). GTP[S], ATP, GDP and p[NH]ppG were purchased from Boehringer, Mannheim, Germany. Cholecystokinin was a gift from Dr. G. Baldwin (Ludwig Institute for Cancer Research); PDGF was kindly provided by Dr. Elaine Raines (University of Washington, Seattle, WA, U.S.A.). Murine EGF was purified from mouse submaxillary glands and radioiodinated in accordance with a published procedure (Burgess *et al.*, 1983). The specific radioactivity of the ^{125}I-labelled EGF ranged from 2×10^6 to 5×10^6 c.p.m./pmol in different experiments.

Treatment with phenylarsine oxide

Confluent monolayers of Balb/c 3T3 cells were rinsed once with iso-osmotic phosphate-buffered saline (PBS; 150 mM-NaCl/20 mM-Na_2HPO_4/4 mM-NaH_2PO_4, pH 7.4), phenylarsine oxide was added, and the cells were incubated at room temperature for 5–30 min. From the time course and dose/response analysis, an incubation time of 10 min in 10 μM-phenylarsine oxide was chosen for subsequent experiments. In the presence of phenylarsine oxide, internalization was decreased to < 10% (Wiley & Cunningham, 1982), and the cells were completely viable.

Permeabilization

Confluent monolayers of Balb/c 3T3 cells were rinsed once with PBS. Lysophosphatidylcholine (1%, w/v, in PBS) was added to a final concentration of 0.004% (w/v), and the cells were kept at room temperature for 3 min. The lysophosphatidylcholine/PBS was washed away and replaced with a cytosolic buffer (Muldoon *et al.*, 1987) (see below).

Typically over 90% of the cells were permeabilized by this treatment, as assessed by Trypan Blue exclusion.

To maintain the intracellular ionic balance as unperturbed as possible, all experiments involving permeabilized cells were performed in a 'cytosolic buffer' (Muldoon *et al.*, 1987): 10 mM-Hepes, 120 mM-KCl, 10 mM-NaCl, 1 mM-KH_2PO_4, 5 mM-$NaHCO_3$, 1 mM-$CaCl_2$ and 0.5 mM-$MgCl_2$.

Transmodulation

Confluent monolayers of Balb/c 3T3 cells were rinsed once in DME and subsequently incubated for 30 min at 37 °C in DME containing the appropriate modulating agent. At the end of the incubation, the cells were rinsed in DME and the binding of ^{125}I-EGF was assessed.

^{125}I-EGF-binding assay

Unless otherwise specified, binding of ^{125}I-EGF to 3T3-cell monolayers was carried out for 30 min at 37 °C in DME containing bovine serum albumin (1%, w/v) for intact cells, and in cytosolic buffer for permeabilized cells. To obtain data for Scatchard analysis, the cells were incubated with ^{125}I-EGF (5 pM–2 nM) for 45 min at 37 °C. Non-specific binding was determined by adding a 50-fold excess of unlabelled EGF to each incubation (i.e. 250 pM–100 nM). Unbound radioactivity was removed by two washes in ice-cold PBS, and cell-associated radioactivity determined by lysing the cells in NaOH (1 M) and quantitatively transferring the lysates to tubes for γ-radiation counting.

RESULTS

Scatchard analysis of ^{125}I-EGF binding in the absence of internalization

One major problem encountered in determining the true affinity constants for hormone receptors at physiological temperatures is the difficulty of reaching equilibrium when there is internalization and cellular processing of both the ligand and its receptor. It has been shown that, under the conditions needed for ^{125}I-EGF and its receptor to reach equilibrium, up to 90% of the ligand is internalized and rapidly degraded by untreated cells (Wiley *et al.*, 1985). The analysis is further complicated by the fact that some degradation products are still capable of binding to the EGF receptor (Wiley *et al.*, 1985). In our experimental system, analysis of EGF binding to its receptor at 4 °C has proved difficult, owing to the loss of the high-affinity component (Bowen-Pope *et al.*, 1983). An alternative approach is to block endocytosis chemically and perform the binding experiments at physiological temperatures.

