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Prostaglandin $F_{2\alpha}$ decreases the affinity of epidermal growth factor receptors in Swiss mouse 3T3 cells via protein kinase C activation

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Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) selectively decreases the binding of ^{125}I-labelled epidermal growth factor ([^{125}I]EGF) to intact Swiss 3T3 cells. Scatchard analysis reveals that $PGF_{2\alpha}$ decreases the number of high-affinity EGF binding sites without changing the total number of receptors. Prostaglandins E_1 (PGE_1), E_2 (PGE_2) or $F_{2\beta}$ ($PGF_{2\beta}$) do not alter the EGF binding to these cells and do not enhance the $PGF_{2\alpha}$ effect. R-59022 and R-59949, two diacylglycerol kinase inhibitors, enhance the inhibitory effect of $PGF_{2\alpha}$, whereas down-modulation of protein kinase C (PKC) abolishes the effect. These results indicate that $PGF_{2\alpha}$ decreases EGF binding in Swiss 3T3 cells via PKC activation.

Epidermal growth factor receptor; Prostaglandin $F_{2\alpha}$; Protein kinase C; Diacylglycerol; Swiss 3T3 cells; DNA synthesis

1. INTRODUCTION

Much evidence has shown that in confluent resting Swiss mouse 3T3 cells, mitogens such as bombesin, platelet derived growth factor or vasopressin cause increases in phosphatidylinositol metabolism, cellular diacylglycerol (DAG) content and protein kinase C (PKC) activity [1–6]. The stimulation of the latter mechanism leads to the occurrence of a complex array of biochemical changes followed by the initiation of DNA replication and cell division [7].

Among these events it has been shown that in these cells either bombesin or vasopressin, as well as the PKC activators OAG or TPA, alter the [^{125}I]EGF binding properties in these cells [8–11]. This phenomenon is characterized by a decrease in the number of high-affinity binding sites without changes in total apparent number of EGF receptors [8–10]. PKC down-modulation prevents the effect of these mitogens on [^{125}I]EGF binding, indicating that PKC activation is involved in the alteration of this cellular membrane property [4,11,12]. Other results reveal that the addition of either insulin or insulin-like growth factor-I (IGF-I) produce a decrease in [^{125}I]EGF binding without PKC involvement [13].

Abbreviations: PGE_1, prostaglandin E_1; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; $PGF_{2\beta}$, prostaglandin $F_{2\beta}$; OAG, 1-oleoyl-2-acetylglycerol; DAG, diacylglycerol; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate; R-59002, (6-[2-(4-[(4-fluorophenyl) phenylmethylene]-1-piperidinyl]-7-methyl)-5*H*-thiazolo[3,2-α]-pyrimidin-5-one; R-59949, (3-[2-[4-[bis(4-fluorophenyl)methylene]-1-piperidinyl]ethyl] 2,3-dihydro-2-thioxo-4(1*H*)-quinazolinone).

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Previous findings have shown that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) stimulates changes in phosphoinositide metabolism, PKC activation and cell growth in Swiss 3T3 cells [14–16]. In contrast, PGE_1, PGE_2 or $PGF_{2\beta}$ had little or no effect in stimulating these events [14,16]. Here we have shown that in these cells $PGF_{2\alpha}$ selectively decreases the number of [^{125}I]EGF high-affinity receptors. Only PGE_2 at high concentration (which has a little effect on phosphoinositide turnover and PKC activation) has a marginal effect on the [^{125}I]EGF binding to these cells. In addition, neither PGE_1 nor PGE_2, which potentiate the $PGF_{2\alpha}$ mitogenic effect, further decrease the $PGF_{2\alpha}$ reduction of [^{125}I]EGF binding. PKC down-modulation prevented the inhibitory effect of $PGF_{2\alpha}$. These results indicate that the decrease in [^{125}I]EGF binding triggered by $PGF_{2\alpha}$ involves PKC activation.

