Anthralin, a Non-TPA Type Tumor Promoter, Synergistically Enhances Phorbol Ester-Caused Prostaglandin E₂ Release from Primary Cultured Mouse Epidermal Cells

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ABSTRACT—Primary cultures of mouse epidermal cells (i.e., target cells of skin tumor promotion) stimulated by 12-O-tetradecanoylphorbol-13-acetate (TPA) released prostaglandin E₂ within 30 min. Anthralin, a non-TPA type tumor promoter, also stimulated PGE₂ release; however, no release was detectable at least up to 4 hr after the addition of anthralin. When the cells were incubated with TPA plus anthralin, both PGE₂ and arachidonic acid release were synergistically enhanced. Other non-TPA type tumor promoters, i.e., chrysarobin, 7-bromomethylbenz[a]anthracene, benzoylperoxide, okadaic acid and palytoxin, did not potentiate the TPA-caused PGE₂ release. In protein kinase C-down regulated cells, the synergistic stimulation of PGE₂ and arachidonic acid release by TPA plus anthralin were not detected. Anthralin plus TPA did not alter the incorporation of arachidonic acid into cellular phospholipids. Cellular cyclooxygenase activity was increased 2 hr after TPA stimulation. Anthralin-caused increase in cyclooxygenase activity was detected at 6 hr after the addition of anthralin. Cyclooxygenase activity was synergistically increased by treating the cells with TPA plus anthralin. Cycloheximide and actinomycin D inhibited the increase in cyclooxygenase activity caused by anthralin or TPA plus anthralin. These results indicate that anthralin synergistically stimulates TPA-caused PGE₂ release by synergistically increasing arachidonic acid release and cellular cyclooxygenase activity.

Keywords: Phorbol ester, Anthralin, Prostaglandin E₂ release, Epidermal cell, Cyclooxygenase

We and others have proposed that an arachidonic acid metabolite(s) plays a role(s) in phorbol ester-caused skin tumor promotion in mice (1–6). The well-known tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) actually stimulates the arachidonic acid metabolism in mouse epidermis (1, 7–10) and murine keratinocytes (11–14). Protein kinase C (PKC) may mediate this stimulatory effect of TPA (14). Other types of tumor promoters which do not interact with PKC, i.e., non-TPA type tumor promoters, such as anthralin (15) and okadaic acid (16), also stimulate prostaglandin E₂ (PGE₂) production in primary cultured mouse epidermal cells (14), the target cells of skin tumor promotion. Other non-TPA type tumor promoters such as chrysarobin (17), benzoylperoxide (18), palytoxin (19) and 7-bromomethylbenz[a]anthracene (BrMBA; refs. 20 and 21) did not stimulate PGE₂ production in these cells (14).

During the course of the above-described studies, we observed that anthralin markedly and synergistically stimulated TPA-caused PGE₂ release in epidermal cells. This finding prompted us to investigate the mechanism of synergistic stimulation of PGE₂ release by these two compounds.

Our present study revealed that anthralin synergistically stimulates TPA-caused PGE₂ release by synergistically increasing arachidonic acid release and cellular cyclooxygenase activity. Such an enhancing effect was not observed with other non-TPA type tumor promoters, and the effect was anthralin-specific.

MATERIALS AND METHODS

Chemicals

The sources of materials used in this study were as follows: TPA from Chemicals for Cancer Research,
Inc., Chicago, IL, U.S.A.; PGE₂, arachidonic acid, anthralin, mezerein, cycloheximide, actinomycin D, indomethacin and fatty acid-free bovine serum albumin (BSA) from Sigma Chemical Co., St. Louis, MO, U.S.A.; palytoxin from Calbiochem, La Jolla, CA, U.S.A.; benzoyl peroxide from Wako Pure Chemical Industries, Ltd., Tokyo, Japan; minimum essential medium (MEM) from Gibco, Grand Island, NY, U.S.A.; fetal calf serum (FCS) from Mitsubishi Kasei, Co., Tokyo, Japan; Chelex-100 from Bio-Rad, Richmond, CA, U.S.A.; [¹²⁵I]PGE₂ radioimmunoassay (RIA) kit from New England Nuclear, Boston, MA, U.S.A.; [³H]arachidonic acid (230 Ci/mmol) and ACS II from Amersham Laboratories, Buckinghamshire, U.K. The other chemicals used were of reagent grade.