The thiol-group-blocking reagent phenylarsine oxide has been known for many years to be a remarkable inhibitor of fluid-phase endocytosis (Wallace & Ho, 1972). Despite this inhibition, phenylarsine oxide does not affect the short-term viability of the cells, nor the extent or rate of binding of ^{125}I-EGF to its receptor. We used phenylarsine oxide to inhibit receptor-mediated endocytosis of ^{125}I-EGF by Balb/c 3T3 cells, and compared the apparent binding constants for EGF in untreated 3T3 cells (Fig. 1*a*) with cells rendered incapable of internalization with phenylarsine oxide (Fig. 1*b*).

Under the conditions chosen for the binding of ^{125}I-EGF to cells (30 min, 37 °C), 55–70% of the radioactivity associated with untreated 3T3 cells was internalized (results not shown), as assessed by the acid-wash method of Haigler *et al.* (1980). The phenylarsine oxide treatment decreases the proportion of ^{125}I-EGF internalized by the 3T3 cells to 8–10%, which is sufficiently low to allow quantitative assessment of the equilibrium binding of EGF to its receptor.

Analysis of the binding data by the LIGAND program (Munson, 1981) gave the best fit for a two-site model in both cases. Within the limits of the experimental system, there was no significant difference between either the low-affinity or the high-affinity dissociation constants of treated and untreated cells. Furthermore, the ratio of high-affinity binding sites to total sites was similar (2.9%

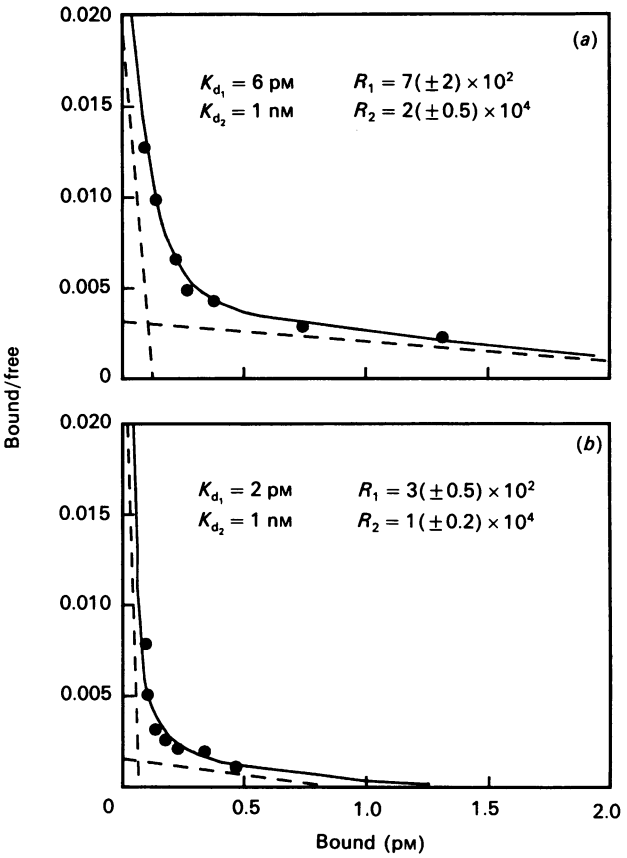

Fig. 1. ^{125}I-EGF binding to phenylarsine oxide-treated cells

Scatchard analysis of EGF binding to control cells (*a*) and to cells treated with 10 μM-phenylarsine oxide for 10 min at 23 °C (*b*). See the Experimental section for details of the binding assay. Binding constants (K_d) and receptor number/cell (R) were obtained from least-squares analysis [22].

in treated cells, 3.3% in the controls). The difference in the estimated receptor number between these two protocols can be explained in terms of the accumulation of internalized radioactivity by the control cells.

