Inhibition by Prostaglandin Synthesis Inhibitors of the Induction of Epidermal Ornithine Decarboxylase Activity, the Accumulation of Prostaglandins, and Tumor Promotion Caused by 12-O-Tetradecanoylphorbol-13-acetate

Ajit K. Verma, Curtis L. Ashendel, and R. K. Boutwell

McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

Application of 12-O-tetradecanoylphorbol-13-acetate (TPA) to mouse skin results in a large and rapid induction of epidermal ornithine decarboxylase (ODC; EC 4.1.1.17) activity, a phenotypic change proposed to be essential for skin tumor promotion. Induction of ODC activity by TPA was inhibited by prior treatment of skin with inhibitors of prostaglandin synthesis in the order, indomethacin > naproxen > flufenamic acid > acetylsalicylic acid. In contrast, dexamethasone, a steroidal anti-inflammatory drug, was ineffective at a 280-nmol dose.

The inhibitory effect of indomethacin on ODC induction was found to be specific. The 280-nmol dose of indomethacin that inhibited the induction of ODC activity by 80% did not inhibit the induction by TPA of S-adenosylmethionine decarboxylase and cyclic adenosine 3':5'-monophosphate phosphodiesterase (EC 3.1.4.17) activities. Furthermore, indomethacin treatment affected neither normal epidermal protein nor DNA synthesis.

The inhibition by the prostaglandin synthesis inhibitors of the induction of ODC activity by TPA was completely overcome by concurrent application of about 25 to 100 nmol of prostaglandins E1, E2, or D2, or the 6,9-thio analog of prostaglandin I2 with TPA. In contrast, prostaglandins F1α and F2α, 6-keto-prostaglandin F1α, or arachidonic acid at doses of as much as 100 nmol were ineffective. Application of 17 nmol of TPA led to about a 3-fold increase in epidermal levels of prostaglandins E and F, and that increase was blocked by pretreatment with 280 nmol of indomethacin.

Application of 280 nmol of indomethacin before each TPA treatment significantly inhibited the formation of skin papillomas. Prostaglandin E2 alone could neither induce epidermal ODC activity nor promote skin tumors in an initiation/promotion experiment. However, potentiation of TPA-induced ODC activity by prostaglandin E2 was observed.

The findings that the application of indomethacin prior to TPA treatment inhibits the accumulation of prostaglandins, the induction of ODC activity, and the formation of skin papillomas suggest that (a) prostaglandins E1, E2, D2, and I2 may play a role in the induction of ODC activity by TPA and that (b) TPA-induced ODC activity may be an important component of the mechanism of skin tumor promotion.

INTRODUCTION

Application of the potent tumor-promoting agent TPA3 to mouse skin leads to a pronounced increase (about 200-fold) in mouse epidermal ODC (EC 4.1.1.17) activity within about 5 h after treatment. Recent evidence indicates that this phenotypic change is one of the essential components of the mechanism of skin tumor promotion (2, 22, 23, 34, 38).

The biochemical mechanism of induction of ODC by TPA is unclear. The role of the microtubule-containing system in regulation of ODC induction has been suggested. Thus, addition of colchicine and vinblastine in micromolar concentrations inhibits the induction of ODC activity of mouse leukemia L1210 cells by fresh medium and serum (5). Similarly, i.p. administration of colchicine or other microtubule-disrupting agents, such as vinblastine or vincristine, prior to topical application of TPA suppressed the induction of mouse epidermal ODC activity (24).

In most cell and tissue systems stimulated to proliferate, a rapid, transient increase in ODC activity is preceded by an increase in the level of cyclic AMP and an increase in the activity of cyclic AMP-dependent protein kinase. Examples include liver following treatment with 3-methylcholanthrene or phenobarbital and lymphocytes following treatment with mitogen (30). This relationship between an increase in cyclic AMP level and ODC induction was not clear in the epidermis of intact mice (20).

