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Effects of Phorbol Ester Tumor Promoters on Arachidonic Acid Metabolism in Chick Embryo Fibroblasts

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SUMMARY

MUFSON, R. A., DEFEO, D. & WEINSTEIN, I. B. Effects of phorbol ester tumor promoters on arachidonic acid metabolism in chick embryo fibroblasts. *Mol. Pharmacol.* **16**: 569-578 (1979).

The phorbol ester tumor promoter (12-0-tetradecanoyl-phorbol-13-acetate (10^{-7}-10^{-9} M) caused a rapid (1-3 hr after addition) release of arachidonic acid and prostaglandins E_2 and $F_{2\alpha}$ from chick embryo fibroblasts. This effect was inhibited by cycloheximide and puromycin. Prostaglandin release was more sensitive to inhibition than was arachidonic acid release. Indomethacin, a cyclooxygenase inhibitor, completely blocked TPA-induced prostaglandin synthesis and slightly enhanced arachidonic acid release. Despite the complete suppression of prostaglandin synthesis, indomethacin caused only a 20-30% inhibition of TPA induction of plasminogen activator. Phorbol 12,13-didecanoate, phorbol-12,13-dibenzoate and mezerein were also potent inducers of arachidonic acid and prostaglandin release, while phorbol and 4α phorbol didecanoate were inactive. All trans retinoic acid (10^{-5}-10^{-6} M) inhibited TPA-induced arachidonic acid and prostaglandin release; retinyl palmitate and β-carotene were less effective inhibitors. The effects of the phorbol compounds and retinoids on arachidonic acid release in this cell culture system correlate with their known effects on tumor promotion in mouse skin. Deacylation of membrane phospholipids may, therefore, be an important concomitant in the action of this class of tumor promoters.

INTRODUCTION

The ubiquitous occurrence of membrane bound phospholipases has prompted speculation as to their role in the action of membrane active hormones. Investigation of the action of several hormones including ACTH[1] (1), TSH (2), prolactin (3) and epidermal growth factor (4), have revealed that the interaction of these molecules with their target cells results in the release of arachidonic acid from phospholipids and increased prostaglandin synthesis, as part of a biochemical stimulus-response mechanism. Recently Ohuchi and Levine have shown that the phorbol ester tumor promoter TPA stimulated arachidonic acid release and prostaglandin synthesis in a canine kidney cell line MDCK (5). Further, Moroney and co-workers (6) have shown that certain prostaglandins can mimic the

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[1] The abbreviations used are: ACTH, adrenocorticotropic hormone; TSH, thyroid stimulating hormone; TPA, 12-0-tetradecanoyl phorbol-13-acetate; CEF, chick embryo fibroblasts; DMSO, dimethyl sulfoxide; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PDD, phorbol-12,13-didecanoate; PDB, phorbol-12,13-dibenzoate.

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effects of phorbol esters on cell membrane properties. In cell culture systems the phorbol ester tumor promoters induce highly pleiotropic effects which resemble in several respects the effects of certain hormones (7). The possibility that phospholipid deacylation may be a critical event in the mediation of these effects has prompted us to examine the effects of TPA and related compounds on arachidonic acid metabolism in CEF cultures. We have studied these cells because they are very sensitive to the phorbol esters, and several of their biochemical responses to these tumor promoters have been previously characterized (8–10).

EXPERIMENTAL PROCEDURES

Cell culture. Chick embryo fibroblasts were prepared from 9–10 day old chicken embryos as previously described (11). Cells were maintained in a culture medium composed of Dulbecco's modified Eagle's medium containing 5% fetal calf serum, 1% chicken serum, and 10% tryptose phosphate broth. 0.25% trypsin in Ca^{++} and Mg^{++}-free phosphate buffered saline was used to remove the cells from the plates for counting and passaging. Cells were not used beyond the eighth passage.