We conclude therefore that the two affinity states of the EGF receptor on 3T3 cells are intrinsic to the receptor and are not due to differential rates of internalization or to artefactual binding of ^{125}I-EGF degradation products.

Transmodulation of the high-affinity EGF receptor

The mechanism of receptor transmodulation has not yet been fully elucidated, although a role for intracellular Ca^{2+} (Korc *et al.*, 1984) and/or the activated protein kinase C (Zachary *et al.*, 1986) has been proposed. In particular, Korc *et al.* (1984) have suggested that modulation by cholecystokinin of EGF binding to pancreatic acinar cells is due to inhibition of EGF-receptor internalization. It was therefore appropriate to test the effects of phenylarsine oxide on receptor transmodulation.

We tested three different agents capable of transmodulating the EGF receptor (Table 1). TPA, PDGF and, to a lesser extent, cholecystokinin were effective in decreasing ^{125}I-EGF binding to both the control and the phenylarsine oxide-treated 3T3 cells. The transmodulating agents were more effective at the lowest concentration of ^{125}I-EGF (Table 1), where the proportional occupancy of the high-affinity binding sites is greater. The data presented in Table 1 are representative of several experiments.

To confirm that transmodulation was affecting the high-affinity EGF receptors, we performed Scatchard analyses for the binding of ^{125}I-EGF to phenylarsine oxide-treatment 3T3 cells in the presence and the absence of PDGF (Fig. 2). Preincubation of phenylarsine oxide-treated cells with PDGF totally abolished the high-affinity EGF-binding sites, and, within the limits of error, the low-affinity sites appeared to be unaffected. Thus, in contrast with previous reports with 3T3 cells at 4 °C (Bowen-Pope *et al.*, 1983), we did not observe a large change in the K_d of the low-affinity receptors. The decrease in binding at low ^{125}I-EGF concentration appears to be due to the specific disappearance of the high-affinity receptors. Thus high-affinity binding can be abrogated by transmodulation without the receptor leaving the cell surface.

Effect of nucleotides

The transition between the high- and low-affinity state of some cell-surface receptors is controlled by the association with regulatory proteins (G-proteins). The classical receptors coupled to G-proteins are the

Table 1. Transmodulation of EGF receptors on the surfaces of Balbc/3T3 cells treated with phenylarsine oxide

Confluent 3T3 cells were incubated at 23 °C for 10 min in medium alone or in 10 μM-phenylarsine oxide. After rinsing the cells, the modulating agents were added to a final concentration of 10 nM and the incubation was continued at 37 °C for 30 min. ^{125}I-EGF was then added to a final concentration of 20 pM or 100 pM, and the cell-associated radioactivity was determined after 30 min. Control values (c.p.m./10^4 cells) for ^{125}I-EGF binding were: a, 390±40; b, 720±10; c, 730±30; d, 1190±30.

		Binding (% of control)			
	[^{125}I-EGF] (pM)...	20		100	
Modulator	Phenylarsine oxide...	+	−	+	−
Nil		100±13[a]	100±2[b]	100±4[c]	100±3[d]
PDGF		17±10	37±7	60±7	84±2
TPA		6±2	5±2	20±13	23±4
Cholecystokinin		26±15	32±8	59±15	54±21