2. MATERIALS AND METHODS

2.1. *Cell culture, [^{125}I]EGF binding and initiation of DNA synthesis assay conditions*

Swiss mouse 3T3 cells [17] were propagated in Dulbecco's modified Eagle's medium as previously described [14]. For [^{125}I]EGF binding and initiation of DNA synthesis, cells were plated into 35-mm Petri dishes in conditions as described before [14]. OAG and prostaglandins were dissolved in ethanol such that the final ethanol concentration added to the culture medium was not greater than 0.5%. R-54022 and R-59949 were dissolved as a 5 mM solution in ethanol and stored at −20°C, and then diluted in 0.005 M HCl before use. Initiation of DNA synthesis was measured as reported previously [14].

2.2. *[^{125}I]EGF binding determination*

For [^{125}I]EGF binding, the cells were washed twice at 37°C with binding medium and the assay was then performed as described by Corps and Brown [13]. The [^{125}I]EGF concentration and incubation time are described for each experiment. Total and non-specific

[^{125}I]EGF binding (the latter carried out in the presence of 200-fold excess unlabelled EGF) were 5% and 0.3%, respectively, of the total radioactivity.

2.3. *Chemicals*

Purified EGF binding grade [18] was labelled with ^{125}I according to a described procedure [19]. Prostaglandins, R-59022 and R-59949 were the generous gift of Dr. J. Meringh, Upjohn Company, Kalamazoo and Dr. D. Chaffoy de Courcelles, Janssen Life Science Products, Beerse, Belgium. All remaining chemicals were purchased from Sigma. [*methyl*-^{3}H]Thymidine (18 Ci/mmol) was purchased from New England Nuclear.

3. RESULTS

3.1. *[^{125}I]EGF binding affinity is reduced by $PGF_{2\alpha}$*

The addition of [^{125}I]EGF to confluent resting Swiss 3T3 cells results in a time-dependent increase in the amount of mitogen bound to the cells, reaching to a plateau after 1 h of exposure (Fig. 1A). In contrast, in the presence of $PGF_{2\alpha}$ (300 ng/ml) the cell-associated [^{125}I]EGF was decreased by 46% at 1 h, although at later times $PGF_{2\alpha}$ had less inhibitory effect (Fig. 1A). Scatchard analysis of [^{125}I]EGF binding revealed that $PGF_{2\alpha}$ decreased the number of high-affinity receptors but did not alter the total number of receptors or the affinity of either class (K_d approximately 6×10^{-11} M and 2×10^{-9} M, respectively; Fig. 1B).

3.2. *Specificity of $PGF_{2\alpha}$ as an inhibitor of [^{125}I]EGF binding*

The action of $PGF_{2\alpha}$ in decreasing the high-affinity [^{125}I]EGF binding sites is specific among other closely related prostaglandins. PGE_1, PGE_2, $PGF_{2\alpha}$ or $PGF_{2\beta}$, added at 1–1000 ng/ml, produced different effects on the [^{125}I]EGF bound to these cells (Fig. 2A). $PGF_{2\alpha}$ induced a dose-dependent decrease in [^{125}I]EGF binding, reaching to a plateau of 50% inhibition at 20 ng/ml (Fig. 2A). In contrast, PGE_1, and $PGF_{2\beta}$ had no effect, whilst

Table I

Differential effect of PGE_1 or PGE_2 on $PGF_{2\alpha}$-induced decrease in [^{125}I]EGF binding affinity and mitogenic response in Swiss 3T3 cells

Additions	% Inhibition of [^{125}I]EGF binding	% Labelled nuclei
Control	0	0
PGE_1	0	0.7
PGE_2	8.0	0.6
PGE_1+PGE_2	10.0	0.8
$PGF_{2\alpha}$	51.0	19.1
$PGF_{2\alpha}$+PGE_1	52.0	47.0
$PGF_{2\alpha}$+PGE_2	53.0	49.0
$PGF_{2\alpha}$+PGE_1+PGE_2	53.0	48.0

Additions were as follows: PGE_1 (100 ng/ml); PGE_2 (100 ng/ml); $PGF_{2\alpha}$ (100 ng/ml). [^{125}I]EGF binding was determined for 60 min as in Fig. 2A. Initiation of DNA synthesis was measured after 28 h of stimulation as described in Section 2. The percentage of labelled nuclei in the presence of EGF (1 ng/ml) was 5% in agreement with previous results [25].

