EFFECT OF EPIDERMAL GROWTH FACTOR ON PROSTAGLANDIN E_1-STIMULATED ACCUMULATION OF CYCLIC AMP IN FIBROBLASTIC CELLS

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1. Introduction

Cells growing in culture ordinarily require factors present in serum for multiplication. A number of peptide hormones have been identified that will partially replace the growth factors present in serum. These include insulin, fibroblast growth factor, epidermal growth factor (EGF), and the family of polypeptides known as somatomedins or insulin-like growth factors (IGF) (reviewed in [1]). EGF is a low molecular weight peptide of M_r 6045 which can act as a mitogen for a variety of cell types including epidermal cells [2], fibroblasts [3–5], and glial cells [6]. The initial event in EGF-mediated stimulation of cellular DNA synthesis involves an interaction between EGF and its specific plasma membrane receptor [7,8]. Specific EGF receptors have been detected in a wide variety of mammalian cells, including normal rat kidney (NRK) cells [9]. Yet, the events mediated by EGF–receptor interaction which lead to a proliferative response have not been elucidated.

One substance that may regulate the growth of certain fibroblastic cells in culture is cyclic AMP (reviewed in [10,11]). Factors or conditions that cause a decrease in cyclic AMP levels (i.e., serum, insulin, proteases) tend to stimulate cell division [12–15]. Addition of cyclic AMP analogues or other agents which elevate cyclic AMP levels often inhibits DNA synthesis and growth [16–18]. For example, the stimulatory effect of EGF on DNA synthesis in human fibroblasts is reversed by dibutyryl cyclic AMP [19]. Thus, we thought it important to determine if EGF might alter cyclic nucleotide metabolism. Here we present evidence that EGF is able to decrease the accumulation of cyclic AMP in response to prostaglandin E_1 (PGE_1) stimulation.

2. Materials and methods

2.1. *Materials*

EGF was purified to electrophoretic homogeneity from male mouse submaxillary glands by the method in [20]. Prostaglandin E_1 was a generous gift from Dr John Pike, Upjohn Co., Kalamazoo, MI. The cyclic AMP antigen was from Collaborative Research.

2.2. *Cell culture*

Fibroblastic NRK-Cl_2-T cells were obtained from Dr George Todaro. Cells were grown to confluency in Dulbecco-Vogt modified Eagle's medium with 10% calf serum (Colorado Serum Co.) in a 5% CO_2-humidified atmosphere at 37°C. Medium was changed 24 h before use. On the day of use cells were washed 2 times with serum-free medium at 37°C and 3 ml serum-free medium were added 20–60 min prior to the addition of hormones. EGF was added 2–20 min prior to the addition of PGE_1 and cyclic AMP was extracted after a period of incubation, usually 10 min, as indicated. The time interval between the addition of EGF and the addition of PGE_1, usually 2–20 min, did not significantly alter the EGF effect on the hormone-stimulated accumulation of cyclic AMP. PGE_1 was dissolved in ethanol and EGF was made up in growth medium containing 1 mg/ml bovine serum albumin.

2.3. *Cyclic AMP analysis*

Cyclic AMP was extracted and quantitated by the acetylation modification of the radioimmunoassay [21] as in [15,22].

2.4. *Protein determination*

Total cellular protein was determined in identically treated companion cultures by the Lowry method [23] with bovine serum albumin as standard.

3. Results

3.1. *Effect of EGF on PGE_1-stimulated cyclic AMP accumulation in NRK cells*

The results presented in fig.1 show the effect of increasing EGF concentration on cyclic AMP levels. EGF decreases the accumulation of cyclic AMP-induced by PGE_1 addition to the cells. Half-maximal inhibition is noted with 1.5 ng/ml EGF, with maximal inhibition observed between 10 and 20 ng/ml EGF. The inhibitory effect on the PGE_1-stimulated accumulation of cyclic AMP is diminished at higher concentrations of EGF (50–100 ng/ml). While the inhibition of cyclic AMP accumulation mediated by EGF was consistently observed, the maximal extent of inhibition was variable from experiment to experiment (i.e., from 20–60%) for reasons which are not clear. When tested alone EGF occasionally causes a slight increase in the basal concentration of cyclic AMP.