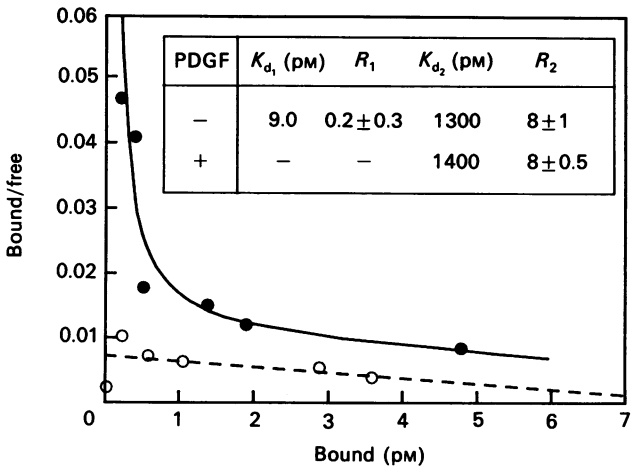

Fig. 2. PDGF-induced transmodulation of the high-affinity EGF receptor

Phenylarsine oxide-treated cells were incubated for 30 min at 37 °C with PDGF (○) or medium alone (●). The cells were exposed to increasing concentrations of ^{125}I-EGF for 30 min at 37 °C. Binding parameters were obtained from computer-assisted analysis of the Scatchard plot: $R_1 = 4.8 \times 10^2$ receptors/cell, $R_2 = 1.8 \times 10^4$ receptors/cell.

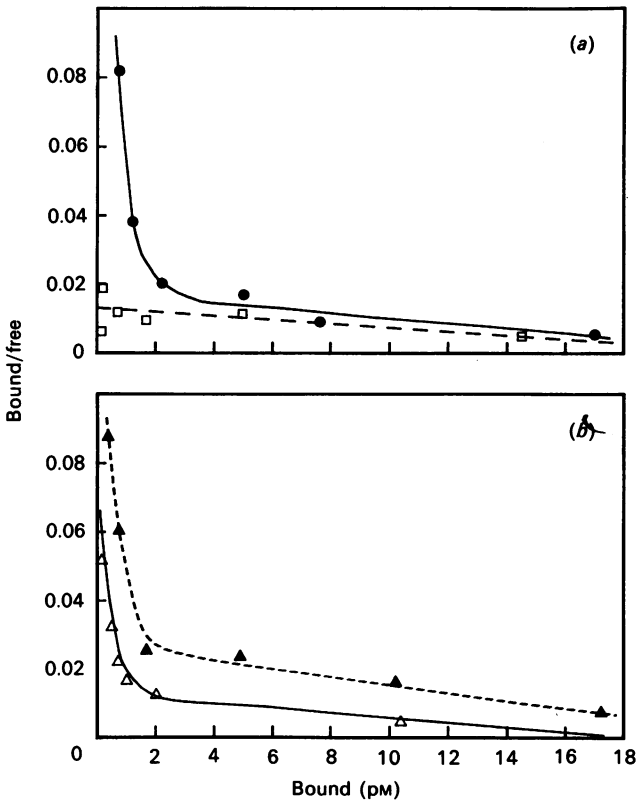

Fig. 3. Effect of nucleotides on binding of ^{125}I-EGF to cells

Permeabilized cells were incubated with medium alone (●) or with 100 μM-GTP[S] (□), -GDP (▲) or -ATP (△) for 20 min at 37 °C before the ^{125}I-EGF-binding assay, which was done as detailed in the Experimental section.

β-adrenergic receptor and the muscarinic receptors, but evidence is accumulating that tyrosine kinase-type receptors can also interact with G-proteins (Gawler *et al.*, 1987).

We decided to investigate the effects of non-hydrolysable GTP analogues on the two affinity states of the EGF receptor. Cells were permeabilized with lysophosphatidylcholine (Nomura *et al.*, 1986) to allow the introduction of nucleotides. In preliminary experiments it was found that the choice of buffer was critical to the maintenance of high-affinity receptors in permeabilized cells; we used the 'cytosolic buffer' described by Muldoon *et al.* (1987) in all experiments involving permeable cells. Preincubation of permeabilized 3T3 with GTP[S] removes the high-affinity components as effectively as does PDGF or TPA (Fig. 3*a*). A second non-hydrolysable GTP analogue, p[NH]ppG, has an identical effect on ^{125}I-EGF binding (Table 2); however, the binding of ^{125}I-EGF to ATP- and GDP-treated cells was not significantly different from that in untreated controls (Fig. 3*b*). The binding constants and the receptor numbers derived by computer analysis of the Scatchard plots are listed in Table 2. We concluded that the effects of GTP[S] and p[NH]ppG on the high-affinity EGF receptor were specific, and pointed to the involvement of a G-protein in the transition between the high- and low-affinity states