In our preliminary communication (37), we reported that indomethacin, flufenamic acid, or acetylsalicylic acid (prostaglandin synthesis inhibitors) applied to mouse skin prior to application of TPA remarkably depressed the induction of ODC activity by TPA. The inhibition of the induction of ODC activity by the prostaglandin synthesis inhibitors was completely overcome by treatment with either PGE1 or PGE2. This suggests that prostaglandins may play a role in ODC induction by TPA in mouse epidermis. The role of prostaglandins in ODC induction and in skin tumor promotion by TPA is elucidated further in this paper.

MATERIALS AND METHODS

Materials. Female Charles River CD-1 mice were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass. and were used for experimentation at 7 to 9 weeks of age. TPA was obtained from Dr. Peter Borchert, Eden Prairie, Minn. DMBA was purchased from Eastman Organic Chemicals, Rochester, N. Y., indomethacin was from Sigma Chemical Co., St. Louis, Mo., and flufenamic acid and acetylsalicylic acid were from Aldrich Chemical Co., Milwaukee, Wis. Naproxen
was a generous gift from Dr. Harold C. Anderson, Syntex Laboratories, Palo Alto, Calif. Prostaglandins were generously supplied by Dr. J. E. Pike, The Upjohn Company, Kalamazoo, Mich. DL-[1-14C]Ornithine hydrochloride (specific activity, 49.9 mCi/mmol), S-adenosyl-L-[carboxyl-14C]methionine (specific activity, 54.6 mCi/mmol), [5,6-3H]PGE, (specific activity, 74 Ci/mmol), [5,6-3H]PGF2a (specific activity, 74 Ci/mmol), L-[4,5-3H]Leucine (specific activity 50 Ci/mmol), and [methyl-3H]Thymidine (specific activity, 20 Ci/mmol) were purchased from New England Nuclear, Boston, Mass. Rabbit antisera to PGE and PGF were purchased from Calbiochem, La Jolla, Calif.

Treatment of Mice. All mice were housed in stainless steel cages. Food and water were available ad libitum. Mice were kept in a normal rhythm of 12-hr light and 12-hr dark periods. The dorsal skin of the mice was shaved 3 to 4 days before experimentation, and only those mice not exhibiting hair regrowth over this period were used. The solutions of all agents to be applied topically were prepared in acetone and were delivered to the shaved areas of individual mice in a volume of 0.2 ml. Control mice were treated with the same volume of acetone.

Assay of ODC and S-Adenosyl-L-methionine Decarboxylase Activity. At appropriate times after treatment, mice were killed by cervical dislocation, and epidermis from individual mice was separated by a brief heat treatment (19). ODC activity from soluble epidermal extracts (30,000 × g) was determined by measuring the release of 14CO2 from DL-[1-14C]ornithine hydrochloride. Assays were carried out at either 100 μM L-ornithine concentration in a final volume of 2 ml in 25-ml Erlenmeyer flasks (34) or 400 μM L-ornithine in a final volume of 0.25 ml in 15-ml Corex centrifuge tubes (36).

S-Adenosylmethionine decarboxylase activity from soluble epidermal extracts was determined by measuring the release of 14CO2 from S-adenosyl-L-[carboxyl-14C]methionine as reported earlier (34).

Assay of Cyclic AMP Phosphodiesterase Activity. At appropriate times after treatment, mice were killed by cervical dislocation, and epidermal homogenates were prepared. Cyclic AMP phosphodiesterase activity in the whole epidermal homogenates was determined at 400 μM cyclic AMP concentration (35).

Protein content of epidermal extracts was determined by the procedure of Lowry et al. (17).

Measurement of Prostaglandins by Radioimmunoassay. PGE and PGF levels were measured from ethyl acetate extracts of acidified (pH 2.0) mouse epidermal homogenates by radioimmunoassay as reported by Orczyk and Behrman (25). DNA content of the homogenates was measured by the diphenylamine method of Burton (4).