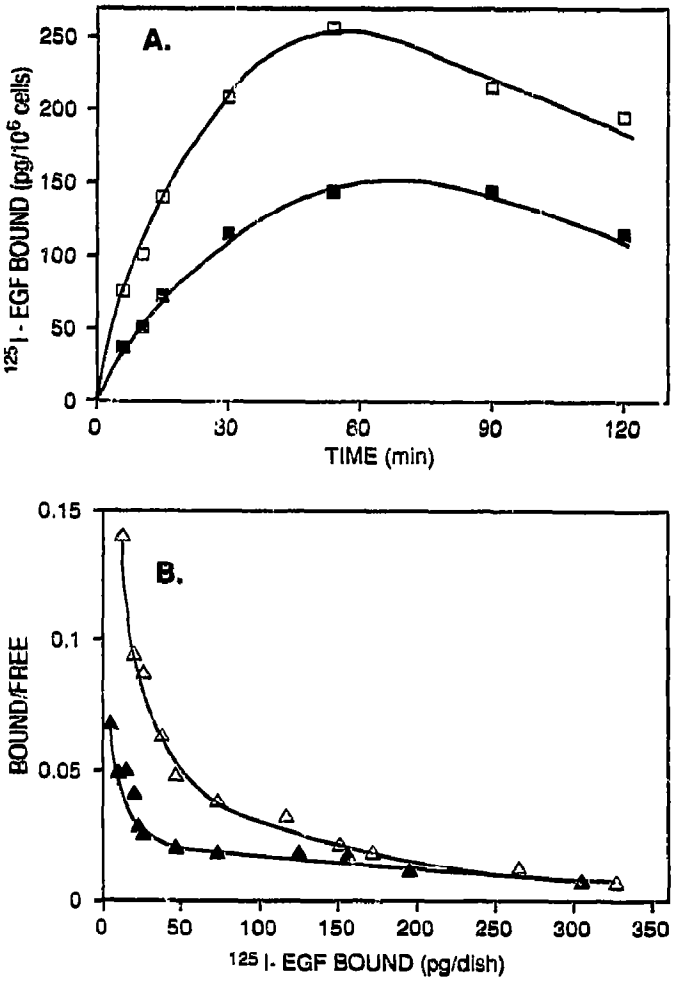

Fig. 1. (A) [^{125}I]EGF binding time course minus (□) or plus (■) $PGF_{2\alpha}$ (300 ng/ml). Cultures after being washed were incubated with 1 ml of binding medium containing 1.0 ng/ml [^{125}I]EGF (130,000 cpm/ng) at 37°C. Cell-associated radioactivity was determined as indicated in section 2. (B) Scatchard analysis of [^{125}I]EGF binding without (△) or with (▲) $PGF_{2\alpha}$ (300 ng/ml) at 37°C for 60 min. [^{125}I]EGF was added from 0.1 to 40 ng/ml. Samples of medium were removed at the end of incubation to determine the free [^{125}I]EGF and specific binding as indicated in section 2.

PGE_2 produced a similar effect to that of $PGF_{2\alpha}$ only at concentrations 20- to 50-fold greater (Fig. 2A). Neither PGE_1 nor PGE_2, alone or together, enhance the [^{125}I]EGF binding decrease induced by $PGF_{2\alpha}$, but both PGE_1 and PGE_2 potentiate the $PGF_{2\alpha}$ mitogenic response (Table I).

3.3. *$PGF_{2\alpha}$ reduction at [^{125}I]EGF binding affinity requires PKC*

In common with the PKC activator TPA, the effect of $PGF_{2\alpha}$ on [^{125}I]EGF binding was temperature-dependent, being much reduced at 4°C (Fig. 2B). The requirement for PKC in the $PGF_{2\alpha}$-induced [^{125}I]EGF binding inhibition is shown in Fig. 3. $PGF_{2\alpha}$, as well as bombesin and the PKC activators OAG or TPA,