Of course, this putative G-protein could either be directly coupled to the EGF receptor or be located upstream along the transmodulation pathway. In view of the involvement of a G-protein in phosphoinositide hydrolysis (Berridge & Irvine, 1984; Michell & Kine, 1986), which results in activation of protein kinase C, and of the known role of protein kinase C in the transmodulation of the EGF receptor, we decided to investigate the coupling between this G-protein and the protein kinase C system.

Table 2. Analysis of high- and low-affinity binding of ^{125}I-EGF to permeabilized 3T3 cells in the presence of nucleotides

Dissociation constants (K_d) and receptor concentration (R) at equilibrium were obtained by least-square analysis (Munson, 1981) of the Scatchard plots presented in Fig. 3.

Nucleotide	K_{d_1} (pM)	R_1 (pM)	K_{d_2} (nM)	R_2 (pM)
–	5±0.5	0.6±0.02	1±0.1	15±6
GTP[S]	–*	–	2±1	14±1
p[NH]ppG	–*	–	4±3	24±1
GDP	6±2	0.4±0.1	2±0.9	15±1
ATP	5±0.8	0.4±0.05	1.5±0.5	50±20

* The best fit to the binding data was obtained with the one-site model.

Effects of protein kinase C and phospholipase C inhibitors on transmodulation

Although chlorpromazine is a potent inhibitor of protein kinase C, its mechanism is still poorly understood (Mori *et al.*, 1980). We tested the ability of chlorpromazine to inhibit transmodulation of the EGF receptor