Chemicals. Phorbol esters were purchased from Consolidated Midland Corp. (Brewster, N. Y.). All trans retinoic acid was a gift from Dr. Michael Sporn; similar results were obtained with all trans retinoic acid (Type xx) purchased from Sigma Chemical Company. All other biochemicals and retinoids were purchased from Sigma Chemical Company (St. Louis, Missouri). [^{3}H]Arachidonic acid (60–100 Ci/mmole), [^{3}H]PGE_2 (100–200 Ci/mmole) and [^{3}H]-$PGF_{2\alpha}$ (100–150 Ci/mmole) were from New England Nuclear (Boston, Mass.). Other chemicals were analytical or reagent grade. Phorbol esters or retinoids were dissolved in DMSO. When added to the cell cultures the final concentration of DMSO did not exceed 0.1%. This concentration did not affect growth, arachidonic acid or prostaglandin release, or plasminogen activator induction. Solutions of phorbol esters were stored in the dark at −20°. Solutions of retinoids were made up immediately before use and handled in subdued light.

Labelling cultures with [^{3}H]arachidonic acid. 2–3 × 10^5 cells were plated per 50 mm tissue culture dish. In each experiment each plate received exactly the same number of cells per plate. Twenty-four hours after plating, the medium was changed and 4 ml of fresh medium containing 5 μCi of [^{3}H] arachidonic acid was added. After a 24 hour incubation with the labelled arachidonic acid the labelling media was removed, and the cultures were washed three times. They were then used as "prelabelled cells" for the various experiments described. Analysis of cellular lipids revealed that 60–70% of the arachidonic acid was incorporated by the cells; 85–90% was bound to phospholipid and 3% or less was present as free arachidonic acid. Test compounds were added to the cultures in 2 ml of serum containing medium.

Analysis of released arachidonic acid metabolites. At the required time the media were removed from the cultures and 400 μl was taken to determine total radioactivity released. The remaining media were acidified with 0.5 ml of 0.1 M HCl and extracted twice with 2.5 ml of ethyl acetate. The ethyl acetate was evaporated under vacuum. The recovery of arachidonic acid and prostaglandins was 55% and 65%, respectively. The residue was applied to plastic-backed silica gel G thin layer plates (Merck, Darmstadt) with pure standards to locate prostaglandins E_2, $F_{2\alpha}$; arachidonic acid; and the phospholipids phosphatidyl-choline, -ethanolamine and -serine. Plates were developed in 2,6-dimethylheptanone, glacial acetic acid, and 0.9% NaCl (80:40:6) as described by Marinetti (12). Standard spots were visualized with iodine vapor, and cut out. After the iodine color faded, the radioactivity associated with the standard spots was determined by liquid scintillation spectrometry. Channels ratio analysis demonstrated that the liquid scintillation quench associated with the arachidonic acid and prostaglandin spots was the same. Therefore, the cpm presented under RESULTS are corrected only for extraction recovery.

The ethyl acetate residue was also analyzed using high pressure liquid chromatog-

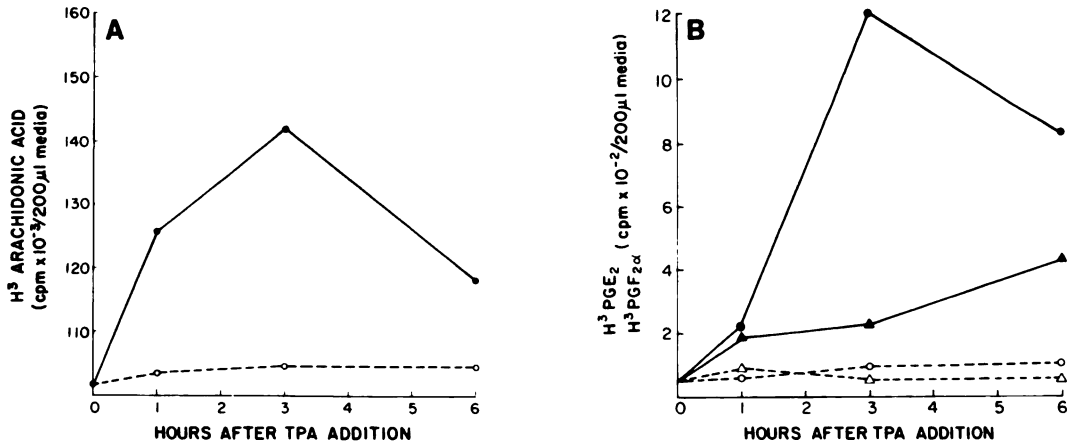

FIG. 1. *The accumulation of arachidonic acid and prostaglandins E_2 and $F_{2\alpha}$ in culture medium from CEF*

