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INDOMETHACIN INHIBITION OF CELL PROLIFERATION INDUCED BY THE PHORBOLESTER TPA IS REVERSED BY PROSTAGLANDIN E_2 IN MOUSE EPIDERMIS IN VIVO

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SUMMARY

The proliferative response of mouse epidermis to the phorbolester TPA (10 nmoles) in vivo is completely inhibited by a single topical application of indomethacin one hour before TPA. DNA labeling in normal mouse epidermis is not significantly depressed by the drug. The inhibition can be reversed by applying prostaglandin E_2 (>3 nmoles) simultaneously with TPA, whereas prostaglandin $F_{2\alpha}$ (100 nmoles) is ineffective. The indomethacin-sensitive event is restricted to the first hour after phorbol ester treatment. TPA-induced skin inflammation is not influenced by the drug. It is proposed that prostaglandin E_2, or a closely related compound, mediates the mitogenic effect of TPA in mouse skin.

INTRODUCTION

One of the most efficient methods of evoking enhanced epidermal cell proliferation is to treat mouse skin locally with such phorbol esters as 12-O-tetradecanoylphorbol-13-acetate, or TPA[+] (1-4). A single application produces a strong inflammatory effect (5) and pronounced epidermal hyperplasia. When chronically applied to carcinogen-treated skin, TPA exerts a strong tumour-promoting effect (5). The molecular mechanisms of both the mitogenic and the tumour-promoting actions are not understood. The theoretical proposal has been made that phorbol ester TPA fits a tentative prostaglandin receptor, i.e. that it acts as a prostaglandin agonist (6). This hypothesis is consistent with observations that E-type prostaglandins can stimulate epidermal cell proliferation both in vivo and in vitro, whereas F-prostaglandins exhibit no effect (7-10). On the other hand TPA was shown recently to stimulate prostaglandin pro-

[+]Abbreviation: TPA, 12-O-tetradecanoylphorbol-13-acetate; PG, prostaglandin; TLC, thin layer chromatography.

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duction in canine kidney cells (11). We report here experiments demonstrating that indomethacin, a prostaglandin synthetase inhibitor (12), can prevent the TPA-induced stimulation of DNA-synthesis in mouse epidermis in vivo. Prostaglandins are therefore probably involved in the mitogenic effect of the phorbol ester.

MATERIALS AND METHODS

The experiments were carried out with female albino mice (strain NMRI, age 7-8 weeks), which were kept under an artificial day-night rhythm (4) and fed ad libitum. The back skin of the animals was shaved by means of an electric clipper 3-4 days before the experiment. Only those animals, which did not show regrowth of hair were used.

12-O-Tetradecanoylphorbol-13-acetate (TPA) and indomethacin were dissolved in acetone (o,1 ml) and topically applied to the shaved area by means of a micropipette.

For pulse labeling of epidermal DNA, 30μCi of methyl-^{3}H-thymidine (Amersham-Buchler, Braunschweig, Germany: specific activity 22 Ci/mmole) dissolved in 0,3 ml saline were injected intraperitoneally. The animals were killed one hour later by cervical dislocation. The back skin was dissected, thoroughly wetted with ice-cold 0,8 M perchloric acid, and flattened on a cork plate. The epidermis was scraped off with a scalpel and homogenized in 2 ml ice-cold 0,4M perchloric acid. The isolation of DNA and measurement of radioactivity are described in ref. 4.

For determination of mitotic activity the animals were intraperitoneally injected with 25 μg vincristine each (in 0,3 ml saline). 4 hours later they were sacrificed and the dissected back skin was fixed in 10% formaldehyde solution. 5 μm sections were made and stained with haematoxylin-eosine. Metaphase figures were counted per visual field (magnification x 500).