The addition of 10 μg/ml PGE_1 to NRK cells causes a rapid increase of cyclic AMP levels, with maximal accumulation of cyclic AMP noted at 10 min after PGE_1 addition (fig.2). This is followed by a decline toward basal levels despite the continued presence of hormone. PGE_1-mediated cyclic AMP accumulation is inhibited by the presence of 20 ng/ml EGF at all time points, indicating that the effect of EGF is a rapid event and that EGF does not appear to alter the time course of desensitization toward PGE_1.

Increasing PGE_1 concentration from 1–10 μg/ml causes a progressive increase in the cyclic AMP content of the cells (fig.3). However, the ability of EGF to decrease the PGE_1-response was not altered by increasing the concentration of PGE_1. This suggests that EGF is not competing with PGE_1 for binding to

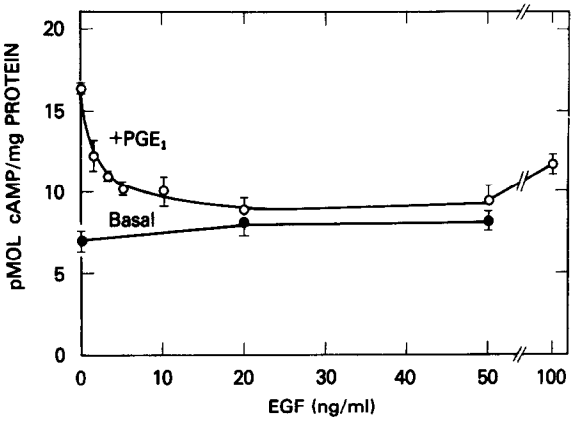

Fig.1. Effect of increasing EGF concentration on basal (•–•) and PGE_1-stimulated (o–o) cyclic AMP levels. NRK cells were grown to confluency (0.55 mg protein/60 mm dish) in 10% serum and changed to serum-free medium 30 min prior to use as in section 2. The indicated concentration of EGF was added 10 min prior to the addition of PGE_1 (10 μg/ml) and 10 min after PGE_1 additional cyclic AMP was extracted. EGF was present for 20 min prior to extraction of cyclic AMP in the basal experiment. Values represent the mean ± SE of triplicate determinations.

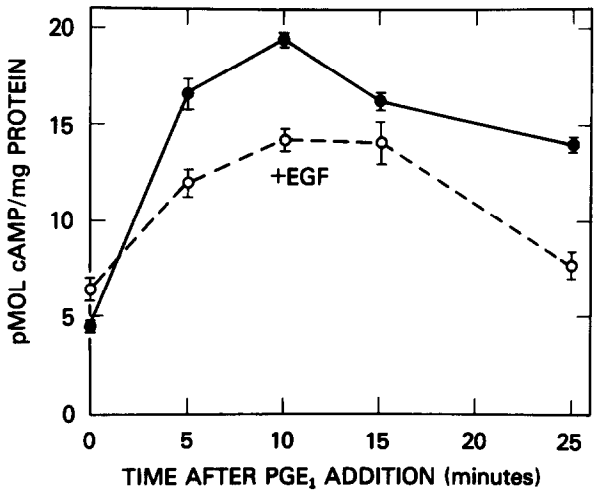

Fig.2. Time course of PGE_1-stimulated accumulation of cyclic AMP in the presence (o–o) or absence (•–•) of EGF. Cells were grown to confluency and placed in serum-free media as in section 2. EGF (20 ng/ml) was added to cells 10 min prior to the addition of PGE_1 (10 μg/ml). Cyclic AMP was extracted at the times indicated after PGE_1 addition. Values represent the mean ± SE of triplicate determinations.

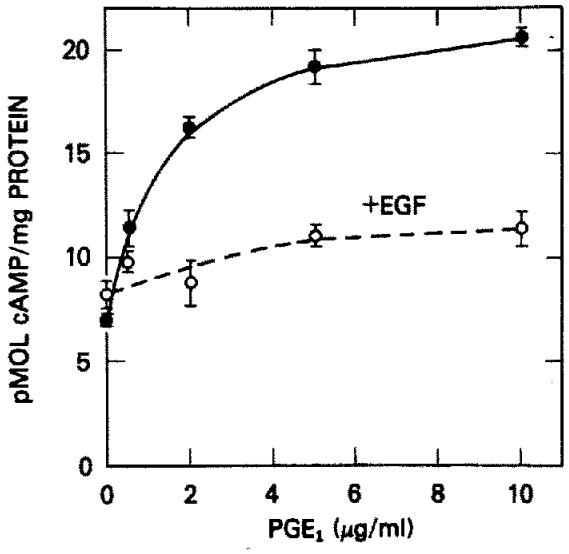

Fig.3. Effect of increasing PGE_1 concentration on intracellular cyclic AMP levels in the presence (○—○) or absence (●—●) of EGF. Cells were grown to confluency and placed in serum-free media as in section 2. EGF (20 ng/ml) was added to cells 10 min prior to the addition of the indicated concentration of PGE_1. Cyclic AMP was extracted 10 min after the addition of PGE_1. Values represent the mean ± SE of triplicate determinations.

