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Research Article

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Antagonism between Epidermal Growth Factor and Phorbol Ester Tumor Promoters in Human Breast Cancer Cells

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ABSTRACT It has been suggested that the phorbol ester tumor promoters act via the receptor-effector system for epidermal growth factor (EGF), since they interact with the EGF receptor system and mimic many of the effects of EGF in cultured cells. We have studied the interaction of phorbol esters with the EGF-responsive MCF-7 human breast cancer cell line. Similar to other systems, phorbol esters inhibit EGF binding in MCF-7 cells in a manner paralleling their potency as tumor promoters in mice. The effect is specific for EGF since the membrane binding of insulin is unaffected. Like EGF, the potent phorbol ester 12-*O*-tetradecanoyl-13-phorbol acetate (TPA) stimulates protein synthesis as indicated by a twofold increase in [^{3}H]leucine incorporation into protein after 24 h in TPA. Cell morphology, however, is significantly different with TPA treatment. After 24–48 h in TPA, cells become markedly enlarged with increased cytoplasmic vacuolization and increased membrane microvilli. This is reflected in a fourfold increase in the protein/DNA ratio (control 13.1; TPA 55.9). Furthermore, TPA inhibits cell division in media with or without serum, and prevents growth stimulation by EGF. Low TPA concentrations (1.0 ng/ml) are active, and 10 ng/ml results in maximal inhibition of cell replication. Other phorbol esters inhibit MCF-7 cells relative to their tumor promoting activity in vivo and their ability to inhibit EGF binding in these cells. After 24 h in TPA, incorporation of [^{3}H]thymidine into DNA is markedly reduced and the thymidine labeling index falls (33% to 2%) indicating very few S-phase cells. Growth inhibition is reversible by removing TPA from the medium. Similar inhibitory effects are seen with the two other human breast cancer cell lines studied, ZR75-1 and MDA-MB-231. In conclusion, phorbol esters may interact with the EGF receptor domain in MCF-7 human breast cancer cells, but they have distinct effects on cell morphology and growth suggesting alternative pathways of action. The antineoplastic activity of these compounds needs further investigation.

INTRODUCTION

The active components of croton oil, diesters of the diterpine alcohol, phorbol, are potent tumor-promoters in the two-stage mouse skin carcinogenesis model (1). The phorbol esters themselves are not carcinogens, but application of these compounds after a suboptimal dose of a chemical carcinogen results in the growth of tumors.

Studies in vitro have revealed a variety of interesting effects of these tumor promoters on cultured cells. In normal cells the phorbol esters induce changes similar to those observed in cells transformed by chemical or viral carcinogens. In transformed cells many of the characteristics of transformation are exaggerated. These include altered cell morphology (2, 3), increased uptake of small molecules (2–4), alterations of the cell membrane (4, 5), increased prostaglandin and polyamine synthesis (6–8), enhanced DNA synthesis and cell division (3, 9–11), and increased production of plasminogen activator (11, 12). Furthermore, in most models studied phorbol esters inhibit terminal differentiation (2, 13). The pleiotypic responses induced by different phorbol esters in vitro are directly proportional to their potency as tumor promoters in vivo suggesting that these events may be important for the activity of these compounds.

The mechanism of tumor promotion is of obvious importance but at present has not been well defined. Lee and Weinstein have postulated that the tumor

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promoting activity and the pleiotypic effects of phorbol esters may be due to the ability of these compounds to interact with the receptor-effector system of an endogenous growth factor such as epidermal growth factor (EGF)[1] (11, 14). Their hypothesis is based on the observations that phorbol esters specifically inhibit receptor binding of EGF, indicating that they interact directly or indirectly with the EGF receptor domain (15, 16) and that phorbol esters share many of the biological properties of EGF in cultured cells (4, 11, 17). Furthermore, EGF has been reported to promote tumor carcinogenesis in mouse skin (18). More recent evidence suggests that the effect of the phorbol esters on EGF receptor binding is indirect and not the result of an interaction with the active binding site (14, 19, 20).

We have recently shown that growth of the cultured human breast cancer cell line, MCF-7, is sensitive to physiological (1–10 ng/ml) concentrations of EGF (21). In fact, EGF may be required for the long-term growth of these cells in defined serumless medium (22). Furthermore, these cells contain specific high affinity receptors for EGF.[2] In the present studies we have used these human-derived cells to further investigate the possibility that these tumor promoters and EGF may have a common mechanism of action.