TPA (80 nM) or 0.1% DMSO were added in 2 ml of serum-containing medium to cultures prelabelled with [^{3}H]arachidonic acid and aliquots of the media collected at the specified times. Control cultures received 0.1% DMSO as vehicle control. Radioactivity was extracted and analyzed by thin layer chromatography for arachidonic acid and prostaglandins E_2 and $F_{2\alpha}$. Results are the means from two different cultures. (A) Arachidonic acid released, plus TPA (●——●) or 0.1% DMSO (○- - -○). (B) Prostaglandin E_2 released, plus TPA (●——●) or 0.1% DMSO (○- - -○); prostaglandin $F_{2\alpha}$ released, plus TPA (▲——▲) or 0.1% DMSO (△- - -△).

raphy (13). Identification of arachidonic acid, PGE_2, and $PGF_{2\alpha}$ and arachidonic acid was confirmed using this chromatographic system.[2]

Plasminogen activator determination. Plasminogen activator activity in cell lysates from subconfluent cultures (approximately 5×10^6 cells cm dish) was assayed by quantitating the lysis of ^{125}I labelled fibrin as described previously (10). Test compounds were added with fresh growth medium 24 hours before enzyme assay.

Cell counts. Cells were removed from tissue culture plates for counting using 0.25% trypsin in phosphate buffered saline. Cell counts were performed using an electronic Coulter counter (Model ZF Coulter Electronics, Hialeah, Florida).

Presentation of data. All experiments were performed either in duplicate or in triplicate. The data presented are representative of the results from two to three separate experiments.

RESULTS

To measure its metabolism, CEF were prelabelled with [^{3}H]arachidonic acid for 24 hr, then washed to remove the unincorporated material and incubated in unlabelled media with and without further additions. Within 1 hr after addition of 80 nM TPA to CEF cultures prelabelled with [^{3}H] arachidonic acid, there was an almost 10-fold increase in the arachidonic acid content of the culture media (Fig. 1A). In addition, there was a 2-3 fold increase in the levels of PGE_2 and $PGF_{2\alpha}$ with a greater increase in the amount of prostaglandin E_2 compared to $F_{2\alpha}$ (Fig. 1B). By three hours the arachidonic acid and prostaglandin accumulation was maximal with PGE_2 still present in greater amounts than $F_{2\alpha}$. Six hours after addition, however, the levels of arachidonic acid and PGE_2 were returning to basal levels. In some experiments, 24 hours after addition of TPA the levels of arachidonic acid and prostaglandins remained somewhat elevated; however, in others the levels had returned to basal by this time. With different batches of embryos the spontaneous release of arachidonic acid and PGE_2 and $PGF_{2\alpha}$ in the absence of TPA varied (see for example Table 2), but in all experiments the addition of TPA enhanced the basal levels. In addi-

[2] Mufson, R. A., A. M. Jeffrey, D. Park and I. B. Weinstein, unpublished data.

tion, TPA stimulated arachidonic acid and PG release from cells in serum free medium. The release also seemed to be specific for arachidonic acid since cells prelabelled with linoleic acid were not stimulated by TPA to release this fatty acid. The maximal stimulations of [^{3}H]arachidonic acid release mediated by 80 nM TPA varied between 3 and 10 fold.

The release of arachidonic acid and prostaglandins was concentration dependent between 10^{-9} and 10^{-7} M TPA (Fig. 2). The arachidonic acid and PGE_2 levels rose in a parallel fashion. Of the total radioactivity released at 3 hours after addition of maximally stimulating concentrations of TPA, arachidonic acid represented 85%, PGE_2 3% and $PGF_{2\alpha}$ 0.5%. Of the remaining radioactivity, about 1.5% was associated with phospholipids and 9.0% was unidentified.

Table 1 compares the abilities of a series of phorbol esters and related compounds to induce arachidonic acid release and stimulate prostaglandin synthesis in CEF cultures. The compound PDD was more active than PDB. The parent alcohol phorbol and the compound 4αPDD, a stereoisomer of PDD, were both inactive (Table 1). These results correlate with previous data on the activities of these compounds as tumor promoters on mouse skin (14), and as mediators of other effects in cell culture systems (7-9). Mezerein, a macrocyclic diterpene with anti-leukemic activity and a structure very similar to phorbol esters (15), was also very active.