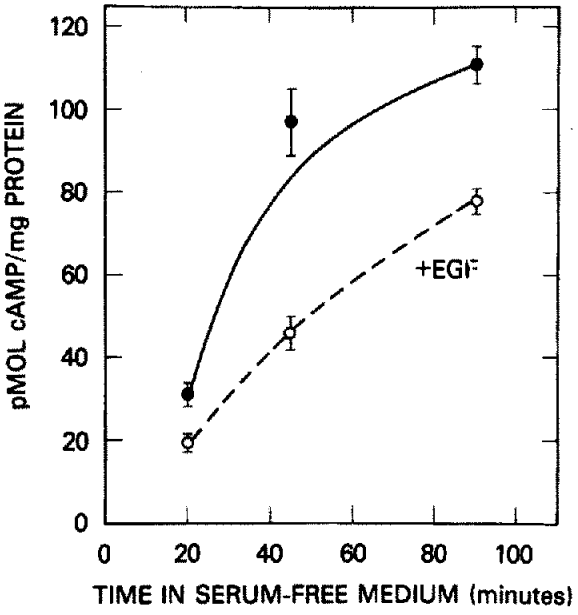

Fig.4. Effect of time of incubation of cells in serum-free medium with (○—○) or without (●—●) EGF on the ability of PGE_1 to stimulate the accumulation of cyclic AMP. Cells were incubated in serum-free medium with or without EGF (20 ng/ml) for the time indicated. PGE_1 (10 μg/ml) was added 10 min prior to termination and extraction of intracellular cyclic AMP. Values represent the mean ± SE of triplicate determinations.

receptor, but rather that EGF acts at a step beyond hormonal binding.

3.2. *Effect of time of incubation in serum-free medium in the presence or absence of EGF on the PGE_1 response*

The addition of serum to a number of different resting cell types has been shown to cause a dramatic fall in intracellular cyclic AMP levels [10,24]. Furthermore, the addition of serum decreases the accumulation of intracellular cyclic AMP in response to hormone [24–26]. For these reasons, the present studies were routinely carried out in the absence of serum. As shown in fig.4, with increasing incubation time of NRK-Cl_2-T cells in the absence of serum, there is a progressive increase in the ability of PGE_1 to stimulate cyclic AMP accumulation. These results also show that incubation of cells in serum-free medium with EGF for 10, 35 and 80 min prior to the addition of PGE_1 does not appreciably alter the extent of EGF inhibition of the PGE_1 response.

4. Discussion

Here we report that EGF exhibits a concentration-dependent inhibition of PGE_1-stimulated accumulation of cyclic AMP in NRK-Cl_2-T cells, a line in which cyclic AMP levels rise as growth slows [11]. Maximal inhibition was observed at 10–20 ng/ml EGF. It has been shown [22] that the somatomedin-like growth factor multiplication stimulatory activity (MSA) inhibits the PGE_1-stimulated accumulation of cyclic AMP in CEF. With other cell types the addition of serum has been shown to decrease the accumulation of intracellular cyclic AMP in response to hormone [24–26].

The decreased PGE_1 response mediated by EGF could be due to inhibition of adenylate cyclase activity, activation of cyclic AMP phosphodiesterase activity, or possibly to increased efflux of newly synthesized cyclic AMP into the extracellular medium. To date

we have not been able to detect any effect of EGF on the adenylate cyclase system of isolated crude plasma membranes. Thus, additional studies are required to elucidate the mechanism of action of EGF alteration of hormonal responsiveness.

The addition of EGF to crude membranes prepared from human epidermal carcinoma cell line A431 was reported [27] to result in a marked stimulation of the phosphorylation of endogenous proteins in the presence of [γ-^{32}P]ATP. Our results indicate that the addition of EGF decreases the PGE_1-stimulated cyclic AMP accumulation, presumably by acting at the membrane level. It seems possible that the enhanced membrane phosphorylation mediated by EGF may regulate the hormonal responsiveness of the plasma membrane adenylate cyclase system.

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