The phorbol ester TPA was kindly supplied by Prof. E. Hecker (Deutsches Krebsforschungszentrum, Institut für Biochemie, D-6900 Heidelberg, Germany). Prostaglandins and indomethacin were purchased from Sigma, Munich, Germany. Prostaglandins were tested for purity before use by TLC.

RESULTS

A single topical application of 10 nmoles TPA to mouse skin in vivo led to an increase in epidermal DNA labeling, with three peaks at 18, 30 and 48 hours (Figure 1).

The effects of indomethacin on normal and TPA-induced epidermal cell proliferation were examined by applying an acetone solution topically. When this treatment was carried out one hour before TPA application, DNA labeling as measured 18 hours after TPA (first peak of Figure 1) was inhibited in a dose-dependent manner (Figure 2). The TPA-induced proliferation could be completely blocked with 560 nmoles ($5.6 \cdot 10^{-3}$M) of indomethacin; at higher doses still,

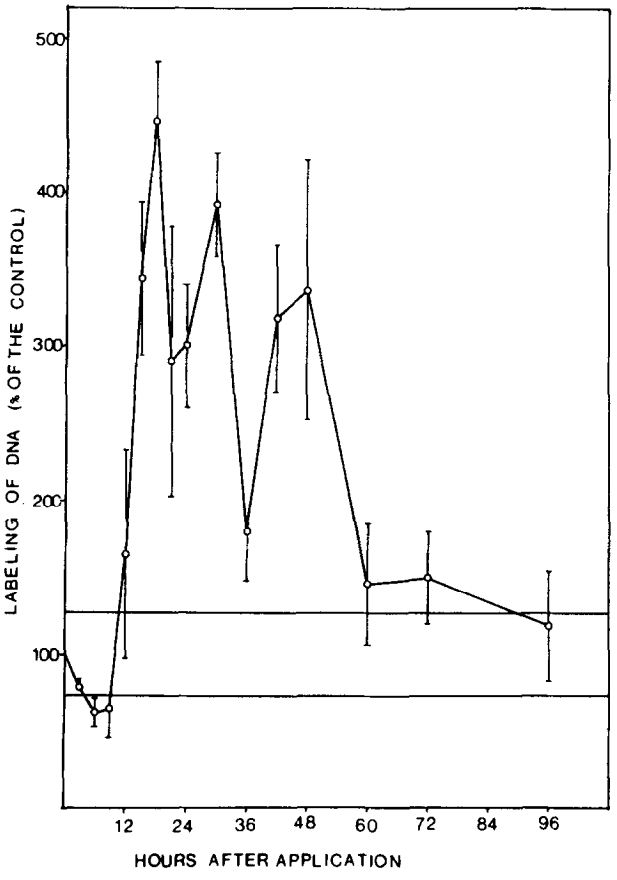

Figure 1. Effect of a single local application of TPA on DNA labeling in mouse dorsal epidermis in vivo.

Mice were treated with either 0,1 ml acetone or a solution of 10 nmoles TPA in 0,1 ml acetone, and killed at the times indicated. Labeled thymidine was injected i.p. one hour prior to sacrifice (see Methods). Each experimental point represents mean ± S.D. for at least 10 mice. Control (acetone-treated), indicated by horizontal lines: 51 ± 13 cpm/μg DNA, N = 60.

epidermal DNA labeling was depressed even below the control level. The increase of epidermal mitotic activity observed 27 hours after TPA application was also prevented by indomethacin (Table 1). No such inhibitory action of the drug could be observed in the skin of acetone-treated control mice (Figure 2, Table 1).