Measurement of Incorporation of Tritiated Precursors into Epidermal Protein and DNA. Incorporation of [3H]thymidine into epidermal DNA was determined by i.p. injection of [3H]-thymidine (1 μCi/g body weight) 30 min before sacrifice as described above (36). For determination of protein synthesis, mice were given [3H]leucine i.p. (1 μCi/g body weight) 30 min before sacrifice. Epidermis from each mouse was separated by a brief heat treatment (19), homogenized in 2 ml of 0.5 M perchloric acid at 4°, and centrifuged. The pellet was washed twice (2 × 2 ml) with ice-cold 0.5 M perchloric acid and once with 100% ethanol. The pellet was dissolved in 0.5 M NaOH by heating at 80° for 30 min. After centrifugation, aliquots of dissolved protein were taken for determination of radioactivity.

Tumor Induction Experiments. Tumors were initiated in all mice by application of 0.2 μmol (51.2 μg) of DMBA in 0.2 ml of acetone. Beginning 2 weeks following initiation, all mice were promoted twice a week (on Days 1 and 4) with either 5 (Table 10, Experiment 1) or 8 (Chart 6, Experiment 2) nmol of TPA for the duration of the experiment. Mice were treated with indomethacin either 2 hr (Table 10) or 5 hr (Chart 6) before each promotion with TPA. Controls were pretreated with acetone only. There were at least 30 mice in each treatment group. Mice were housed 10/cage in screen-bottomed stainless steel cages. The incidence of papillomas was observed weekly.

RESULTS

Effect of Treatment with Indomethacin or Other Inhibitors of Prostaglandin Synthesis on the Induction of Mouse Epidermal ODC Activity by TPA

Application of TPA to mouse skin leads to a rapid, transient induction of ODC with peak activity at about 4.5 hr following TPA treatment. Treatment of mouse skin with 280 nmol of indomethacin 2 hr prior to TPA treatment results in a dramatic inhibition of the induction of ODC activity by TPA (37). The time course of the effect of indomethacin application relative to time of TPA treatment on ODC induction is shown in Chart 1. In this experiment, 280 nmol of indomethacin were applied once to the skin at various times before and after treatment with 5 nmol of TPA, and epidermal ODC activity was determined 4.5 hr following TPA treatment. Indomethacin was virtually ineffective in inhibiting the induction of ODC activity when 24 hr was allowed to elapse between indomethacin and TPA treatment. However, the induction of ODC activity was depressed by 79% when indomethacin was applied 6, 5, or 2 hr before TPA treatment, respectively. The induction of enzyme activity was decreased by 52% when indomethacin was applied 1 hr after TPA treatment, and the inhibition was progressively
less as the interval between the time of its application and time of assay was shortened further. Thus, when indomethacin was applied 2.5 hr after TPA treatment, the inhibition was only 39%.

Inhibition of the induction of ODC activity by indomethacin was dose dependent (Chart 2). Inhibition of enzyme induction was observed at a dose above 25 nmol. Application of 150 and 300 nmol of indomethacin 2 hr before treatment with 5 nmol of TPA inhibited the induction of ODC activity by 54 and 71%, respectively.

A number of other nonsteroidal antiinflammatory drugs which are known to inhibit prostaglandin synthesis were tested for their ability to inhibit the induction of ODC activity, and the data are summarized in Table 1. The relative inhibitory potency was in the order, indomethacin > naproxen > flufenamic acid > acetylsalicylic acid. A single application of 280 nmol of dexamethasone, a steroidal antiinflammatory drug, did not inhibit the induction of ODC activity when applied in 0.2 ml of acetone 2 hr before TPA treatment. However, a detailed dose- and time-response relationship was not determined.

The possibility that treatment with prostaglandin synthesis inhibitors may alter the time course of induction of ODC activity by TPA was explored. In this experiment, mice were treated with either 280 nmol of indomethacin or 1000 nmol of acetylsalicylic acid 2 hr prior to treatment with 5 nmol of TPA, and mice were killed for enzyme assay at various times following TPA treatment (Chart 3). Application of TPA resulted in a pronounced increase in ODC activity with peak activity at 6 hr following TPA treatment. Enzyme activity returned to basal control value at about 13 hr. Indomethacin and acetylsalicylic acid treatment resulted in a sharp reduction in the degree of induction of ODC activity by TPA. Furthermore, there is no indication of an altered time course of the induction of ODC activity following inhibitor pretreatment.