BrMBA and okadaic acid were kindly supplied by Dr. N. Fukazawa, Mitsui Pharmaceutical Co., Ltd., Tokyo, Japan and Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan, respectively.

The drugs were dissolved as follows: indomethacin, in ethanol; cycloheximide and actinomycin D, in distilled water; palytoxin, in MEM; the other promoters, in dimethylsulfoxide (DMSO). The final concentration of each solvent was 0.1%.

Primary cultures of mouse epidermal cells

Epidermal cells were isolated from newborn CD-1 mice and cultured as described previously (22). Briefly, isolated epidermal cells were plated on 35-, 60- or 100-mm plastic dishes at an initial density of 3 X 10⁵ cells/cm² in MEM supplemented with 10% FCS and cultured at 36°C in an atmosphere of 92% air/8% CO₂ for 20 hr. Thereafter, the medium was switched to the low Ca²⁺ culture medium, i.e., Ca²⁺-free MEM supplemented with 50 μM of CaCl₂ and 10% Chelex-100-treated (Ca²⁺-deprived) FCS. The cells were further cultured at 36°C in an atmosphere of 92% air/8% CO₂ for 2 days and then used for the experiments. Cell viability was determined by the trypan blue exclusion test.

Radioimmunoassay (RIA) of PGE₂

Epidermal cells in 35-mm dishes were incubated in 1 ml of the assay medium, i.e., Ca²⁺-free MEM supplemented with 50 μM CaCl₂ and 0.5% BSA but without FCS, in the presence or absence of the indicated drugs at 36°C for 2 hr. After the incubation, the medium was collected and centrifuged at 10,000 X g for 20 min at 2°C. The resultant supernatant was diluted with an appropriate volume of Ca²⁺- and Mg²⁺-free phosphate-buffered saline (PBS(−)) containing 0.005% Triton X-100, 10 mM EDTA, 0.3% bovine-γ-globulin and 0.05% sodium azide. PGE₂ in the diluted medium was assayed by RIA.

Measurement of [³H]arachidonic acid and [³H]PGE₂ release

Epidermal cells in 35-mm dishes were incubated for 24 hr in the low Ca²⁺ culture medium containing 1 μCi of [³H]arachidonic acid. The cells were then washed with PBS(−) containing 0.1% BSA and then incubated in the presence or absence of various drugs at 36°C for the indicated time periods in 1 ml of the assay medium. After incubation, the medium was collected and centrifuged; and the resultant supernatant was transferred to another test tube, and 4 ml of ethyl acetate / methanol / 0.4 M citric acid (15:2:1, v/v) was added. The mixture was vortexed for 1 min and centrifuged. The organic phase was transferred to another test tube and dried under an N₂ gas stream. For the detection of PGE₂ and arachidonic acid on thin-layer chromatography (TLC), authentic PGE₂ and arachidonic acid (5 μg each) were added to each sample. The dried residue was dissolved in a small amount of chloroform / methanol (1:1, v/v) and subjected to silica gel TLC (Whatman LK6D). The plates were developed in the solvent system of ethyl acetate / n-hexane / acetic acid / water (57:26:6:60, v/v, upper layer). The fractions of PGE₂ and arachidonic acid were detected by gas and scraped into the counting vials. Radioactivity was eluted with 0.1 ml methanol and determined by the addition of 3 ml scintillation cocktail (ACSII) in a liquid scintillation counter.

Release of [³H]arachidonic acid and [³H]PGE₂ from PKC-down regulated epidermal cells

When the low Ca²⁺ culture medium was changed to a fresh one, one group of the cells in 35-mm dishes was treated with TPA (50 nM) and the other group was treated with vehicle. At 24 hr after the addition of TPA or vehicle, the medium was changed to a fresh one containing either TPA (50 nM) or vehicle. The cells were further cultured for 24 hr in the presence of 0.1 μCi of [³H]arachidonic acid. Thereafter, the cells were washed with PBS(−) containing 0.1% BSA and were incubated in the presence or absence of the indicated drugs for 2 hr in 1 ml of the assay medium. The amounts of [³H]PGE₂ and [³H]arachidonic acid released into the medium were measured as described above.