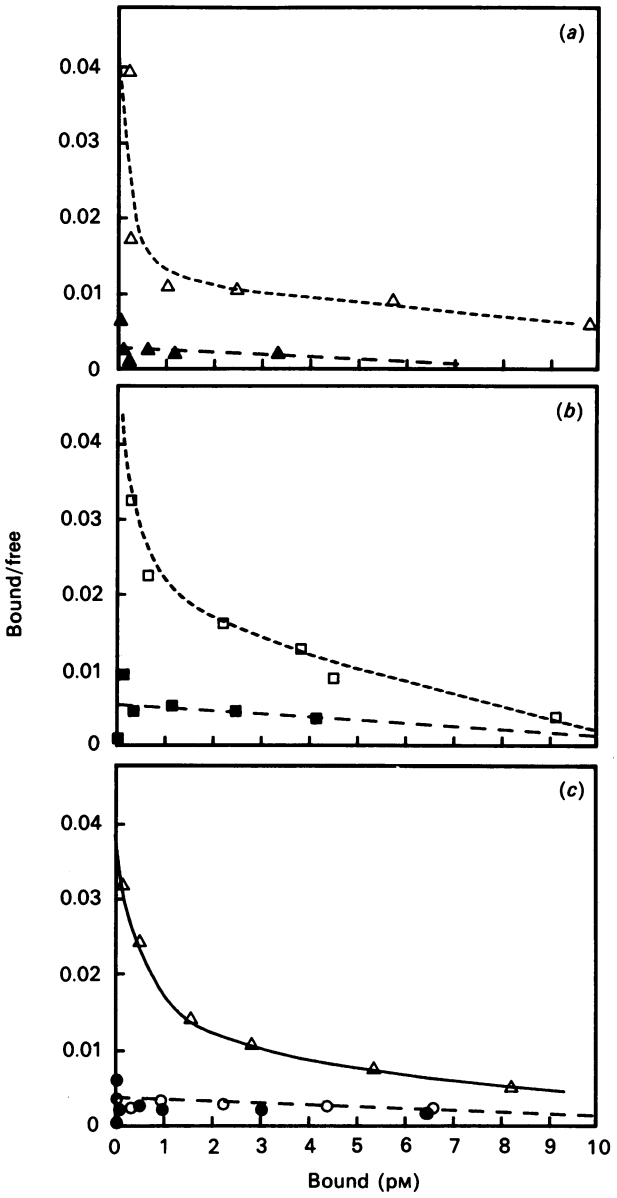

Fig. 4. Effect of chlorpromazine on transmodulation of EGF receptors

Preincubation of the cells with 10 nM-TPA (△,▲: panel *a*), 0.1 μM-GTP[S] (□,■: panel *b*) and 1 nM-PDGF (○,●: panel *c*) was carried out in the presence (△, □, ○) or absence (▲, ■, ●) of 1 μM-chlorpromazine for 30 min at 37 °C before measurement of the binding of ^{125}I-EGF. Binding of ^{125}I-EGF to control cells is also shown in panel (*c*) (△).

in permeabilized 3T3 cells (Fig. 4). Chlorpromazine causes a complete blockade of both TPA- (Fig. 4*a*) and GTP[S]- (Fig. 4*b*) mediated loss of high-affinity EGF receptors. In parallel experiments using PDGF as the transmodulator, chlorpromazine had no effect (Fig. 4*c*; see also Table 3). It seems therefore that the putative G-protein involved in the transmodulation of EGF-receptor affinity is located along the protein kinase C activation pathway, and may correspond to Gp (Cockcroft, 1987). Fig. 4 shows that PDGF induces transmodulation of the high-affinity EGF receptor by a

Table 3. Analysis of kinetic binding parameters for ^{125}I-EGF binding to permeabilized 3T3 cells treated with chlorpromazine (CPZ)

Kinetic binding parameters were obtained by least-squares analysis (Munson, 1981).

Treatment	K_{d_1} (pM)	R_1 (pM)	K_{d_2} (nM)	R_2 (pM)
–	20±4	0.6±0.3	1.2±0.5	1.2±0.3
TPA*	–	–	1.9±0.2	1±0.5
TPA+CPZ	6±0.4	0.2±0.05	1.8±0.9	2±0.9
GTP[S]*	–	–	2±1	1.8±0.9
GTP[S]+CPZ	8±0.6	0.3±0.2	0.6±0.02	1.3±0.2
PDGF*	–	–	2±0.1	0.9±0.6
PDGF+CPZ*	–	–	5±3.2	1.8±0.3

* The best fit to the binding data was obtained by using the one-site model.

Table 4. Neomycin does not affect the PDGF-induced transmodulation of the EGF receptor

Permeabilized Balb/c 3T3 were incubated with neomycin (0.2–5 mM) for 3 h at 37 °C. PDGF (1 nM) was added to the cells and the incubation continued for further 30 min before addition of ^{125}I-EGF (100 pM) in the presence or absence of excess unlabelled EGF. Specific binding to control cells (no neomycin, no PDGF) was 780±5 c.p.m.

Neomcyin (mM)	PDGF...	Binding of ^{125}I-EGF (% of control) +	–
0		37±5	100
0.2		33±2	94±8
2.0		39±4	105±4
5.0		33±3	100±1

pathway divergent from that of TPA, and hence independent of protein kinase C activation.

It has been reported that PDGF is able to stimulate phosphatidylinositol hydrolysis in fibroblasts (Berridge *et al.*, 1984). Breakdown of phosphoinositides by the activation of a phospholipase C yields two important second messengers: diacylglycerol, which stimulates protein kinase C, and inositol 1,4,5-trisphosphate, involved in the release of Ca^{2+} from intracellular stores. Neomycin selectively blocks phosphoinositide metabolism by binding to phosphatidylinositol mono- and bis-phosphate (Carney *et al.*, 1985; Schibechi & Schact, 1977) and thus preventing the generation of diacylglycerol and inositol trisphosphate. We therefore tested the effects of neomycin on the PDGF transmodulation of high-affinity EGF receptors. In our experimental system, addition of neomycin to the cells did not alter PDGF-induced transmodulation even at the highest concentrations used (Table 4). Neomycin on its own had no effect on binding of ^{125}I-EGF to Balb/c 3T3 cells.