Other Effects of Indomethacin Pretreatment

Is the Effect of Indomethacin on the Induction of ODC Activity Due to General Cytotoxicity? The possibility that indomethacin may inhibit the induction of ODC activity due to general cytotoxicity is ruled out by a number of findings.

Application of 280 nmol of indomethacin inhibited neither normal nor TPA-stimulated mouse epidermal protein synthesis. Furthermore, indomethacin treatment did not affect normal DNA synthesis. However, indomethacin application 2 hr prior to treatment with 17 nmol of TPA depressed TPA-enhanced incorporation of [3H]thymidine into DNA (Table 2). These results are in accord with previous findings (7, 8).

Chart 2. Effect of indomethacin dose on the induction of epidermal ODC activity by TPA

Table 1

Inhibition by the inhibitors of prostaglandin synthesis of the induction of ODC activity by TPA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (nmol)</th>
<th>ODC activity (% of control)</th>
<th>Median inhibitory dose (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>280</td>
<td>26</td>
<td>110</td>
</tr>
<tr>
<td>Naproxen</td>
<td>250</td>
<td>36</td>
<td>160</td>
</tr>
<tr>
<td>Flufenamic acid</td>
<td>280</td>
<td>36</td>
<td>180</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1000</td>
<td>49</td>
<td>1100</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>280</td>
<td>95</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

Chart 3. Effect of indomethacin or acetylsalicylic acid pretreatment on the induction of ODC activity by TPA

Table 2

Effect of indomethacin on incorporation of tritiated precursors into epidermal protein and DNA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[3H]Leucine incorporation (dpm/µg protein)</th>
<th>[3H]Thymidine incorporation (dpm/µg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone—acetone</td>
<td>4.49 ± 0.69</td>
<td>77 ± 6</td>
</tr>
<tr>
<td>Indomethacin—acetone</td>
<td>4.92 ± 0.55</td>
<td>84 ± 10</td>
</tr>
<tr>
<td>Acetone—TPA</td>
<td>15.90 ± 1.00</td>
<td>431 ± 22</td>
</tr>
<tr>
<td>Indomethacin—TPA</td>
<td>14.60 ± 0.5</td>
<td>224 ± 34</td>
</tr>
</tbody>
</table>

310

CANCER RESEARCH VOL. 40
The dose of indomethacin that dramatically inhibited the induction of ODC activity failed to inhibit the induction of S-adenosylmethionine decarboxylase activity by TPA (Table 3).

Addition of as much as 560 nmol of indomethacin to soluble epidermal homogenates prepared 4.5 hr after TPA treatment of mouse skin did not alter enzyme activity when assayed under normal assay conditions (ODC activity was 3.84 nmol/30 min/mg protein in the absence and was 3.99 nmol/30 min/mg protein in the presence of 560 nmol of indomethacin).

Effect of Indomethacin Pretreatment on TPA-induced Cyclic AMP Phosphodiesterase Activity. Indomethacin inhibits prostaglandin synthesis by irreversibly inactivating cyclooxygenase (6). In addition, indomethacin has been shown to inhibit a number of enzymes including cyclic AMP phosphodiesterase. However, a higher concentration of indomethacin is required for the latter effects (6). The possibility that indomethacin treatment may inhibit the induction of cyclic AMP phosphodiesterase activity by TPA was examined, and the results are shown in Table 4. Application of 10 nmol of TPA led to a 3-fold increase in cyclic AMP phosphodiesterase activity measured at 6 hr following TPA treatment in accord with our previous findings (35). Treatment with 280 nmol of indomethacin 2 hr before TPA treatment did not affect the degree of enzyme induction (Table 4). In contrast, epidermal ODC activity was inhibited by 80% following indomethacin pretreatment (Table 3).

Does Indomethacin Pretreatment Lead to the Production of an Inhibitor of TPA-induced Activity? Mixing of epidermal extracts prepared from acetone- and indomethacin-pretreated mouse skin resulted in simple additive ODC activity (Table 5); this suggests that indomethacin treatment does not lead to the production of inhibitor(s) of TPA-induced ODC activity.