METHODS

Materials. Mouse EGF was purchased from Collaborative Research, Inc. (Waltham, Mass.). Porcine insulin (lot 615-D63-10, 25.4 U/mg) was the gift of Dr. M. Root, Lilly Research Laboratories (Indianapolis, Ind.). The phorbol esters 12-*O*-tetradecanoylphorbol 13-acetate (TPA), phorbol 12,13-didecanoate (PDD), and phorbol 12,13-diacetate (PDA) were purchased from P-L Biochemicals, Inc. (Milwaukee, Wis.). Tritiated-thymidine (47 Ci/mmol), L-[U-^{14}C]leucine (342 mCi/mmol), and ^{125}I-labeled sodium iodide (>300 μCi/ug) were obtained from The Radiochemical Centre (Amersham, England).

Cells and culture techniques. The MCF-7 breast cancer cell line was originally provided by Dr. H. Soule of the Michigan Cancer Foundation (23). The ZR75-1 and MDA-MB-231 cell lines were provided by L. Engel and M. Lippman, respectively, at the National Cancer Institute. The characteristics of these cell lines and the tissue culture techniques employed by us for the routine maintenance of the lines have been summarized (24–26). The cells remained free of mycoplasma contamination and were subcultured weekly by suspension in 0.05% trypsin: 0.02% EDTA in 150 mM NaCl.

Receptor binding studies. Binding studies were performed under conditions similar to the biological activity studies described later (physiologic conditions). MCF-7 cells were plated in multiwell culture dishes (FB-4-TC or FB-6-TC, Linbro Scientific, New Haven, Conn.) in Richter's improved MEM-Z0 medium (IMEM, Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) supplemented with glutamine (0.6 g/liter), penicillin (62 mg/liter), streptomycin (130 mg/liter), insulin (1.0 nM), and 5% calf serum, and grown to near confluence. The medium was replaced with serum and hormone-free medium for 24 h, and then changed again to IMEM without or with phorbol esters solubilized in acetone. An equivalent amount of acetone (final concentration, 0.1%) was added to controls and had no effect on binding. After 2 h at 37°C, the medium was discarded and the monolayers washed twice with a total of 8 ml of binding medium (IMEM, 0.1% bovine serum albumin, 10 mM Hepes). Fresh binding medium was then added (1.4 ml in four well dishes or 0.4 ml in six well dishes).

^{125}I-labeled porcine insulin (130–200 μCi/ug) or mouse EGF (40–100 μCi/ug) were prepared by described modifications of the chloramine T method (27–29). Labeled insulin (120 pg/ml) or EGF (60 pg/ml) were added to the cells in a 50 μl vol for 15 and 30 min, respectively, in a humidified incubator with 5% CO_2 at 37°C. Under these conditions insulin binding reaches a maximum between 15 and 30 min and EGF binding reaches a maximum between 30 and 60 min. After the incubation, the binding medium was rapidly aspirated and the monolayers washed three times with a total of 12 ml of iced phosphate-buffered saline with 0.1% bovine serum albumin to remove unbound ligand. The cells were harvested by suspension in 10.02% EDTA in phosphate-buffered saline and the cell bound radioactivity determined in a Searle autogamma counter (Searle Radiographics Inc., Des Plaines, Ill.). Specific binding was defined as the total radioactivity bound minus the amount bound in the presence of excess unlabeled hormone (insulin, 6 μg/ml; EGF, 0.6 μg/ml) and was expressed per 10^6 cells. Nonspecific binding for EGF and insulin represented <3 and 20% of the total counts bound, respectively. Medium from cells incubated with labeled hormone only was saved for estimation of degradation by TCA precipitability (insulin) or by column chromatography on Sephadex G-50 (EGF).

Other characteristics of the insulin receptor in these cells have been published (26). EGF receptor binding in these cells demonstrates temperature dependence, saturability, reversibility, and high affinity:[2] half-maximal displacement of labeled tracer EGF is observed with a concentration of unlabeled EGF that results in approximately half-maximal biological activity (2.0 ng/ml, 0.33 nM). Insulin, fibroblast growth factor, prolactin, and growth hormone do not compete for EGF binding.