The duration of the indomethacin-sensitive period is shown in Figure 3. When applied either one hour before or simultaneously with TPA, 1,1 μmoles of indomethacin inhibited thymidine incorporation into epidermal DNA 18 hours

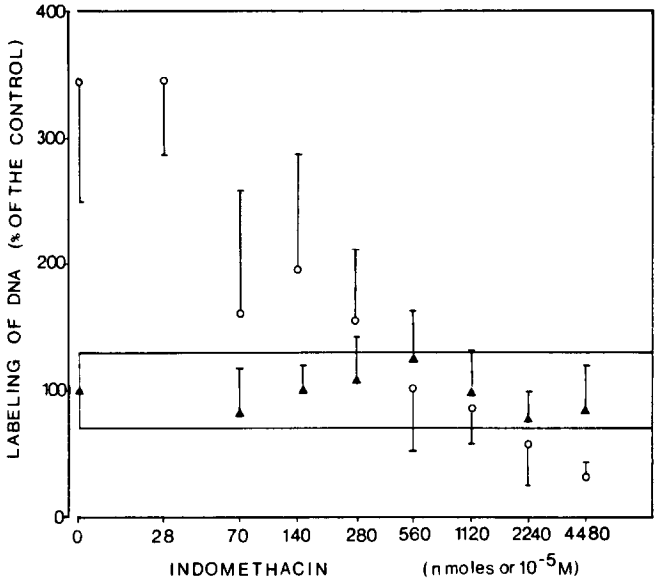

Figure 2. Effect of indomethacin on normal (▲) and on TPA-induced (O) epidermal DNA-synthesis.

Mice were treated with indomethacin, dissolved in 0,1 ml acetone, one hour prior to acetone (0,1 ml) or TPA (10 nmoles in 0, 1 ml acetone) application, and killed 18 hours after TPA treatment. For details see Figure 1. N = 10 (± S.D.).

Table 1. Effect of indomethacin on normal and TPA-induced mitotic activity in mouse epidermis

Treatment	Metaphase figures per visual field	Metaphase figures per visual field % of the control
acetone (control)	0.21 ± 0.06	100 ± 28
acetone, indomethacin	0.22 ± 0.05	105 ± 24
TPA	1.81 ± 0.49	862 ± 233
TPA, indomethacin	0.16 ± 0.05	76 ± 24

Mice were treated with acetone (0.1 ml) or 1.1 µmoles indomethacin in 0.1 ml acetone one hour before application of 0.1 ml acetone or 10 nmoles TPA in 0.1 ml acetone and killed 27 hours after TPA treatment. Vincristine was injected i.p. 4 hours prior to sacrifice (see Methods). Each value is the mean ± S.D. of metaphase figures per visual field of at least 16 sections prepared from 4 to 5 mice.

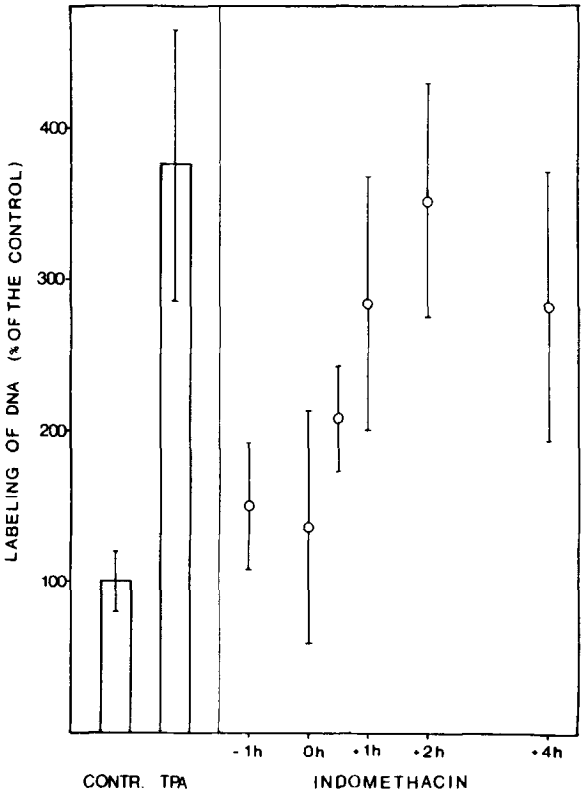

Figure 3. Effect on epidermal DNA labeling of varying the time interval between indomethacin and TPA treatment.