Effects of Prostaglandins on Indomethacin-caused Inhibition of the Induction of Epidermal ODC Activity by TPA

Inhibition of the induction of ODC activity by indomethacin can be completely overcome by concurrent application of either PGE1 or PGE2 with TPA. Counteraction of the indomethacin inhibition was dependent on the dose of prostaglandins. Application of 70 nmol of PGE2 completely counteracted inhibition of the induction of ODC activity by 280 nmol of indomethacin (37). As shown in Table 6, application of 70 nmol of PGE2 concurrently with TPA also completely counteracted inhibition of the induction of ODC activity by various prostaglandin inhibitors. As reported earlier (37), application of as much as 70 nmol of PGE2 alone did not induce epidermal ODC activity. In contrast, PGE2 applied with TPA potentiated the induction of ODC activity by TPA. The time course of the effect of application of PGE2 alone or concurrently with TPA to normal mouse epidermis resulted in simple additive ODC activity (Table 5); this suggests that indomethacin treatment does not lead to the production of inhibitor(s) of TPA-induced ODC activity.

Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC activity (nmol CO2/30 min/mg protein)</th>
<th>SAMD° activity (nmol CO2/30 min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone + acetone</td>
<td>0.02 ± 0.04</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Acetone + TPA</td>
<td>3.14 ± 0.48</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>Indomethacin + TPA</td>
<td>0.59 ± 0.05</td>
<td>0.28 ± 0.03</td>
</tr>
</tbody>
</table>

* SAMD, S-adenosylmethionine decarboxylase.

Table 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cyclic AMP phosphodiesterase activity (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
</tr>
<tr>
<td>Acetone + acetone</td>
<td>292 ± 6</td>
</tr>
<tr>
<td>Acetone + TPA</td>
<td>883 ± 85</td>
</tr>
<tr>
<td>Indomethacin + TPA</td>
<td>850 ± 105</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
</tr>
<tr>
<td>Acetone + acetone</td>
<td>281 ± 35</td>
</tr>
<tr>
<td>Acetone + TPA</td>
<td>906 ± 78</td>
</tr>
<tr>
<td>Indomethacin + TPA</td>
<td>1089 ± 137</td>
</tr>
</tbody>
</table>

Table 5

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC activity (nmol CO2/30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Indomethacin + acetone</td>
<td>0.00</td>
</tr>
<tr>
<td>B. Indomethacin + TPA</td>
<td>0.85</td>
</tr>
<tr>
<td>C. Acetone + TPA</td>
<td>0.50</td>
</tr>
<tr>
<td>A + C</td>
<td>0.50</td>
</tr>
<tr>
<td>B + C</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Table 6

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC activity (nmol CO2/30 min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>4.1 ± 0.68</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.86 ± 0.10</td>
</tr>
<tr>
<td>Naproxen</td>
<td>1.75 ± 0.10</td>
</tr>
<tr>
<td>Flufenamic acid</td>
<td>1.92 ± 0.24</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>2.52 ± 0.24</td>
</tr>
</tbody>
</table>
or indomethacin-pretreated mouse skin on epidermal ODC activity is shown in Chart 4. Inhibition of the induction of ODC activity by indomethacin was completely counteracted by application of 70 nmol of PGE2 concurrently with TPA. Application of PGE2 alone did not induce PDC activity at any of the time points (Chart 4) or doses (Chart 4, inset) studied.

A number of other prostaglandins (Chart 5) were tested for their ability to overcome inhibition of ODC induction by indomethacin, and the data are summarized in Table 7. Application of 25 or 100 nmol of PGE2, PGD2, or 6,9-thio-PGI2 completely overcame the inhibition of ODC induction by indomethacin. In contrast, 100 nmol of 6-keto-PGF1α or arachidonic acid were ineffective (Table 7). PGF1α or PGE2α was also unable to counteract indomethacin-caused inhibition of the induction of ODC activity (37).