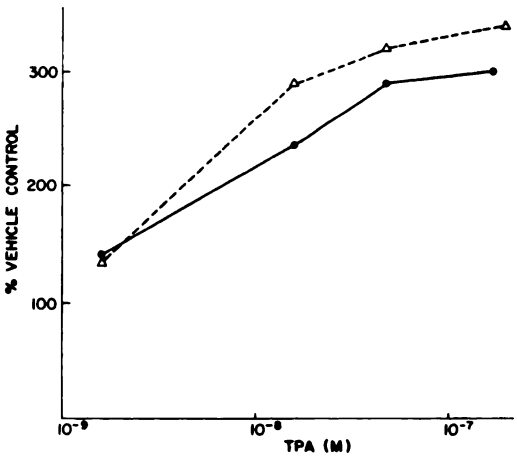

FIG. 2. *The effect of TPA concentration on the accumulation of arachidonic acid and prostaglandins E_2 and $F_{2\alpha}$* from prelabelled CEF

The indicated concentrations of TPA in 2 ml of serum-containing medium were added to CEF cultures prelabelled with [^{3}H]arachidonic acid. Three hours later the media were collected and radioactivity was extracted and analyzed by thin layer chromatography for arachidonic acid and prostaglandin E_2. Results are the means from three different cultures. Arachidonic acid content (●——●) and prostaglandin E_2 content (△——△) of TPA-treated cultures are presented as percent of that in control cultures exposed to 0.1% DMSO.

TABLE 1

The effect of phorbol esters and mezerein on arachidonic acid and prostaglandin E_2 release from chick embryo fibroblasts

Compounds were added at the specified concentrations to cultures prelabelled with [^{3}H] arachidonic acid and three hours later the media was collected. Radioactivity was extracted and analyzed by thin layer chromatography. Results are the means ±SEM from three different culture dishes.

Addition		Arachidonic acid (cpm × 10^{-3}/200 μl)	PGE_2 (cpm/200 μl)
DMSO	0.1%	19.5 ± 1.7	0
PDD	1.5×10^{-7} M	87.5 ± 6.1	395 ± 45
4αPDD	1.5×10^{-7} M	21.4 ± 1.9	0
PDB	1.5×10^{-7} M	65.1 ± 2.0	111 ± 19
Phorbol	2.6×10^{-7} M	24.4 ± 2.0	0
Mezerein	1.5×10^{-7} M	82.8 ± 3.8	307 ± 30

Cycloheximide was used to determine whether the deacylation response required protein synthesis. A concentration of 40 μg/ml of cycloheximide was found to reduce [^{3}H]leucine incorporation into protein by 95–100%, when measured three hours after addition to cells. This concentration when added simultaneously with 80 nM TPA markedly suppressed both arachidonic acid release and prostaglandin synthesis (Fig. 3). Reducing the concentration of cycloheximide resulted in increasing arachidonic acid release and prostaglandin synthesis (Table 2). It appeared that TPA-induced prostaglandin synthesis was more sensitive to cycloheximide inhibition than arachidonic acid release. Puromycin (20 μg/ml) also inhibited TPA-induced arachidonic acid release and PGE_2 synthesis (Table 2).

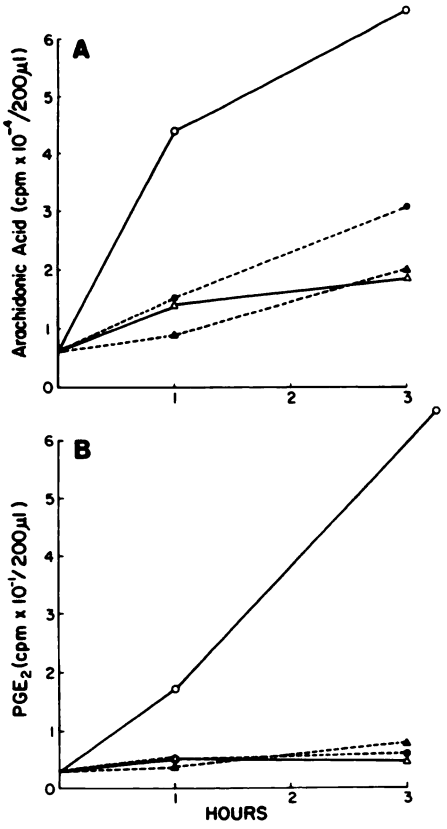

FIG. 3. *The effect of cycloheximide on arachidonic acid and prostaglandin release from CEF·TPA (80 nM) or TPA (80 nM) plus cycloheximide (40 µg/ml) were added in 2 ml of serum-containing medium to prelabelled cultures, and the media was collected at specified times*