Cell growth studies. Cells were plated replicately in 100-mm petri dishes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) at concentrations of 0.5–1.1 × 10^5 cells/dish in growth medium containing 5% calf serum plus insulin (1.0 nM). 24–48 h later the medium was changed to serum and hormone-free medium for an additional 24 h. Hormones and/or phorbol esters were then added directly to the dishes at the indicated concentrations. An equivalent volume of acetone was added to controls and had no effect on growth. Cells were fed with identical fresh medium every 2 or 3 d. Total DNA (30), protein (31), and hemocytometer cell counts were performed as parameters of cell growth. In some experiments the effect of phorbol esters on cell growth was measured in cells growing in medium supplemented with 5% calf serum.

Studies of macromolecular synthesis and thymidine labeling index (TLI). The rates of protein and DNA synthesis were estimated by the incorporation of labeled precursors into TCA-precipitable material as described (25, 26). MCF-7 cells were replicately plated to a subconfluent density (3 × 10^5 cells per dish) in multiwell plates as described above. 24 h later the medium was changed to serum-free IMEM with 0.1% BSA for an additional 24 h. TPA or acetone (controls)

[1] *Abbreviations used in this paper*: EGF, epidermal growth factor; IMEM, Richter's improved minimal essential medium; PDA, phorbol 12, 13-diacetate; PDD, phorbol 12, 13-didecanoate; TLI, [^{3}H]thymidine labeling index; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

[2] C. K. Osborne and B. Hamilton. Manuscript in preparation.

were then added directly to the wells, and 22 h later [^{3}H]thymidine (0.5 μCi/ml) or [^{14}C]leucine (0.5 μCi/ml) were added for a 2-h pulse. The cells were harvested on ice, an aliquot taken for DNA determination (30), and the remaining cells disrupted by sonicating for 3 s in a Heat Systems-Ultrasonics (Plainview, N. Y.) sonicator with a microtip at the lowest setting. Precursor incorporation was determined by measuring the radioactivity precipitated in 10% TCA after collection on Millipore filters (0.45 μm, type HA; Millipore Corp., Bedford, Mass.). Incorporation was linear under the conditions employed.

The TLI was determined autoradiographically by the described (21) modification of the method of Livingston et al. (32). The TLI (labeled/unlabeled cells) was calculated by counting the fraction of intact cells containing eight or more grains over the nucleus. Background counts were always <5 grains/nucleus.

Ultrastructural studies. Transmission and scanning electron microscopy were performed by published methods (33) except that 3% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4, was used for primary fixation.

RESULTS

EGF and insulin binding. Similar to reports studying other cell types, a 2-h preincubation with phorbol esters inhibited the binding of EGF but not insulin to MCF-7 breast cancer cells (Fig. 1). PDA, a weak tumor promoter, had no effect on EGF binding, but the two potent tumor promoters, TPA and PDD, were potent inhibitors. Binding was markedly reduced with a PDD or TPA concentration of only 10 ng/ml, a concentration which also resulted in maximal biological effects (see below).

Inhibition of binding by TPA was apparent when TPA was added simultaneously to the binding assay (Fig. 2). In this experiment in which unlabeled EGF was used to compete with tracer EGF for receptor, a 2-h preincubation of the cells with TPA reduced binding to that comparable to nonspecific binding at all concentrations of EGF. Addition of TPA at the start of the binding assay also significantly reduced binding but to to a lesser extent. Thus, the effect of TPA on binding occurred long before the TPA-induced changes in cell morphology (see below). The phorbol esters had no effect on degradation of EGF or insulin. Media degradation, estimated by measuring the TCA-soluble fraction (insulin) or by calculating the fraction appearing in the small molecular weight peak after Sephadex G-50 chromatography (EGF), were <5% in both control and TPA-treated dishes.