Does PGE2 Counteract Retinoic Acid-caused Inhibition of the Induction of ODC Activity?

Retinoic acid is a potent inhibitor of the induction of mouse epidermal ODC activity by TPA (34). It was of interest to determine whether PGE2 could also counteract the inhibition of the induction of ODC activity by retinoic acid. As shown in Table 8, application of 0.17 nmol of retinoic acid 2 hr prior to TPA treatment inhibited by 85% TPA-induced ODC activity. Application of PGE2 concurrently with TPA failed to counteract enzyme inhibition caused by retinoic acid. Furthermore, simultaneous application of 280 nmol of indomethacin and 0.17 nmol of retinoic acid to mouse skin resulted in inhibition of the induction of ODC activity greater than that obtained with retinoic acid or indomethacin alone. These results suggest that inhibition of the induction of ODC activity by retinoic acid may not involve inhibition of synthesis of prostaglandins.

Effect of Indomethacin Pretreatment on Accumulation of Prostaglandins by TPA

The possibility that indomethacin may inhibit the induction of ODC activity by inhibiting the accumulation of prostaglandins was examined, and the results are shown in Table 9. In this experiment, mice were treated with either acetone or 280 nmol of indomethacin in acetone 2 hr before treatment with either 5 nmol of TPA or 5 nmol of TPA containing various doses of prostaglandin. Mice were killed 4.5 hr after the last treatment. ODC activity from the soluble epidermal extracts was measured at 400 μM L-Ornithine concentration. Each value represents the mean ± S.E. of determinations carried out on 3 groups of mice with 3 mice/group.

Table 7

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (nmol)</th>
<th>Pretreated with acetone</th>
<th>Pretreated with indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7.00 ± 1.29</td>
<td>2.21 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>PGE2</td>
<td>25</td>
<td>8.51 ± 1.36</td>
<td>4.09 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>8.63 ± 1.26</td>
<td>7.43 ± 1.43</td>
</tr>
<tr>
<td>PGD2</td>
<td>25</td>
<td>8.93 ± 0.55</td>
<td>3.03 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6.96 ± 0.73</td>
<td>5.97 ± 0.67</td>
</tr>
<tr>
<td>6,9-Thio-PGI2</td>
<td>25</td>
<td>8.16 ± 0.34</td>
<td>5.43 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>8.55 ± 1.63</td>
<td>8.22 ± 1.76</td>
</tr>
<tr>
<td>6-Keto-PGF1α</td>
<td>25</td>
<td>6.72 ± 0.94</td>
<td>2.01 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.12 ± 0.09</td>
<td>3.39 ± 0.31</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>100</td>
<td>7.72 ± 1.07</td>
<td>2.18 ± 0.06</td>
</tr>
</tbody>
</table>

Effect of Indomethacin Pretreatment on Accumulation of Prostaglandins by TPA

The possibility that indomethacin may inhibit the induction of ODC activity by inhibiting the accumulation of prostaglandins was examined, and the results are shown in Table 9. In this experiment, mice were treated with either acetone or 280 nmol of indomethacin 2 hr prior to application of 17 nmol TPA. Mice were killed at various times following TPA treatment, and PGE or PGF was quantitated by radioimmunoassay. A 2-fold increase in PGE levels was detected as early as 4 hr following TPA treatment, and a peak level was observed between 6 and 14 hr. PGF levels were also increased at 4, 6, and 14 hr after TPA treatment. Indomethacin pretreatment completely in-
Course of the effect of tumor-promoting phorbol esters on PGE

The time points examined. Recently, a more detailed time

ornithine concentration. Each value represents the mean ± SE. of
determinations carried out on 3 groups of mice with 3 mice/group.

Effect of indomethacin treatment on the formation of skin papillomas

Prostaglandins and Epidermal ODC Induction

The inhibition of formation of skin papillomas was not due to toxicity. The average weight of mice

treated with indomethacin did not differ from that of controls. Repeated applications of indomethacin at a 560-nmol dose were toxic. Similar results have been observed by others (39).