Incorporation of [³H]arachidonic acid into cellular phospholipids

Epidermal cells in 35-mm dishes were incubated in 1 ml of the assay medium in the presence or absence of the indicated drugs at 36°C for 90 min; thereafter,
[3H]arachidonic acid (0.1 μCi) was added into the medium and cells were incubated for another 30 min. After the incubation, cells were washed with PBS(−) containing 0.1% BSA and scraped. Cellular phospholipids were extracted by chloroform / methanol (1:2; v/v) and subjected to TLC with a solvent system of chloroform / methanol / acetic acid / 0.9% NaCl (50:25:8:4, v/v). Rf values of phosphatidylcholine, phosphatidylethanolamine plus phosphatidylglycerol and phosphatidylethanolamine were 0.53, 0.68 and 0.89, respectively. Each phospholipid fraction was scraped into the counting vial, and its radioactivity was determined by a liquid scintillation counter. Under our experimental conditions, the incorporation of [3H]arachidonic acid into cellular phospholipids increased linearly at least up to 30 min after the addition of [3H]arachidonic acid into the medium.

**Cyclooxygenase activity**

Epidermal cells in 60-mm dishes were incubated in 2.5 ml of the assay medium in the presence or absence of the indicated agents at 36°C for the indicated time periods. After incubation, the cells were washed with PBS(−) and scraped with 100 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA, 2 mM reduced glutathione, and 2 μM hemoglobin. The cells were sonicated for 30 sec at 4°C, and the resultant cellular sonicates were used as the enzyme source.

Cyclooxygenase activity of the cellular sonicates was determined as reported previously (23) with minor modifications. Briefly, the cellular sonicates (15–30 μg of protein) were incubated at 37°C for 5 min in the presence or absence of 10 μM indomethacin. Then 50 μM [3H]arachidonic acid (final concentration; specific activity, 10 mCi/mmol) was added and incubated for another 10 min. The reaction was stopped by the addition of 1 ml of ethyl acetate / methanol / 0.4 M citric acid (15:2:1, v/v). Indomethacin-sensitive formation of [3H]PGE2 was determined as described previously (23).

**Statistical analyses**

Statistical analyses were done by the t-test.

**RESULTS**

Epidermal cells cultured in the low Ca2+ (20–50 μM) medium proliferate rapidly as a monolayer and are characterized as basal cells (24). When the medium Ca2+ was raised to a normal level, squamous differentiation ensued rapidly (24). Therefore, all the experiments were performed under the low Ca2+ (50 μM) condition.

When the epidermal cells were incubated with TPA (30 nM) for 2 hr, a marked stimulation of PGE2 release was observed (Table 1). During this observation period, significant stimulation of PGE2 release was not detected by non-TPA type tumor promoters, i.e., anthralin, chrysarobin, BrMBA, benzoyleperoxide, okadaic acid and palytoxin (Table 1). These results were consistent with our previous data (14). Higher concentrations of non-TPA type tumor promoters caused obvious reduction in cell viability. Among these non-TPA type tumor promoters, only anthralin synergistically stimulated TPA-caused PGE2 release (Table 1). BrMBA rather suppressed TPA-caused PGE2 release (Table 1). When the cells were incubated with mezerein, a TPA type stage 2 tumor promoter (25), which is known as a potent PKC activator like TPA, PGE2 release was stimulated (Table 1). The effects of mezerein and TPA were not additive (Table 1), supporting the contention that TPA and mezerein stimulate PGE2 release through a common mechanism, i.e., PKC activation.

When [3H]arachidonic acid-prelabeled epidermal cells were incubated with TPA (30 nM), the stimulation of [3H]PGE2 release was detected within 30 min and observed for at least up to 8 hr after TPA stimulation (Fig. 1A). Anthralin also stimulated [3H]PGE2 release; however, the stimulation was not detected until 6 hr after the addition of anthralin (Fig. 1A). These results were consistent with our previous observations (14). When [3H]arachidonic acid-prelabeled cells were incubated with anthralin plus TPA, [3H]PGE2 release was markedly and synergistically stimulated (Fig. 1A). Anthralin tended to stimulate [3H]arachidonic acid release at 6 hr after the addition of this agent. Eight hours after the addition of anthralin, [3H]arachidonic acid release was 2.56 ± 0.16**.