Cell morphology. Although the phorbol esters interacted (directly or indirectly) with the EGF receptor, their effects on cell morphology and growth (see below) were markedly different from that of EGF (Fig. 3). EGF (Fig. 3B) had a minimal effect on cell size and shape compared to controls (Fig. 3A). The stimulatory effect of EGF on cell proliferation was evidenced by the more confluent cell density clearly visible after 6 d in culture (compare Figs. 3B and 3A). TPA had a

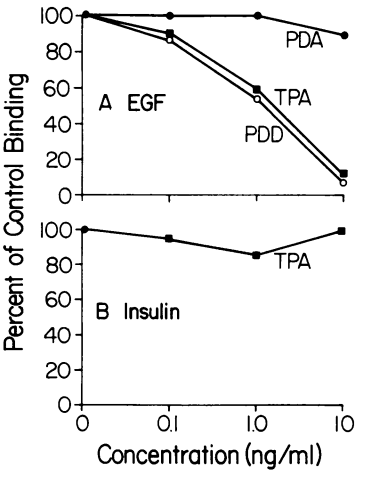

FIGURE 1 Effect of phorbol esters on EGF and insulin binding. MCF-7 cells were replicately plated in small culture dishes as described in Methods. When the cells were nearly confluent the medium was changed to fresh serum and hormone-free medium for 24 h. The cells were then preincubated with fresh medium containing the indicated concentrations of phorbol esters or an equivalent amount of acetone (0.1%, controls) for 2 h. The cells were washed twice with 8 ml of binding medium and fresh binding medium added. After 30 min at 37°C in 5% CO_2, ^{125}I-insulin (120 pg/ml) or EGF (60 pg/ml) were added to the cells for 15 and 30 min, respectively. After the binding incubation, the monolayers were washed three times with a total of 12 ml of iced phosphate-buffered saline with 0.1% bovine serum albumin. The cell bound radioactivity was determined by suspending the cells in .02% EDTA in phosphate-buffered saline and counting the suspension in an autogamma counter. Nonspecific binding was determined by the addition of excess unlabeled hormone (insulin 6 μg/ml; EGF 0.6 μg/ml) and was subtracted from the total counts bound to determine the specific binding shown. Results are expressed as a percentage of control binding obtained in the absence of phorbol esters. Values are the average of duplicate determinations.

dramatic effect on cell morphology (Fig. 3C). The cells markedly enlarged and became more flattened in shape with prominent intercellular attachments. The enlargement was mainly due to an increase in the cytoplasm to nucleus ratio, which is consistent with the increased protein: DNA ratio described below. Striking vacuolization was present in some cells but no dome formation was seen. Although the cells had reached a confluent density, in the presence of TPA a significant reduction from controls in cell number was observed. These data are quantified in Fig. 4. Scanning electron microscopy (Figs. 3D–F) revealed a dramatic increase in microvilli in the TPA-treated cells suggesting increased secretory or adsorptive activity. Transmission electron microscopy, (not shown) confirmed the presence of numerous microvilli in TPA-treated cells and, in addition, revealed a more intimate relationship between endoplasmic reticulum and mitochondria in these cells compared to that in controls or with EGF. These

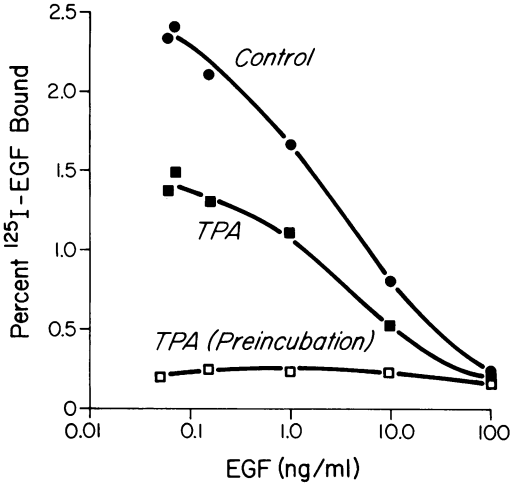

FIGURE 2 Effects of TPA and EGF on ^{125}I-EGF binding. Cells were plated and EGF binding measured as described in Fig. 1 with increasing concentrations of unlabeled EGF in controls, or in cells exposed to TPA (10 ng/ml) during the binding assay only (■) or after a 2 h preincubation with TPA (□). Values are the means of triplicates.

morphological changes were observed as little as 24–36 h after the addition of TPA to the culture medium. Incubation of cells with both EGF and TPA resulted in morphologic changes identical to cells incubated with TPA alone.