Mice were treated with 1,1 µmoles indomethacin in 0,1 ml acetone and 10 nmoles TPA in 0,1 ml acetone, and killed 18 hours after TPA treatment. Left diagram: control experiments with acetone or TPA alone. Right diagram: combined treatment with TPA and indomethacin; the negative sign refers to indomethacin application before, the positive sign after TPA treatment. $N \geq 10 (\pm$ S.D.).

after TPA treatment. When given 1/2 hour after TPA, only a partial inhibition could be achieved, whereas indomethacin administration 2 hours after TPA did not depress DNA labeling at all.

The results described suggest that biosynthetic products from the arachidonic acid cascade may play a role in TPA-induced epidermal DNA-synthesis. It should be possible, therefore, to overcome the indomethacin effect by topical application of prostaglandins. The outcome of such an experiment is shown in Figure 4. Prostaglandin E_2 completely restored the TPA effect when applied

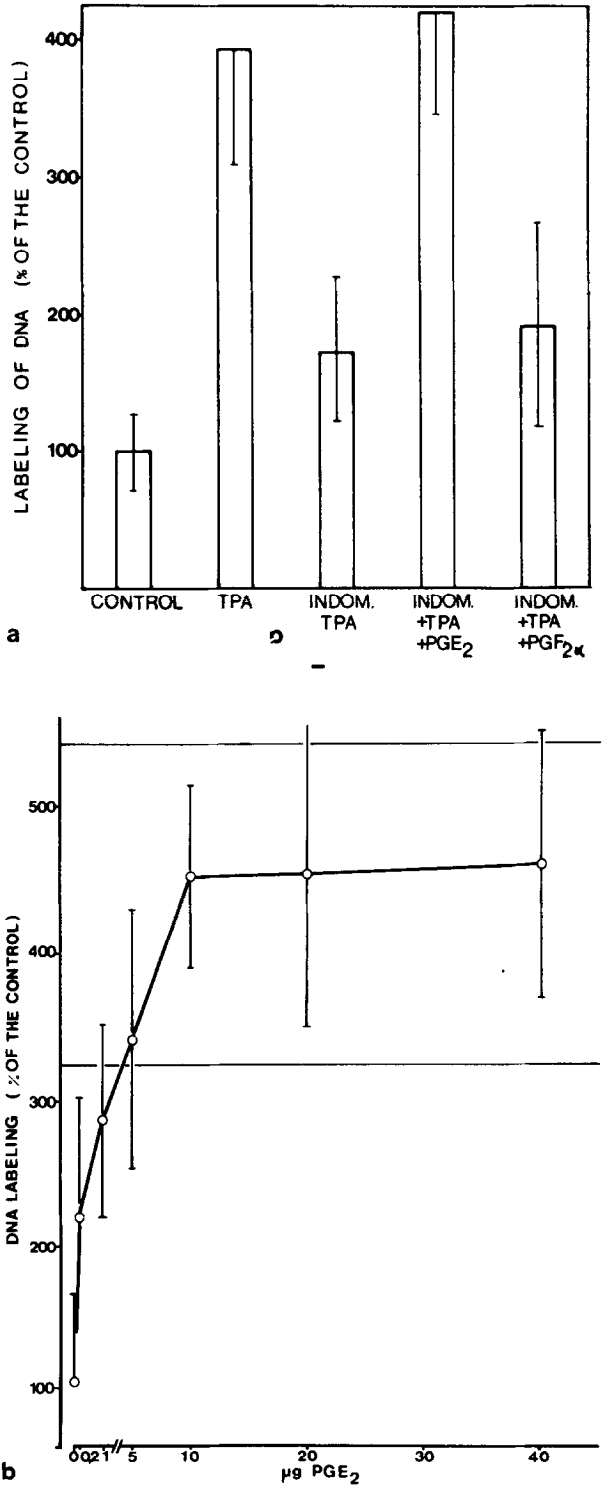
LABELING OF DNA (% OF THE CONTROL)
400
300
200
100
CONTROL
TPA
INDOM. TPA
INDOM. +TPA +PGE2
INDOM. +TPA +PGF2α
a
DNA LABELING (% OF THE CONTROL)
500
400
300
200
100
0 0.2 1 5 10 20 30 40
μg PGE2
b