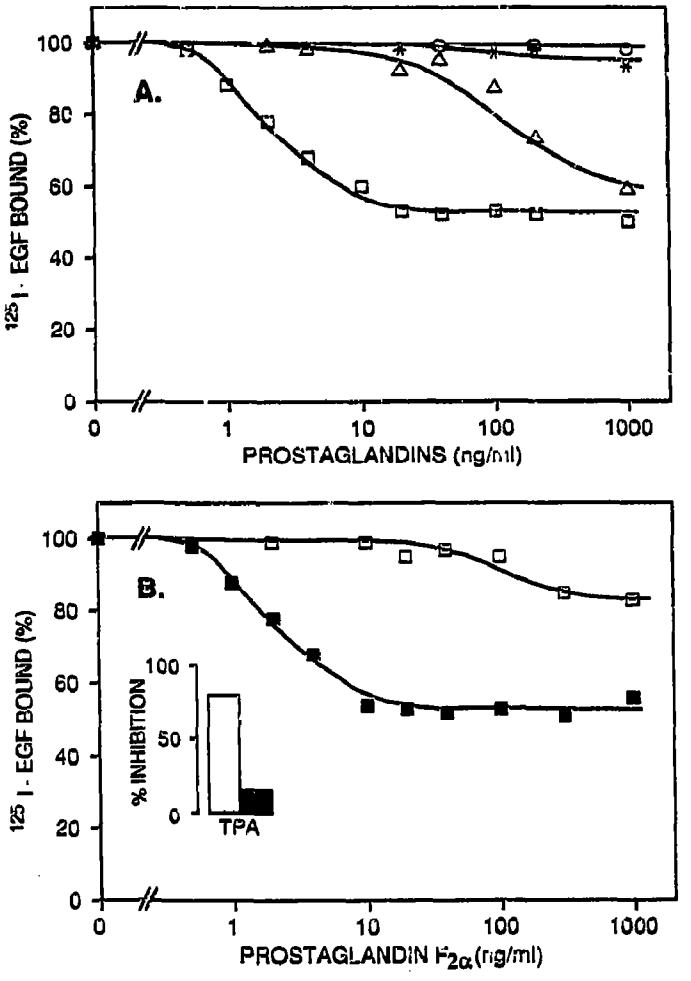

Fig. 2. (A) Differential effects of various concentrations of PGE_1, PGE_2, $PGF_{2\alpha}$ and $PGF_{2\beta}$ on [^{125}I]EGF binding at 37°C. Additions were as follows: (○) PGE_1; (△) PGE_2; (□) $PGF_{2\alpha}$; and (*) $PGF_{2\beta}$. (B) Inhibition of [^{125}I]EGF binding by increasing concentrations of $PGF_{2\alpha}$ at 37°C (■) or 4°C (□). The inset shows the effect of TPA (30 ng/ml) at 37°C (open bar) and 4°C (filled bar). Binding conditions were as in Fig. 1A. Incubation time at 37°C was 60 min and at 4°C was 150 min.

produced a decrease in the percentage of [^{125}I]EGF associated with the cells (Fig. 3). Cells pretreated with TPA to down-modulate PKC exhibited a substantially

Table II

Effect of R-59022 and R-59949 on the $PGF_{2\alpha}$ and Bombesin-induced decrease in [^{125}I]EGF binding affinity in Swiss 3T3 cells

Additions	% Inhibition of [^{125}I]EGF binding		
Additions	–	+R-59022	+R-59949
Control	0	1.4	5.0
$PGF_{2\alpha}$ (3 ng/ml)	25	43.0	46.0
$PGF_{2\alpha}$ (300 ng/ml)	47	66.0	65.0
Bombesin (17 ng/ml)	60	80.0	74.0

R-59022 (8 μM) and R-59949 (0.3 μM) were added to the conditioned medium for 60 min before the assay. Cells were rapidly washed with binding medium without or with R-59022 or R-59949, and [^{125}I]EGF binding was assayed as in Fig. 2A.