### Table 1. Effect of non-TPA type tumor promoters and mezerein on PGE2 release caused by TPA

| Treatment       | PGE2 release (ng/dish) |
|-----------------|------------------------|
|                 | TPA (−)                | TPA (+)             |
| Vehicle         | 0.28 ± 0.04            | 2.56 ± 0.16**       |
| Anthralin (10 μM)| 0.36 ± 0.02            | 4.78 ± 0.59†       |
| Chrysarobin (10 μM)| 0.24 ± 0.02      | 2.37 ± 0.18*       |
| BrMBA (3 μM)    | 0.35 ± 0.09            | 0.34 ± 0.02*       |
| Benzoylperoxide (10 nM)| 0.35 ± 0.03  | 2.91 ± 0.32*       |
| Okadaic acid (10 nM)| 0.28 ± 0.04      | 2.69 ± 0.09*       |
| Palytoxin (30 pM)| 0.23 ± 0.01            | 2.33 ± 0.14*       |
| Mezerein (30 nM)| 2.37 ± 0.20*           | 2.27 ± 0.43*       |

Epidermal cells were incubated for 2 hr with a tumor promoting agent in the presence or absence of TPA (30 nM); thereafter, the amount of PGE2 released into the medium was determined by RIA. Values are means ± S.E. (n = 3). *P < 0.01 vs. vehicle and TPA (−). †P < 0.05 vs. vehicle and TPA (+). ‡not significant vs. vehicle and TPA (+).
lease was significantly increased (Fig. 1B). Although TPA rather reduced [3H]arachidonic acid release at 4–8 hr after the addition of TPA, combined addition of TPA plus anthralin markedly and synergistically stimulated the release of [3H]arachidonic acid (Fig. 1B).

Figure 2 shows the effects of various concentrations of TPA on [3H]PGE2 and [3H]arachidonic acid release from [3H]arachidonic acid-prelabeled epidermal cells either in the presence or absence of 10 μM anthralin. In the absence of anthralin, the EC50 value of TPA for [3H]PGE2 release was 7 nM. Anthralin synergistically enhanced TPA-caused PGE2 release in a TPA concentration-dependent manner (Fig. 2). The EC50 value (5 nM) of TPA, however, was not changed even in the presence of 10 μM anthralin. Maximal effects were obtained at 30 nM TPA, both in the presence or absence of anthralin. Similar results were obtained with [3H]arachidonic acid release (Fig. 2).

Figure 3 shows the effects of anthralin on PGE2 release from epidermal cells either in the presence or absence of 30 nM TPA. Anthralin synergistically enhanced TPA-caused PGE2 release in a concentration-dependent manner.

As described previously (14), TPA stimulates PGE2 release at least partly through the activation of PKC. Although the mechanism of PGE2 and arachidonic acid release by anthralin is unknown, PKC is not involved in the above actions of anthralin (14). We next examined whether the synergistic stimulation of PGE2 and arachidonic acid release by TPA plus anthralin is dependent on PKC. When the epidermal cells are treated with 50 nM TPA for 48 hr, cellular PKC is down-regulated (26). In the PKC-down-regulated epidermal cells, the stimulation of [3H]PGE2 release by TPA was not detected (Fig. 4). Synergistic stimulation of [3H]PGE2 and [3H]arachidonic acid release by TPA plus anthralin were also no longer observed in the PKC-down-regulated cells (Fig. 4).

We next examined whether an apparent increase in [3H]arachidonic acid release by TPA plus anthralin is due to a true enhancement of arachidonic acid release or a decrease in the arachidonic acid incorporation into cellular phospholipids. As shown in Fig. 5, the amounts of [3H]arachidonic acid incorporated into cellular phospholipids were not altered by treating the cells with TPA plus anthralin.

Figure 6 shows the effects of TPA and/or anthralin on cellular cyclooxygenase activity. Cyclooxygenase activity in cellular homogenates was determined by measuring the indomethacin-sensitive conversion of [3H]arachidonic acid into [3H]PGE2. When the cells were incubated with TPA (30 nM) for 1 hr or longer,
cyclooxygenase activity in cellular homogenates increased substantially (Fig. 6). Anthralin also induced an increase in the cyclooxygenase activity, but the increase was detected only after a 4-6 hr lag period (Fig. 6). Cyclooxygenase activity was synergistically increased by treating the cells with TPA plus anthralin (Fig. 6).

Effects of cycloheximide and actinomycin D on the anthralin-caused increase in cyclooxygenase activity were also examined. Both cycloheximide and actinomycin D inhibited the anthralin-caused increase in cyclooxygenase activity in a concentration-dependent manner (Fig. 7). Cycloheximide and actinomycin D also inhibited the synergistic increase of cyclooxygenase activity caused by TPA plus anthralin: 30 nM TPA + 10 μM anthralin, 288 pmol [3H]PGE2/mg protein/min; TPA + anthralin + 1 μM cycloheximide, 98 pmol [3H]PGE2/mg protein/min; TPA + anthralin + 0.1 μg/ml actinomycin D, 56 pmol [3H]PGE2/mg protein/min.