DISCUSSION

Two previous studies have attempted to investigate the link between receptor internalization and the transmodulation of the EGF receptor by PDGF (Bowen-Pope *et al.*, 1983; Collins *et al.*, 1983). Although both of these studies observed the transmodulation phenomenon, Collins *et al.* (1983) reported that the transmodulation was strongly inhibited at 4 °C, whereas Bowen-Pope *et al.* (1983) found that transmodulation occurred at both 37 °C and 4 °C. The Scatchard analyses in the latter report indicated a change in the 1–2 nM binding site. However, the high-affinity binding site was not detected in those studies. Our results, using phenylarsine oxide to block endocytosis, indicate that PDGF selectively transmodulates the high-affinity receptor ($K_d \sim 10$ pM). PDGF did not change significantly the apparent number of low-affinity receptors ($K_d \sim 1$ nM). There was no internalization of the EGF receptor in the presence of phenylarsine oxide, nor was the EGF-dependent phosphorylation of the receptor or other cellular proteins in any way affected (F. Walker & A. W. Burgess, unpublished work). This suggests that the transmodulation process can affect the EGF-binding site on the extracellular domain of the high-affinity receptor, even though transmodulation presumably occurs because of the activation of the intracellular PDGF tyrosine kinase. Subsequent events must change the interactions between the EGF receptor and a hypothetical affinity modulator or change the conformation of the EGF receptor.

Our initial attempts to develop a simplified system to study transmodulation of PDGF and EGF receptors by using cell membranes were unsuccessful. Even though our results with phenylarsine oxide indicated that receptor internalization was not required, whenever we prepared membranes the high affinity of the EGF-receptor state was lost. Thus it was impossible to study transmodulation by using membrane preparations. However, the high-affinity receptor could be preserved in cells permeabilized with lysophosphatidylcholine (Nomura *et al.*, 1986). The permeabilized cells allowed the exploration of biochemical pathways on the cytoplasmic surface of the plasma membrane which are involved in the transmodulation process. It was necessary to mimic the ionic conditions of the cytoplasm (Muldoon *et al.*, 1987) to preserve the high-affinity state of the receptor. The Ca^{2+} concentration did not appear to be a critical determinant, as neither EGTA nor ionomycin affected the high-affinity EGF receptor on permeable cells (results not shown).

Our initial investigations indicated that the transmodulation pathway were intact and that both PDGF and TPA transmodulated the high-affinity EGF receptor on permeabilized cells. Neither ATP nor GDP altered the number of high-affinity EGF-binding sites; however, two non-hydrolysable GTP analogues (GTP[S] and p[NH]ppG) both decreased the number of high-affinity EGF-binding sites. Either a G-protein is involved in the maintenance of the high-affinity state or a G-protein induces phosphoinositol hydrolysis, with consequent activation of protein kinase C and an inevitable transmodulation. The inhibitor of protein kinase C, chloropromazine, blocked the effects of GTP[S] and p[NH]ppG, suggesting that these molecules cause their effects on the EGF receptor indirectly, via the protein kinase C system. Interestingly, neither neomycin nor chloropromazine prevents the PDGF-induced transmodulation of the high-affinity EGF receptor on the permeabilized cells. Although we did not directly test the effectiveness of these two agents in inhibiting protein kinase C and phosphoinositide metabolism respectively, their properties have been extensively characterized, and there is no reason to believe that they would be ineffective in our experimental system. We therefore suggest that the signalling between the PDGF receptor and the EGF receptor can occur independently of phosphoinositide metabolism and the activation of protein kinase C.