Figure 4

simultaneously with the phorbol ester, whereas prostaglandin $F_{2\alpha}$ had no effect. A PGE_2 dose as low as 1 µg already caused a measurable effect, and 10 µg were sufficient to overcome entirely the inhibition by indomethacin (Figure 4, right diagram). On the other hand, prostaglandin E_2, in doses up to 80 µg per mouse, neither led to a significant increase of epidermal DNA labeling when applied to normal, i.e. acetone-treated skin, nor intensified the stimulatory effect of 10 nmoles TPA alone, i.e. without previous indomethacin treatment.

Surprisingly, indomethacin, which is classified as an antiinflammatory drug (12), did not exhibit any visible effect on TPA-induced skin inflammation, at least as far as such parameters as oedema, leucocyte infiltration, and epidermal hypertrophy are concerned.

DISCUSSION

The results of our experiments suggest that products of the arachidonic cascade, most probably prostaglandin E_2 or a closely related compound, are involved in the TPA-induced epidermal cell proliferation in mouse skin in vivo. The objection that the observed inhibitory effect of indomethacin was due to cytotoxic effects can be ruled out, since the drug turned out to be completely ineffective when applied one hour after TPA. Furthermore, normal epidermal cell proliferation as well as DNA synthesis induced by mechanical means (skin massage) or by certain other types of mitogens cannot be inhibited at all by indomethacin (M. Bravo, G. Fürstenberger, S. Bertsch and F. Marks, in preparation). Finally, PGE_2 in comparatively low doses was able to overcome specifically the inhibitory effect of the drug.

Our results are consistent not only with recent observations that TPA induced induction of epidermal ornithine decarboxylase activity is prevented by

Figure 4. Effect of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ on epidermal DNA-synthesis following combined treatment with indomethacin and TPA.

Mice were treated with either 0,1 ml acetone or 1,1 µmoles indomethacin in 0,1 ml acetone one hour prior to application of either 0,1 ml acetone, 10 nmoles TPA in 0,1 ml acetone or 10 nmoles TPA plus prostaglandin in 0,1 ml acetone, and killed 18 hours after TPA treatment. For further details see Figure 1. (a) Comparison of the effects of 40 µg PGE_2 and 40 µg $PGF_{2\alpha}$. (b) Dose-response curve of the PGE_2 effect. $N \geq 10$ ($\pm$S.D.).

indomethacin and restored by PGE_2 though not by $PGF_{2\alpha}$(13), but also with the concept that arachidonic acid and its metabolites may play a role in hyperproliferative processes of skin, for example in psoriasis (14,15). Furthermore, in liver after partial hepatectomy prostaglandins also seem to be involved in mediating the proliferative response (16).

The indomethacin-sensitive reaction must be a very early event in the course of TPA-induced epidermal cell proliferation. Whether it involves stimulation by TPA of prostaglandin synthesis or a sensitization of epidermal cells for prostaglandins already present in skin is not clear at present. The fact that TPA-treated skin after pretreatment with indomethacin responds much better to exogenous PGE_2 as compared with normal epidermis may be taken as evidence for the second possibility.

Most surprisingly, indomethacin does not relieve the symptoms of TPA-induced inflammation, which is therefore probably not mediated by prostaglandins. Thus indomethacin offers the interesting possibility of separating epidermal cell proliferation from skin inflammation caused by a tumour-promoting phorbol ester.

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