Fig. 2. Concentration-response relationships of [3H]PGE2 and [3H]arachidonic acid release by TPA: Potentiation of the releases by anthralin. [3H]Arachidonic acid-prelabeled epidermal cells were incubated for 2 hr with various concentrations of TPA in the presence of vehicle (○) or 10 μM anthralin (○). The amounts of [3H]PGE2 (solid line) and [3H]arachidonic acid (broken line) released into the medium were determined. Each point and vertical bar represent a mean ± S.E. (n = 3). *P < 0.05 vs. corresponding vehicle.

Fig. 3. Concentration-response relationship of potentiation of TPA-caused PGE2 release by anthralin. Epidermal cells were incubated with various concentrations of anthralin in the presence of vehicle (○) or 30 nM TPA (○) for 2 hr. The amounts of PGE2 released into the medium were determined by RIA. Each point and vertical bar represent a mean ± S.E. (n = 3). *P < 0.05 vs. vehicle, **P < 0.01 vs. vehicle.
Fig. 4. Effect of TPA, anthralin or TPA plus anthralin on \([\text{3H}]\text{PGE}_2\) and \([\text{3H}]\text{arachidonic acid}\) release from PKC down-regulated epidermal cells. Epidermal cells were pretreated with vehicle (A) or 50 nM TPA (B). At 24 hr after the addition of TPA or vehicle, the medium was changed to a fresh one containing 50 nM TPA or vehicle. The cells were further cultured for 24 hr in the presence of 0.1 μCi \([\text{3H}]\text{arachidonic acid}\). Thereafter, the cells were washed and incubated for 2 hr with 30 nM TPA, 10 μM anthralin or TPA plus anthralin. The radioactivity of \([\text{3H}]\text{PGE}_2\) and \([\text{3H}]\text{arachidonic acid}\) released into the medium were determined. Each point represents mean of duplicate determinations. Similar experiments were repeated twice, and the results obtained were reproducible.

Fig. 5. Effect of TPA plus anthralin on the incorporation of \([\text{3H}]\text{arachidonic acid}\) into cellular phospholipids. Epidermal cells were incubated for 90 min with vehicle or 30 nM TPA plus 10 μM anthralin. Then, \([\text{3H}]\text{arachidonic acid}\) was added to the medium, and the cells were incubated for another 30 min. \([\text{3H}]\text{Arachidonic acid}\) incorporated into phosphatidylcholine (II), phosphatidylserine plus phosphatidylinositol (II) or phosphatidylethanolamine (II) was determined. Each column and vertical bar represent a mean ± S.E. (n = 3).

Fig. 6. Cyclooxygenase activity in the homogenates of the cells treated with TPA, anthralin or TPA plus anthralin. Epidermal cells were incubated with vehicle (○), 30 nM TPA (△), 10 μM anthralin (□) or TPA plus anthralin (△) for the indicated time periods. After the incubation, the cells were homogenized, and cyclooxygenase activity was determined. Each point represents the mean of duplicate determinations. Similar experiments were repeated twice, and the results obtained were reproducible.
Fig. 7. Effects of cycloheximide and actinomycin D on the anthralin-caused increase in cellular cyclooxygenase activity. Epidermal cells were incubated with the indicated concentrations of cycloheximide or actinomycin D in the presence (□) or absence (□) of 10 μM anthralin for 6 hr. After the incubation, the cells were homogenized, and cyclooxygenase activity was determined. Each column represents the mean of duplicate determinations. Similar experiments were repeated twice, and the results obtained were reproducible.

**DISCUSSION**

TPA stimulated PGE₂ release from mouse epidermal cells within 2 hr after the addition of this agent, as reported previously (14). Anthralin stimulated both PGE₂ and arachidonic acid release after a 4–6 hr lag period. When the cells were incubated with TPA plus anthralin, both PGE₂ and arachidonic acid release were synergistically stimulated. Under the same experimental conditions, other non-TPA type tumor promoters, i.e., chrysarobin, BrMBA, benzoylperoxide, okadaic acid and palytoxin, did not show the synergistic stimulation of PGE₂ release. Thus, the synergistic stimulation of PGE₂ and arachidonic acid release observed in the presence of TPA was anthralin specific.