Biological activity. The effects of a 6-d incubation with TPA on MCF-7 cell growth as measured by cell number, total DNA, or total protein per replicately plated culture dish are shown in Figs. 4A, B, and C, respectively. As we have published (21, 25), EGF and insulin significantly stimulated growth two- to threefold above control cultures maintained in serum-free medium alone. In contrast, TPA had a markedly different effect. TPA (10 ng/ml) significantly inhibited cell proliferation and DNA content by more than 50%. Total protein, however, was increased by TPA indicating a striking increase in protein content per cell (see below). Furthermore, TPA totally blocked the stimulatory effect of both EGF and insulin, despite the fact that it had no effect on insulin receptor binding. The effect of TPA was unaltered even by the addition of a large concentration of EGF (1.0 μg/ml). No additional activity was observed with a 10-fold higher TPA concentration.

Similar to the effect on cell morphology, inhibition of DNA synthesis and growth by phorbol esters was clearly evident by 48 h and was proportional to their tumor promoting activity in vivo (Fig. 5). DNA content per dish was significantly reduced in PDD-treated cultures, but not in cultures incubated with the weak tumor promoter PDA. The ability of PDD to inhibit growth paralleled its ability to inhibit EGF receptor binding (Fig. 1).

The phorbol ester effects on MCF-7 cell morphology and growth are consistent with the effects on precursor incorporation into macromolecules and the TLI (Table I). A 24-h incubation with TPA increased the incorporation of labeled leucine into protein twofold above controls suggesting increased protein synthesis and consistent with the increase in protein content noted above (Fig. 4C). In contrast, thymidine incorporation was markedly inhibited after TPA treatment for 24 h, and the TLI indicated that very few cells were active in DNA synthesis after TPA. The reduction in S-phase cells is consistent with either a marked slowing in cell cycle transit time or an actual reduction in the proliferating pool of cells.

The increase in protein synthesis coupled with the block in cell replication are reflected by the marked enlargement of the cells morphologically as well as by a marked increase in protein: DNA ratio induced by TPA (Table II). After 6 d in culture EGF and insulin had only a minor effect on the ratio compared to the increase seen with TPA. Thus, the TPA effect in these cells is not simply a nonspecific toxic effect since protein synthesis is spared, and, in fact, stimulated by the tumor promoter.

The effect of TPA was also examined in two other human breast cancer cell lines (Table III). In this experiment cells were incubated with or without TPA for 3 days. TPA inhibited cell replication in all three lines studied as shown by the reduction in DNA content. The marked change in the protein: DNA ratio, however, was seen only in the MCF-7 and ZR75-1 cells and not in the MDA-MB-231 line.

All of the above experiments were performed with cells growing in serum-free medium. However, identical results were obtained when MCF-7 cells growing under optimal conditions (5% calf serum) were incubated with TPA. In either case cell replication was blocked and protein synthesis was stimulated resulting in a hypertrophied cell. After 10–12 d of continuous TPA treatment, cell size diminished, and the cells began to detach from the dish. We could not reverse the TPA effect by removing the TPA containing medium after 48 h, washing the cells, and refeeding with fresh serum-free medium (Fig. 6). However, cell proliferation was restored by removing the medium with TPA and refeeding with a fresh medium containing 5% serum. Thus, the effects of TPA on the MCF-7 cells are reversible but require removal of TPA from the cell environment and addition of one or more components present in serum.

DISCUSSION

In general, the phorbol esters have been found to promote the characteristic features of a transformed or malignant phenotype in cultured cells. They interact