We thank E. Nice and L. Fabri for preparing the purified EGF and for the determination of the specific radioactivity of the iodinated derivative. We thank Professor Russell Ross and Dr. Elaine Raines for the generous gift of the purified PDGF.

REFERENCES

Berridge, M. J. & Irvine, R. F. (1984) Nature (London) **312**, 315–321
Berridge, M. J., Heslop, J. P., Irvine, R. F. & Brown, K. D. (1984) Biochem. J. **222**, 195–201
Bowen-Pope, D. F., Dicorleto, P. E. & Ross, R. (1983) J. Cell Biol. **96**, 679–683
Burgess, A. W., Lloyd, C. J. & Nice, E. C. (1983) EMBO J. **2**, 2065–2069
Carney, D. H., Scott, D. L., Gordon, E. A. & La Belle, E. F. (1985) Cell **42**, 479–488
Carpenter, G. (1984) Cell **37**, 357–358
Cockcroft, S. (1987) Trends Biochem. Sci. **12**, 75–78
Collins, M. K. L., Sinnett-Smith, J. W. & Rozengurt, E. (1983) J. Biol. Chem. **258**, 11689–11693
Gawler, D., Milligan, G., Speigel, A. M., Unson, C. G. & Houslay, M. D. (1987) Nature (London) **327**, 229–231
Haigler, H. T., Maxfield, F. R., Willingham, M. C. & Pastan, J. (1980) J. Biol. Chem. **255**, 1239–1241
King, A. C. & Cuatrecasas, P. (1982) J. Biol. Chem. **257**, 3053–3060
Korc, M., Matrisian, L. M. & Magun, B. E. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 461–465
Lin, C. R., Chen, W. S., Lazar, C. S., Carpenter, C. D., Gill, G. N., Evans, R. M. & Rosenfeld, M. G. (1986) Cell **44**, 839–848
Massague, J. (1985) J. Cell Biol. **100**, 1508–1514
Michell, B. & Kine, C. (1986) Nature (London) **323**, 112–113
Mori, T., Takai, Y., Minakuchi, R., Yu, B. & Nishizuka, Y. (1980) J. Biol. Chem. **255**, 8378–8380
Muldoon, L. L., Jameson, G. A. & Villereal, M. L. (1987) J. Cell. Physiol. **130**, 29–36
Munson, P. J. (1981) User's Guide to LIGAND: A Program System for Fitting Multiple Ligand, Multiple Binding Site Data, NIH, Bethesda
Nomura, S., Kamiya, T. & Oishi, M. (1986) Exp. Cell Res. **163**, 434–444
Prywes, R., Livneh, E., Ullrich, A. & Schlessinger J. (1986) EMBO J. **5**, 2179–2190
Rozengurt, E. (1986) Science **234**, 161–166
Schibechi, A. & Schacht, J. (1977) Biochem. Pharmacol. **26**, 1769–1774
Todaro, G. J. & De Larco, J. E. (1976) Nature (London) **264**, 26–31

Wallace, R. A. & Ho, T. (1972) J. Exp. Zool. **181**, 303–317
Westermark, B. & Heldin, C. H. (1985) J. Cell. Physiol. **124**, 43–48
Wiley, H. S. & Cunningham, D. D. (1982) J. Biol. Chem. **257**, 4222–4229
Wiley, H. S., Van Nostraud, W., McKinley, D. N. & Cunningham D. P. (1985) J. Biol. Chem. **260**, 5290–5295
Yarden, Y. & Schlessinger, J. (1985) Growth Factors in Biology and Medicine: CIBA Found. Symp. **116**, 23–45
Zachary, I., Sinnett-Smith, J. W. & Rozengurt, E. (1986) J. Cell Biol. **102**, 2211–2222

Received 22 March 1988/20 May 1988; accepted 1 June 1988