Control cultures received 0.1% DMSO as a vehicle control. (A) Arachidonic acid release from TPA (○——○) or TPA plus cycloheximide cultures (●- - -●); arachidonic acid from 0.1% DMSO (△——△) or 0.1% DMSO plus cycloheximide (▲- - -▲) cultures. (B) PGE_2 release from TPA (○——○) or TPA plus cycloheximide (●- - -●) treated cultures; PGE_2 release from 0.1% DMSO (△——△) or 0.1% DMSO plus cycloheximide (▲- - -▲) treated cultures. Results presented are the mean from three different cultures.

Actinomycin D (2.5 µg/ml) which reduced [^{3}H] uridine incorporation into RNA by 70%, when added simultaneously with TPA, did not inhibit arachidonic acid release. Raising the actinomycin concentration to 5 µg/ml, a concentration that completely suppresses TPA induction of plasminogen activator (16), only partially inhibited (50%) TPA-induced arachidonic acid release. Indomethacin, a cyclooxygenase inhibitor, inhibited the stimulation of prostaglandin release induced by TPA and reduced the basal level of prostaglandin found in the medium of CEF cultures (Fig. 4). This inhibition was maintained for 24 hr. Inhibition of the TPA-mediated increase in prostaglandin release was concentration dependent: 50% inhibition was obtained with about 10^{-7} M indomethacin. This was not accompanied by a reduction in arachidonic acid release (Fig. 4). In fact the accumulation of arachidonic acid induced by TPA was slightly enhanced by indomethacin (Fig. 4). Increasing the indomethacin concentration to 100 µM produced a 100% inhibition of TPA-induced prostaglandin synthesis which was maintained for at least 24 hours. Thus it would seem that the appearance of prostaglandins in TPA-treated cultures is secondary to the TPA-induced release of arachidonic acid which provides large amounts of substrate for the cyclooxygenase enzyme. Similar conclusions were reached in previous studies with MDCK cells (4).

TPA has been shown to induce in normal CEF several phenotypic properties characteristic of Rous Sarcoma virus transformed CEF. One of these phenotypic properties is induction of the serine protease plasminogen activator, whose level is maximal 24 hr after TPA addition (10). To determine whether the increase in prostaglandin synthesis is a prerequisite for the increase in plasminogen activator activity, 100 µM indomethacin was added simultaneously with 80 nM TPA and the protease activity was measured in cell lysates 24 hr later. We found that although this concentration of indomethacin completely suppressed the TPA-induced synthesis of PGE_2 it inhibited the TPA induction of plasminogen activator by only 20–30%.

We also examined whether retinoic acid, a known inhibitor of carcinogenesis in several systems (17–20) and an inhibitor of tumor promotion on mouse skin (21), would affect the TPA-stimulated release of arachidonic acid and prostaglandins. Figure 5A illustrates an experiment in which the ad-

TABLE 2

The effects of inhibitors on TPA-induced arachidonic acid and prostaglandin E_2 release from chick embryo fibroblasts

TPA (80 nM) or TPA plus the indicated inhibitor were added to cultures prelabelled with [^{3}H] arachidonic acid and the media was collected 3 hr after addition. Radioactivity was extracted and analyzed by thin layer chromatography. The results are means ± SEM of three to six different culture dishes.

Addition	Arachidonic acid (cpm × 10^{-3}/200 μl)		PGE_2 (cpm/200 μl)
Experiment 1			
DMSO 0.1%	20.6	± 1.0	34 ± 5
TPA 80 nM	50.4	± 4.0	397 ± 60
TPA + cycloheximide 40 μg/ml	27.9	± 3.5	45 ± 6
TPA + cycloheximide 4 μg/ml	37.8	± 2.4	51 ± 11
TPA + cycloheximide 0.4 μg/40	74.2	± 11.1	167 ± 23
Experiment 2			
DMSO 0.1%	5.4	± 0.27	34 ± 7.7
TPA 80 nM	15.4	± 2.20	75 ± 11.9
0.1% DMSO + puromycin 20 μg/ml	6.4	± 0.61	45 ± 6.0
TPA + puromycin 20 μg/ml	8.1	± 0.71	35 ± 3.2
DMSO 0.1% + actinomycin D 2.5 μg/ml	5.2	± 0.32	35 ± 4.0
TPA + actinomycin D 2.5 μg/ml	16.0	± 2.0	70 ± 8.2
DMSO 0.1% + actinomycin D 5 μg/ml	6.3	± 0.68	40 ± 3.3
TPA + actinomycin D 5 μg/ml	11.3	± 1.01	33 ± 2.6