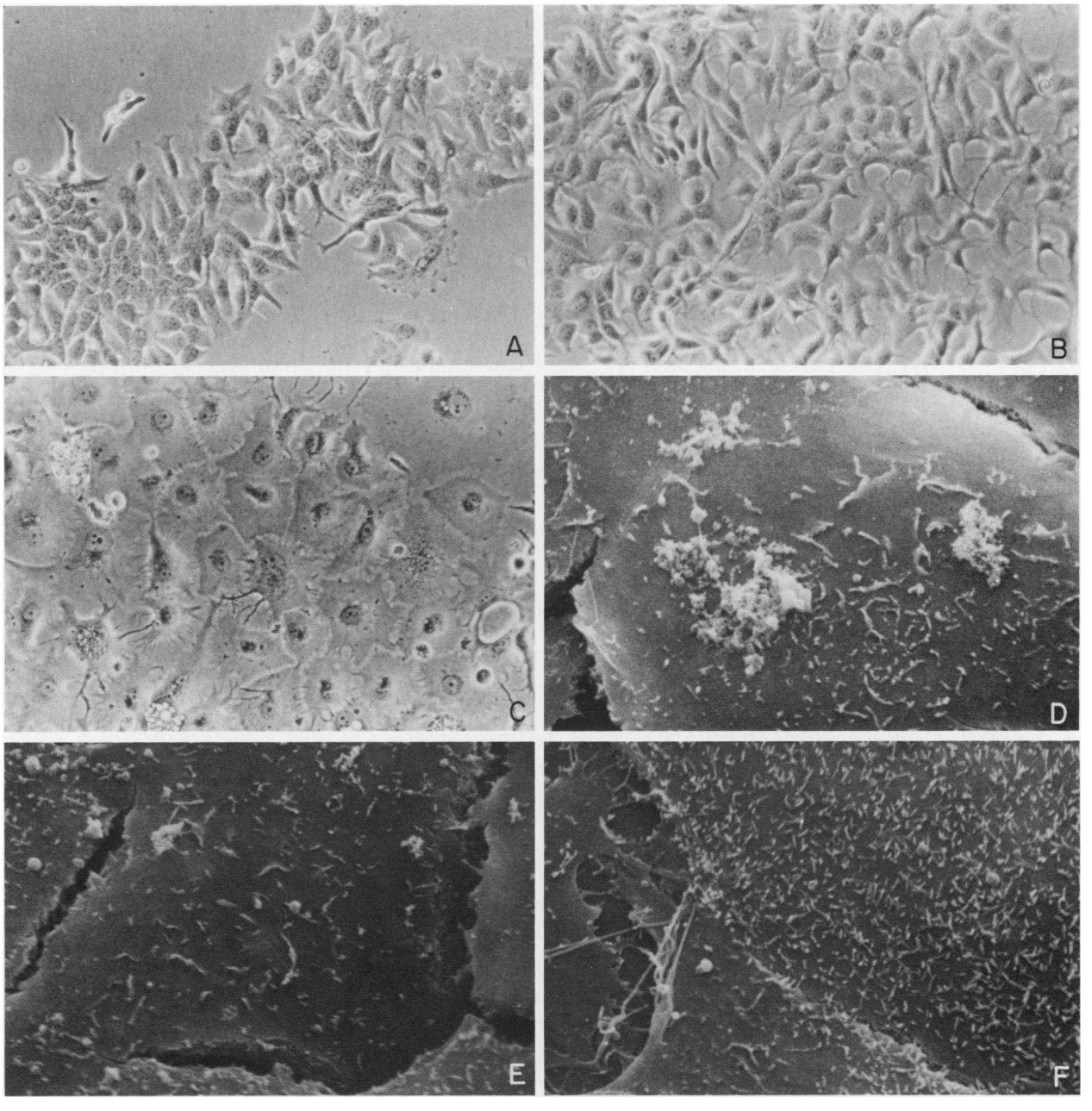

FIGURE 3 Effect of TPA and EGF on MCF-7 cell morphology. Cells were replicately plated and then changed to serum-free medium as described in Methods. Acetone (controls), EGF (10 ng/ml), or TPA (10 ng/ml) were added directly to the culture dishes in 50 μl vol. Cells were fed with identical fresh medium on alternate days. A, B, and C demonstrate the appearance of MCF-7 cells under a polarizing microscope (×220) after 6 d incubation with control, EGF, or TPA, respectively. D, E, and F are scanning electronmicrographs (×2700) of control, EGF-, and TPA-treated cells after 4 d of incubation.

specifically with the receptor system for EGF and share many of the biological properties of this hormone leading to speculation that tumor promotion may be accomplished through the EGF biochemical effector system.