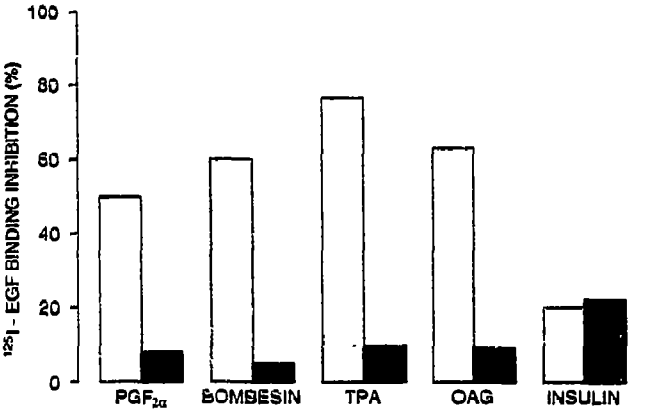

Fig. 3. Effect of PKC down-modulation on the inhibition by insulin (100 ng/ml), bombesin (17 ng/ml), $PGF_{2\alpha}$ (300 ng/ml), OAG (100 μg/ml) and TPA (30 ng/ml) on [^{125}I]EGF binding at 37°C. For PKC down-modulation cells were treated with TPA (480 ng/ml) for 72 h prior to the experiment. [^{125}I]EGF binding was determined at 37°C for 60 min. Conditions for determination were as in Fig. 1A. Standard error was less than 5% of the mean value. Open bars, without TPA; filled bars, with TPA.

smaller decrease of [^{125}I]EGF binding in response to $PGF_{2\alpha}$, bombesin, OAG or TPA. In contrast, insulin decreases [^{125}I]EGF binding to the same extent regardless of PKC down-modulation. In addition, R-59002 and R-59499, two diacylglycerol kinase inhibitors [20,21] which raise cellular DAG content [22] and thereby potentiate PKC-mediated events in the Swiss 3T3 cells [23] further enhance the effect of $PGF_{2\alpha}$ (3 and 300 ng/ml) on [^{125}I]EGF binding (Table II). Similar potentiation of the effect of bombesin also occurred (Table II).

4. DISCUSSION

Several findings show that in Swiss 3T3 cells the binding affinity for [^{125}I]EGF can be altered by the activation of different signalling mechanisms [8–13]. Bombesin and vasopressin, mitogens which increase phosphatidylinositol breakdown and cellular DAG content, decrease the number of high-affinity EGF receptors via PKC activation without alterations in the total number of receptors or the affinity of either class [5,16]. In addition, insulin or IGF-I, which in these cells do not stimulate phosphatidylinositol metabolism or PKC activity, induce similar changes to [^{125}I]EGF binding affinity [13]. Also, in PKC down-modulated cells platelet-derived growth factor induces a decrease in [^{125}I]EGF binding by activating a different kinase [24].

Here we present evidence that in Swiss 3T3 cells $PGF_{2\alpha}$ a mitogen which triggers phosphatidylinositol metabolism, also decreases the number of high-affinity EGF receptors via PKC activation without changing the total number of receptors. The $PGF_{2\alpha}$ effect is specific since neither PGE_1 nor $PGF_{2\beta}$, which do not elicit

changes in PIP_2 or DAG content, fail to induce this alteration. Only PGE_2, at high concentrations which increase phosphatidylinositol turnover, also decreased the EGF binding to these cells. In support for a possible diacylglycerol kinase involvement in modulating the [^{125}I]EGF binding we have shown that two enzyme inhibitors which increase cellular DAG [22] and PKC activity [23], potentiate this $PGF_{2\alpha}$-induced event. In contrast PGE_1 or PGE_2 can only potentiate the mitogenic effect of $PGF_{2\alpha}$, but do not enhance either the PKC-dependent phosphorylation of the 80 kDa protein [5,16] or the decrease in EGF receptor affinity. These results indicate that both PGE_1 and PGE_2 potentiate the $PGF_{2\alpha}$ mitogenic response, possibly by modulating another PKC-dependent event. Other findings reveal that $PGF_{2\alpha}$ enhances the mitogenic action of EGF [25] suggesting that the $PGF_{2\alpha}$ induced decrease of EGF receptor affinity might have implications in the regulation of the different signals triggered by both mitogens. Our future research is directed to unravel further differences in the events elicited by these growth factors.

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