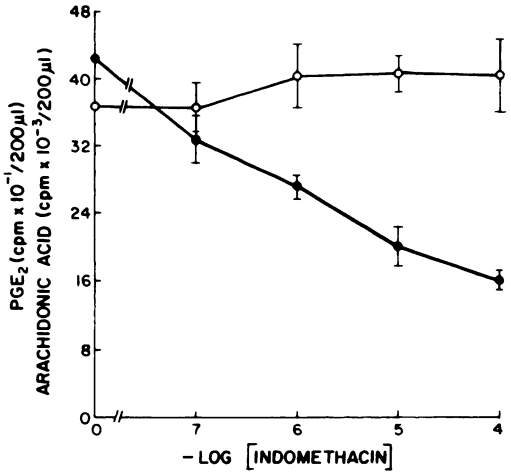

FIG. 4. *The effect of indomethacin concentration on PGE_2 and arachidonic acid release from TPA treated CEF*

Prelabelled cultures were treated with 80 nM TPA or 80 nM TPA plus indomethacin. Three hours after addition the medium was collected for analysis. Arachidonic acid content (○——○) and PGE_2 content (●——●) are shown. Symbols indicate the mean ± SEM from three different cultures.

dition of TPA to CEF cultures resulted in about a five-fold increase in the release of cellular arachidonic acid. In the presence of 10 μM all trans retinoic acid, this response was inhibited about 60%. TPA-induced release of PGE_2 was similarly inhibited by the presence of trans retinoic acid (Fig. 5B). Dose response curves for retinoic acid are given in Fig. 7; the most effective concentrations were between 1 and 30 μM. These dose response curves are rather similar to those reported by Kensler *et al.* (22) for retinoic acid inhibition of TPA-mediated comitogenesis in bovine lymphocytes. For reasons that are not apparent, the dose response curves for inhibition of arachidonic acid and PGE_2 release were somewhat different (Fig. 6). These effects of retinoic acid are not due to cytotoxicity since the growth of CEF in the presence of 80 nM TPA or 80 nM TPA plus 33 μM retinoic acid was the same as that of control cultures.

Retinyl palmitate and β-carotene are closely related in structure to retinoic acid; however, they are only 10–30% as active as retinoic acid as inhibitors of TPA-induced skin tumor promotion (21), TPA-mediated induction of ornithine decarboxylase in mouse epidermis (21), and TPA comitogenesis in lymphocyte culture (22). The data in Fig. 7 indicate that whereas all trans retinoic acid produced about a 60% reduction in TPA-induced arachidonic acid re-

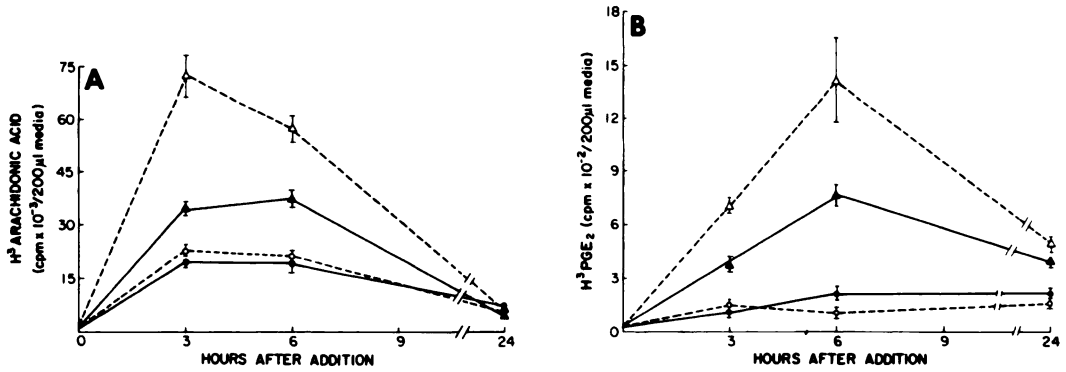

FIG. 5. *Suppression of TPA-induced release of arachidonic acid and prostaglandin E_2 by trans retinoic acid*