Our studies suggest that the relationship between the phorbol esters and the EGF receptor-effector system is much more complex and that alternative pathways of action for the tumor promoters must be considered. Similar to studies of other cultured cells, phorbol

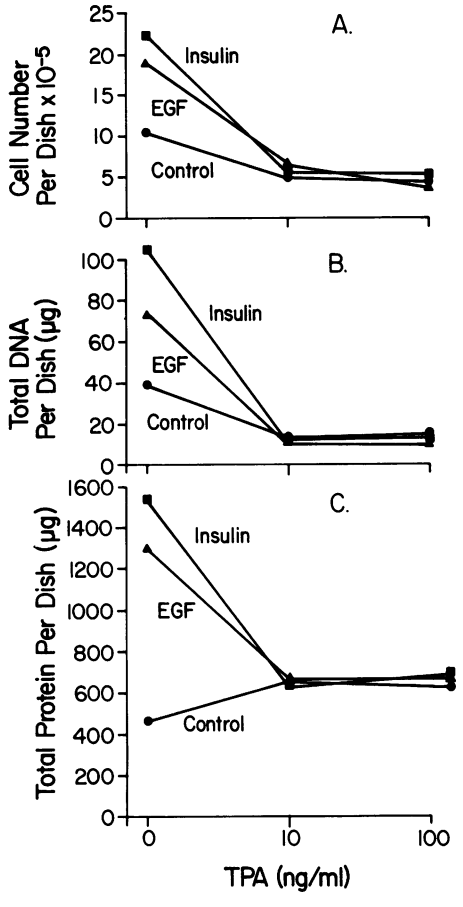

FIGURE 4 Effect of TPA on cell growth. MCF-7 cells were plated as described in Fig. 3 and incubated in serum-free medium with acetone (0.1%, controls), EGF (10 ng/ml), and insulin (6 ng/ml) with and without TPA at the concentrations shown. Fresh identical medium was exchanged for spent medium on alternate days. On day 6 cells were harvested and cell number (A), total DNA (B), and total protein (C) per dish were measured as described in Methods. Values are the means of quadruplicates. SE < 5% in every case. The differences observed with TPA are all highly significant ($P < 0.001$).

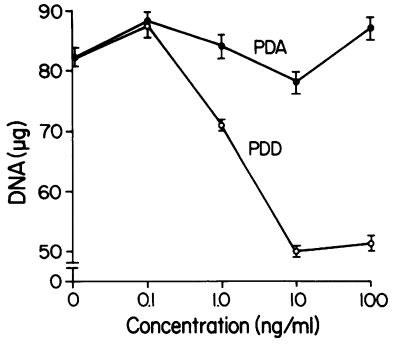

FIGURE 5 Effect of other phorbol esters on MCF-7 cell growth. Cells were plated as described in Fig. 4 and incubated with various concentrations of PDA or PDD. After 2 d cells were harvested and total DNA content per dish determined. Values are the means of quadruplicates±SE.

TABLE I
Effect of TPA on Macromolecular Synthesis

	Precursor incorporation*		
	[^{14}C]Leucine	[^{3}H]Thymidine	TLI‡
	dpm × 10^{-2}/µg *DNA*		%
Control	5.4±1.1	302±50	33
TPA	10.7±0.9	14±1	2

* Values shown are means of quadruplicates±SE.
‡ Means of triplicate determinations.

esters inhibit the binding of EGF but not insulin to MCF-7 human breast cancer cells. Because of the inherent difficulties and controversies in interpreting a curvilinear Scatchard plot, we make no statement as to the mechanism of this effect although the shape of the competition curves (Fig. 2) suggests that the number of receptors has been reduced. Despite this apparent interaction with the EGF receptor and in contrast to other studies, the effects of phorbol esters are quite distinct from the effects of EGF in these cells. Both EGF and insulin stimulate macromolecular synthesis and growth of MCF-7 cells; however, the phorbol esters reversibly block DNA synthesis and cell proliferation and antagonize the stimulatory effects of EGF and insulin, despite the fact that they do not influence insulin receptor binding. It is not likely that these effects are due to nonspecific cell toxicity because (*a*) the effects are observed with very low (hormone-like) concentrations in the nanomolar range, (*b*) growth inhibition is directly proportional to the ability of various phorbol esters to inhibit EGF binding, (*c*) the relative potency of the phorbol esters in our system parallels their potency as tumor promoters in vivo, and (*d*) although cell proliferation is blocked, the phorbol esters induce biochemical and morphological changes in MCF-7 cells indicative of increased protein synthetic and secretory activity.

