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PROSTAGLANDIN E AND F LEVELS IN MOUSE EPIDERMIS ARE INCREASED BY TUMOR-PROMOTING PHORBOL ESTERS

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SUMMARY

After topical application of tumor-promoting phorbol esters, immunoreactive prostaglandins E and F in mouse epidermis were increased several-fold over basal levels. The increases were doubled by 3 hours and lasted until 5 days after phorbol ester treatment. The activities of various phorbol esters for increasing epidermal prostaglandin levels paralleled the tumor-promoting activities of these compounds. Topical pretreatment with non-steroidal anti-inflammatory drugs inhibited the effect of phorbol esters on epidermal prostaglandin levels.

Tumor-promoting phorbol esters have elicited various responses when applied to mouse epidermis in vivo (1,2) or when added to cells in culture (3,18). Two effects, which appear to be related to the tumor-promoting action of these phorbol esters, are increased DNA synthesis (4) and induction of ornithine decarboxylase (ODC)[1] (5). It has been shown in mice that these two responses can be inhibited by non-steroidal anti-inflammatory drugs that inhibit PG synthesis and that this inhibition can be reversed by exogenous PGE but not by PGF (6,7). Furthermore, the enzymatic activity for producing prostaglandins from precursor fatty acids is present in mouse epidermis (8). These facts suggest that one or more PGs play a role in the increased DNA synthesis and ODC activity in mouse epidermis elicited by phorbol esters. In order to further evaluate this possibility, PG levels in mouse epidermis have been determined after treatment with phorbol esters and non-steroidal anti-inflammatory drugs.

[1] Abbreviations used: PG = prostaglandin, TPA = 12-O-tetradecanoylphorbol-13-acetate, ODC = ornithine decarboxylase.

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METHODS AND MATERIALS

Female 7- to 9-week-old CD-1 mice (Charles River Breeding Labs, Wilmington, MA) were housed and shaved as before (9). Phorbol or a phorbol ester (Chemical Carcinogenesis, Eden Prairie, MN), indomethacin (Sigma Chemical Co., St. Louis, MO), flufenamic acid (Aldrich Chemical Co., Milwaukee, WI), or naproxen (Syntex Corp., Palo Alto, CA) was applied in 0.2 ml of acetone to the backs of mice. At the appropriate time after treatment, the mice were killed and immediately frozen flat on solid CO_2. The epidermes were scraped off with a scalpel, and the scrapings from 4 mice were placed in 5 ml of ice-cold 0.01 N HCl containing 0.25 ml isopropyl alcohol, 0.25 ml ethyl acetate, and 0.9% NaCl. The tissue suspension was immediately frozen in liquid N_2 and stored at -20°C until homogenized. An additional 4.5 ml of ethyl acetate:isopropanol:water (1:1:1, v/v/v) was added, and each sample was homogenized for 30 sec at 0°C using a Polytron homogenizer. Each homogenate was roughly divided in half, 4000 DPM of [5,6-^{3}H]PGE_1 was added to one half, and an equal amount of [5,6-^{3}H]-$PGF_{1\alpha}$ (both PGs < 60 Ci/mmol, New England Nuclear, Boston, MA) was added to the other half to allow determination of extraction recovery. All samples were extracted by the addition of 2 ml of 0.9% NaCl and 4 ml ethyl acetate, vigorous shaking, and centrifugation. For each sample, the ethyl acetate layer was removed and dried under a stream of nitrogen, and the acidic lipids were removed from the residue by 1 to 3 ml of phosphate-buffered saline containing 0.1% gelatin (Calbiochem, La Jolla, CA). Part of this solution was assayed for PGE or PGF by radioimmunoassay (10) with PGE- or PGF-specific rabbit antiserum (Calbiochem, La Jolla, CA). The radioactivity was measured in the remainder of the solution to determine the percentage recovery, which was typically 50-90%. The macromolecules in the aqueous layer were precipitated by the addition of 1 ml of 3N perchloric acid. The DNA in the precipitate was hydrolyzed in 4 ml of 0.5N perchloric acid at 90°C for 10 min, and the DNA content of the soluble hydrolyzate was determined (11). The results were expressed as pmoles PGE or PGF per mg DNA.

RESULTS

The PGE and PGF levels in the epidermis after a single topical application of a promoting dose (17 nmoles) of the potent tumor-promoting phorbol ester, TPA[1], are depicted in Figure 1. Peak PGE levels were attained at about 6 hours and 24 hours after TPA treatment. At both times PGE levels were increased approximately sixfold over levels at zero time. PGF levels peaked twice, increasing threefold at 9 hours and tenfold by 72 hours after TPA treatment. The PG levels returned to normal between the fifth and seventh days after TPA treatment.