TPA (80 nM) plus or minus trans retinoic acid (10 μM) were added in 2 ml of serum-containing media to prelabelled cultures. The medium was collected at the specified times and radioactivity was extracted and analyzed. Results are the means ± SEM from three different cultures. (A) Arachidonic acid content in TPA (Δ- - -Δ) and TPA plus retinoic acid (▲——▲) cultures; arachidonic acid content in 0.1% DMSO (O- - -O) and retinoic acid plus 0.1% DMSO (●——●) treated cultures. (B) Prostaglandin E_2 content in TPA (Δ- - -Δ) and TPA plus retinoic acid (▲——▲) cultures; prostaglandin E_2 content in control (O- - -O) and retinoic (●——●) and treated cultures.

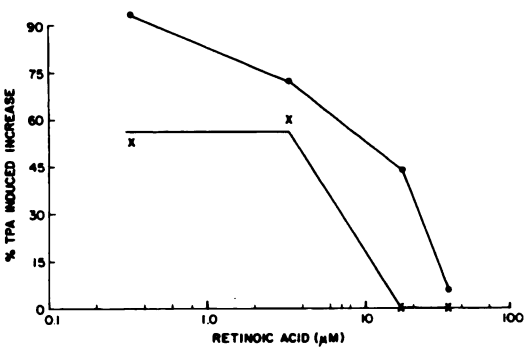

FIG. 6. *The effect of trans retinoic acid concentration on TPA-induced release of arachidonic acid and PGE_2*

Prelabelled CEF cultures were treated with 0.1% DMSO or TPA (80 nM) in 2 ml of serum-containing medium plus or minus the specified concentration of retinoic acid. Three hours after treatment the media was collected and analyzed. Results are presented as a percent of the TPA induction in the absence of retinoic acid. The arachidonic acid (●——●) and PGE_2 (×——×) values shown are the means from three different cultures.

lease, retinyl palmitate and β-carotene produced only about a 20% inhibition.

DISCUSSION

It appears that in diverse cell types phorbol ester tumor promoters induce the release of arachidonic acid from phospholipids and a consequent increase in prostaglandin synthesis. Ohuchi and Levine (5) have demonstrated this in a canine kidney fibroblast cell line MDCK and in the present report we have described this phenomenon in normal chick embryo fibroblasts.We have also examined C3H10T½ mouse embryo fibroblasts, in which TPA enhancement of cell transformation has been demonstrated (23), and have observed similar TPA-induced changes in arachidonic acid metabolism in that system.[3] The identification of arachidonic acid, PGE_2, and $PGF_{2\alpha}$ in the present studies is based on separations in a thin layer chromatography system used by Levine and co-workers (5) to identify these compounds in medium from fibroblast cultures. Moreover, the radioactive materials obtained from the cell cultures also co-chromatographed with authentic standards in a high pressure liquid chromatography system (13). Our results, however, do not exclude the possibility that other arachidonic acid metabolites were produced in small amounts in CEF cultures in the presence or absence of TPA. This aspect requires further study.

It is likely that TPA-induced arachidonic

[3] Mufson, R. A. and I. B. Weinstein, unpublished data.

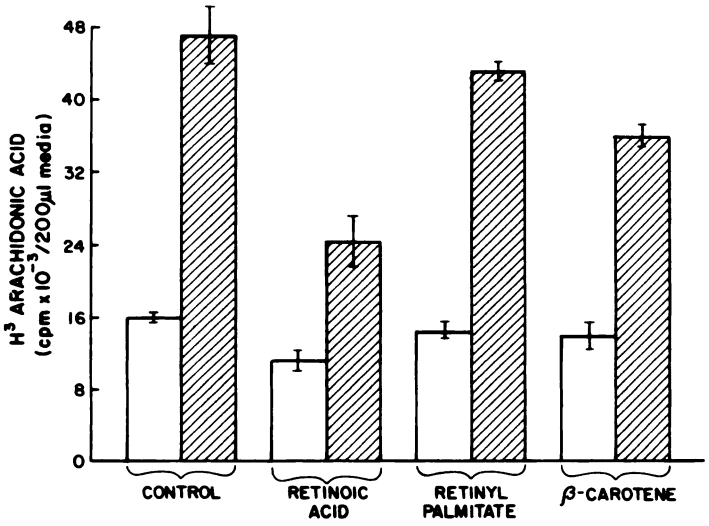

FIG. 7. *Suppression of TPA-induced arachidonic acid release by various retinoids*