It is apparent also that the effects of the phorbol esters are not confined to the MCF-7 cells. Very similar changes were noted in the ZR75-1 cells. Interestingly these cells are similar to the MCF-7 line in terms of hormonal responsiveness (24). Inhibition of growth

TABLE II
Effect of TPA, EGF, and Insulin on Protein and DNA Accumulation

	Protein/DNA ratio
Control	11.6
EGF	17.7
Insulin	14.7
TPA	50.2

Mean of quadruplicate determinations; adapted from Fig. 4.

TABLE III
Effect of TPA on Growth of MCF-7, ZR75-1, and MDA-MB-231 Breast Cancer Cells

Cells	Protein*	DNA*	Protein/DNA
		μg	
MCF-7			
Control	707	62	11
TPA	788	35	22
ZR75-1			
Control	387	20	19
TPA	399	12	33
MDA-MB-231			
Control	799	71	11
TPA	613	51	12

* Values shown are means of triplicates after a 3-d incubation with TPA (10 ng/nl).

was also seen with the MDA-MB-231 cells, but increased protein synthesis and the accompanying morphological changes were lacking. Unlike the other two lines, these cells do not contain estrogen receptors and do not respond to insulin or EGF (21, 24, 26). A possible relationship between "hormonal responsiveness" and sensitivity to phorbol esters will require further study.

Our studies indicate that the biological activity of the phorbol ester tumor promoters does not result from the simple direct recognition of these compounds by the active site of the EGF receptor. There is now considerable evidence that the phorbol esters do not bind directly to EGF receptors. In addition to our studies, it has been previously reported that the mechanism of inhibition of labeled EGF binding by TPA and native EGF are different (13, 19, 20). These studies suggest that phorbol esters interact indirectly with the EGF receptor system. They could bind to a nonactive site on the EGF receptor leading to a confirmational change with a resultant decrease in the receptor number or affinity for EGF. Alternatively, the effects on EGF binding could result from a more generalized membrane alteration with secondary effects on the EGF receptor.

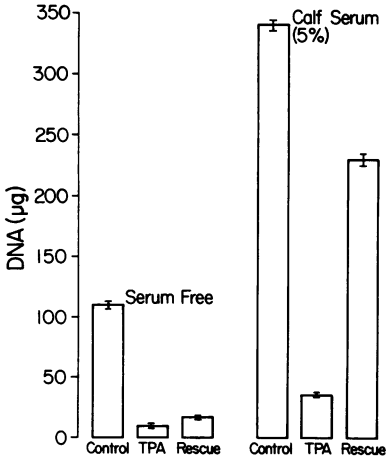

FIGURE 6 Reversal of TPA effect. Cells were plated as described and divided into three groups. One group (controls) was incubated with serum-free medium only for 11 d; another group was incubated with TPA (10 ng/ml) for 11 d; and a third group was incubated with TPA for 48 h, washed three times with 12 ml of medium, and then fed with fresh serum-free medium without TPA for 9 d (rescue). Identical medium was exchanged for spent medium on alternate days. Cells were analyzed for DNA content on day 11. A parallel set of dishes was treated in an identical fashion except that medium was supplemented with 5% calf serum. Values are the means of quadruplicates ± SE.

It is interesting to speculate that the inhibition of cell proliferation and stimulation of protein synthesis observed in our human breast cancer cells in response to these tumor promoters is due to activation of a pathway leading to differentiation. The phorbol esters appear to interact with a mechanism involved with control of differentiation in other in vitro animal model systems, but inhibition of spontaneous and induced differentiation has usually resulted (34–42). Different results have been reported with human-derived cells. Human promyelocytic leukemia cells (HL60) and human melanoma cells have been shown to acquire differentiated functions and to slow their proliferative rate in response to phorbol esters (43–47). Further investigation will be required to determine whether the morphological and biochemical changes induced by phorbol esters in these human breast cancer cells represent features of a more differentiated phenotype.

These studies suggest that the phorbol ester tumor promoters interact with a site on the plasma membrane of cells that indirectly alters the binding of EGF to its receptor. This plasma membrane-phorbol ester interaction can then alter cell morphology, cell replication, the degree of differentiation, and other cell functions in a manner partially dependent on the tissue and species involved. The growth inhibition and stimulation of differentiation of several human neoplastic cell types induced by these compounds suggests that the antineoplastic properties of these "tumor promoters" deserve additional study.

ACKNOWLEDGMENTS

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