Anthralin neither affects the binding of TPA to PKC (27) nor the TPA-caused PKC translocation from the cytosol to the membrane in epidermal cells (E. Aizu et al., unpublished data). In the PKC-down regulated cells, synergistic stimulation of both PGE₂ and arachidonic acid release caused by TPA plus anthralin was not detected. These results suggest that the PKC pathway is essential to induce the synergistic stimulation of PGE₂ and arachidonic acid release by these agents.

TPA rather suppressed [³H]arachidonic acid release at 4–8 hr after the addition of TPA. Since under the same experimental condition, TPA stimulated [³H]PGE₂ release, it seems unlikely that TPA actually suppressed [³H]arachidonic acid release. Conversion of [³H]arachidonic acid into other metabolites may explain the apparent decrease of [³H]arachidonic acid release. This, however, is unlikely, since we could not detect any major metabolite of [³H]arachidonic acid except PGE₂ (E. Aizu et al., unpublished data). TPA may increase the turnover rate (release and incorporation into phospholipids) of arachidonic acid and consequently induce the apparent decrease in [³H]arachidonic acid release.

Although TPA alone apparently suppressed [³H]arachidonic acid release, anthralin plus TPA synergistically stimulated [³H]arachidonic acid release at 2–8 hr after the addition of these compounds. Since obvious changes in the incorporation of arachidonic acid into phospholipids were not detected at 2 hr after the addition of these two compounds, it is likely that the apparent increase in arachidonic acid release by TPA plus anthralin (at least 2 hr after the stimulation) is mainly due to an increase in the release of free arachidonic acid from cellular phospholipids.

It has been reported that anthralin inhibits the lipoxygenase activity of mouse epidermis (28) and leukotriene production in human neutrophils (29). If that is the case, synergistic enhancement of TPA-caused PGE₂ release by anthralin may result from an increase in an available free arachidonic acid for the cyclooxygenase pathway. However, none of the lipoxygenase inhibitors that we tested enhanced TPA-caused PGE₂ release (E. Aizu et al., unpublished data), indicating that the lipoxygenase inhibitory effect of anthralin does not contribute to the enhancement of PGE₂ release.

Anthralin is widely used as a therapeutic agent for psoriasis (30, 31). At present, it is not known whether the enhanced PGE₂ release is related to the anti-psoriatic effect of anthralin. It is well-known that anthralin induces an inflammatory reaction as a side-effect of psoriasis therapy (30, 31). The enhanced release of PGE₂ may at least in part contribute to this inflammatory reaction, since indomethacin is effective in the suppression of anthralin-induced inflammation (32).

Treatment of epidermal cells with TPA increased indomethacin-sensitive cellular PGE₂ synthase activity, i.e., cyclooxygenase activity. It has been reported that TPA stimulated PGE₂ release through *de novo* synthesis of cyclooxygenase (33). When the epidermal cells were treated with anthralin, cyclooxygenase activity was also increased after 4–6 hr lag period. Since cycloheximide and actinomycin D inhibited anthralin-caused increase in cyclooxygenase activity, it is possible that anthralin induced *de novo* synthesis of cyclooxygenase.
TPA and anthralin synergistically induced cyclooxygenase activity, and this synergistic increase was also inhibited by cycloheximide and actinomycin D. These results suggest that synergistic stimulation of PGE$_2$ release caused by TPA and anthralin is due to the synergistic stimulation of arachidonic acid release and the synergistic induction of cyclooxygenase activity by these agents. At present the significance of this synergistic release of PGE$_2$ on skin tumor promotion is not known. It is unlikely that the synergistic release of PGE$_2$ relates to an enhancement of tumor promotion, since anthralin does not augment the tumor-promoting action of TPA but rather inhibits it (34).

Similar synergistic stimulation of PGE$_2$ release is also observed with epidermal growth factor (EGF) and anthralin in the same primary cultured epidermal cells (35). Similar to the case of TPA plus anthralin, synergistic stimulation of PGE$_2$ release by EGF plus anthralin is due to a synergistic stimulation of arachidonic acid release and a synergistic induction of cyclooxygenase activity (35). Although at present, the mechanism of synergism remains to be elucidated, the above findings suggest that anthralin exerts its synergistic effects by acting on common steps of TPA and EGF actions.

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