TPA (80 nM) or 0.1% DMSO (vehicle control), with or without the specified retinoid (33 µM), were added to prelabelled cultures. Three hours later the medium was collected and radioactivity was extracted and analyzed by thin layer chromatography. Open bars represent 0.1% DMSO treated cultures and hatched bars TPA treated cultures. Each bar is the mean ± SEM from three different cultures.

acid release results from the hydrolysis, by phospholipase A (24), of acyl groups in membrane phospholipids. Curiously, the response in chick embryo fibroblasts is inhibited by compounds that interfere with the synthesis of new proteins, and this has also been observed by Ohuchi and Levine in their system (5). Thus the release of arachidonic acid may not be due simply to a direct interaction of phorbol esters with cell membranes, a phospholipase, or other enzymes, since it appears to require *de novo* protein synthesis. It is not clear whether protein synthesis is required to mediate the phospholipid deacylation or the secretion of metabolites from the cell. The requirement for a protein factor has been demonstrated for the release of arachidonic acid induced by immunologic stimuli (25).

It does not appear that the increased synthesis of prostaglandins is causally associated with the TPA-mediated induction of plasminogen activator. Although TPA-induced prostaglandin synthesis could be completely inhibited by indomethacin this led to only a partial (20–30%) inhibition of plasminogen activator induction. As expected, although indomethacin blocked prostaglandin synthesis it did not inhibit the marked release of arachidonic acid induced by TPA. It is possible, therefore, that the deacylation of phospholipids could play a more important role than the synthesis of prostaglandins in the mechanism of action of TPA. In separate studies, we have found that phorbol ester-induced platelet aggregation is not associated with increased release of cyclooxygenase products and could not be blocked by indomethacin,[4] thus providing additional evidence that all of the diverse effects of TPA are not secondary to prostaglandin synthesis. Deacylation of membrane phospholipids is associated with the action of several hormones (see INTRODUCTION and references 1, 2, 3, 26). Changes in the state of acylation of membrane phospholipids (i.e., accumulation of lysophospholipids) could, therefore, play a role in a stimulus-response cascade triggered by phorbol ester tumor promoters and certain hormonal agents.

The present studies indicate that TPA-induced release of arachidonic acid and

[4] Mufson, R. A., P. Kulkarni, K. E. Eakins and I. B. Weinstein, Platelet aggregating activity of phorbol esters correlates with tumor promoting activity and is independent of cyclooxygenase products, *Cancer Research*, in press 1979.

prostaglandins in CEF cultures was inhibited by all trans retinoic acid. The effective concentration in this study (about 10^{-5} M) is similar to that used previously to inhibit carcinogen-induced hyperplasia in organ cultures of mouse prostate (17, 18) and hamster trachea (18), and to influence epidermal cell differentiation in cell culture (26). The concentrations required for an inhibitory effect on arachidonic acid release are also the same as those observed previously for retinoid inhibition of TPA-mediated comitogenesis in lymphocytes (22). It has been suggested that the broad dose response curve is indicative of a requirement for metabolic activation of retinoic acid, perhaps by epoxidation of the β ionone ring; it may also reflect the binding of retinoids to serum proteins (28).

With a series of six phorbol esters, we found a correlation between ability to induce arachidonic acid release in CEF cultures and published data on tumor promoting activity on mouse skin. In addition, trans retinoic acid was more effective than retinyl palmitate or β carotene in inhibiting TPA-induced arachidonic acid release in CEF cultures. Trans retinoic acid is also a more effective inhibitor of tumor promotion on mouse skin (21). This cell culture system may, therefore, be an excellent model for studying the relationship between lipid metabolism and the mechanism by which phorbol esters act as tumor promoters.

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