The increase in PGE levels was dependent on the dose of TPA (Figure 2). Also shown in Figure 2 are the epidermal PGE levels 6 hours after treatment with other phorbol compounds. The activities of these agents for increasing PGE levels were TPA > phorbol-12,13-didecanoate > phorbol-12,13-dibenzoate>

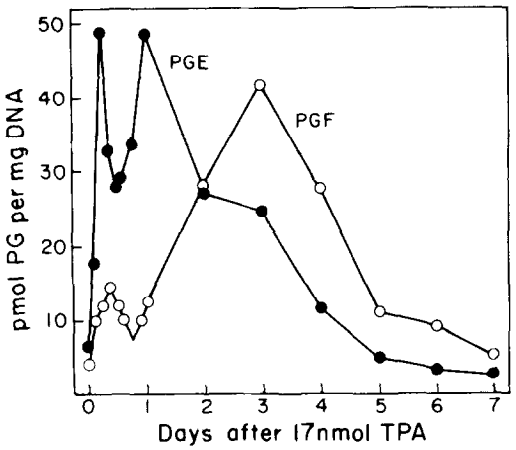

Figure 1. PGE (●) and PGF (○) levels in mouse epidermis after a single topical application of 17 nmoles of TPA.

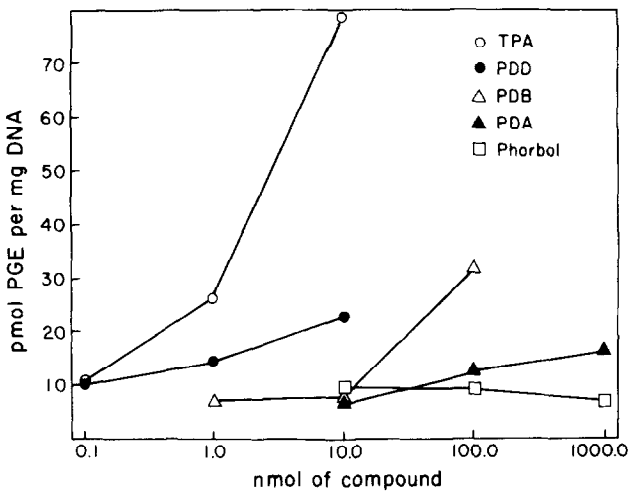

Figure 2. Increased PGE levels in mouse epidermis 6 hours after a single topical application of various doses of 12-O-tetradecanoylphorbol-13-acetate (TPA), phorbol-12,13-didecanoate (PDD), phorbol-12,13-dibenzoate (PDB), phorbol-12,13-diacetate (PDA), and phorbol.

phorbol-12,13-diacetate ≅ phorbol. Similar results were found for PGF levels 6 and 48 hours post treatment and also for PGE levels measured 48 hours after treatment.

The non-steroidal anti-inflammatory drug, indomethacin, has been shown to be a potent inhibitor of PG biosynthesis (19). When 280 nmoles of indomethacin were topically applied to mice 2 hours prior to treatment with 17 nmoles of TPA, no changes in the epidermal PGE and PGF levels were detected at the

times tested (up to 14 hours after TPA). Various doses of indomethacin and two other non-steroidal anti-inflammatory drugs, flufenamic acid and naproxen, were applied topically to mice 2 hours prior to treatment with 17 nmoles of TPA, and epidermal PGE levels were measured 6 hours after TPA. The doses of indomethacin, flufenamic acid, and naproxen that inhibited the TPA-increased PGE levels by 50% were 46, 110, and 135 nmoles, respectively.

DISCUSSION

Many investigators have used non-steroidal anti-inflammatory drugs to study the involvement of PGs in pathophysiological conditions. At least three of the many responses of mouse epidermis to TPA — edema (12,13), ODC induction (6), and increased DNA synthesis (7) — can be inhibited by treatment with non-steroidal anti-inflammatory drugs, and it has been suggested that PGs may be involved in the mechanism of tumor promotion (6). Additional support for this proposition has come from studies that show that TPA increased the release of PGs from canine kidney cells *in vitro* (14).

We have shown that TPA and other phorbol compounds elicited a dose-dependent increase in immunoreactive PGE and PGF levels in the mouse epidermis. For the five phorbol compounds tested, the increased epidermal PG levels paralleled ODC induction (5), increased DNA synthesis (4), and skin papilloma formation in initiated mice (15). Moreover, the relative potencies for indomethacin and other non-steroidal anti-inflammatory drugs for inhibition of increased epidermal PG levels after TPA correlated well with similar data for anti-inflammatory activity in mouse ear skin (12), inhibition of PG synthetase (19), and inhibition of ODC activity and DNA synthesis induced by TPA in mouse epidermis (6,7). Also, others have found that indomethacin and other non-steroidal anti-inflammatory drugs partially inhibit tumor promotion by TPA in mouse skin (16,17). While these results have added more evidence for the involvement of PGs in the mechanism of action of tumor-promoting phorbol esters, several important points remain unresolved. PGs may either mediate

or modulate the tissue responses to tumor promoters, and it is not known which of the responses involving PGs are critical to tumor promotion. Further investigation is required to determine exactly which PGs are increased and by what molecular mechanism this response to TPA occurs.

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