Effect of Indomethacin on Formation of Skin Papillomas

In order to establish further the correlation between induction of epidermal ODC activity and skin tumor promotion by TPA, the effect of indomethacin treatment on the formation of skin papillomas was investigated, and the results of the first experiment are summarized in Table 10. In this experiment, mice were initiated with 0.2 μmol of DMBA in 0.2 ml of acetone. Two weeks after initiation, mice were treated twice a week with either 0.2 ml of acetone or 280 nmol of indomethacin in 0.2 ml of acetone 2 hr before each application of 5 nmol of TPA for the tumor induction experiment and of 8 nmol of TPA for determination of ODC and S-adenosylmethionine decarboxylase activities. Mice were killed 4.5 and 18 hr after the 11th application of TPA for determination of ODC and S-adenosylmethionine decarboxylase activities, respectively. Each value represents the mean ± S.E. of determinations of enzyme activities from 6 individual mice. Incidence of skin papillomas was recorded after the 20th week of promotion, and there were 30 mice in each treatment group.

Table 8

Effect of PGE2 on indomethacin- or retinoic acid-caused inhibition of the induction of ODC activity

Groups of mice were treated with acetone, 280 nmol of indomethacin, or 0.17 nmol of retinoic acid 2 hr prior to treatment with 5 nmol of TPA or 5 nmol of TPA containing 70 or 140 nmol of PGE2. Mice were killed 4.5 hr after the last treatment, and soluble epidermal ODC activity was measured at 400 μM L-ornithine concentration. Each value represents the mean ± S.E. of determinations carried out on 3 groups of mice with 3 mice/group.

Table 9

Effect of indomethacin on TPA-stimulated epidermal PGE and PGF levels

Groups of mice were treated with either 0.2 ml of acetone or 280 nmol of indomethacin in 0.2 ml of acetone 2 hr prior to application of 17 nmol of TPA. Mice were killed at the indicated times. Epidermal PGE and PGF were quantitated by radioimmunoassay (see “Materials and Methods”). Each value is the mean of 4 determinations carried out on 4 mice (variation, <15%).

Table 10

Effect of indomethacin pretreatment on the induction of ODC and S-adenosylmethionine decarboxylase activities and formation of skin papillomas

All mice were initiated with 0.2 μmol of DMBA in 0.2 ml of acetone. Two weeks after initiation, mice were treated twice a week with either 0.2 ml of acetone or 280 nmol of indomethacin in 0.2 ml of acetone 2 hr before each application of 5 nmol of TPA for the tumor induction experiment and of 8 nmol of TPA for determination of ODC and S-adenosylmethionine decarboxylase activities. Mice were killed 4.5 and 18 hr after the 11th application of TPA for determination of ODC and S-adenosylmethionine decarboxylase activities, respectively. Each value represents the mean ± S.E. of determinations of enzyme activities from 6 individual mice. Incidence of skin papillomas was recorded after the 20th week of promotion, and there were 30 mice in each treatment group.

Chart 6. Effect of indomethacin treatment on formation of skin papillomas. All mice were initiated with 0.2 μmol of DMBA and 2 weeks later were treated twice a week with 0.2 ml of acetone ( ), 28 nmol of indomethacin ( ), or 280 nmol of indomethacin ( ) in 0.2 ml of acetone 5 hr before application of 8 nmol of TPA for the duration of the experiment. There were 30 mice/cage, and the incidence of papillomas was observed weekly.
DISCUSSION

Prostaglandins are naturally occurring cyclic metabolites of unsaturated fatty acids that have numerous physiological functions (10, 27, 28, 31, 32). A number of prostaglandins in tissues of various species are biosynthesized from their precursor, unsaturated fatty acids present in cell membranes in the form of phospholipids (Chart 5). Biosynthesis of PGE2 and PGF2 from the precursor, arachidonic acid, has been shown in the skin of various species including mice and humans. The prostaglandin synthetase is mostly microsomal (14, 41) and is present in the epidermis (40). Prostaglandins are implicated as mediators of cutaneous inflammation as well as various pathological skin conditions (9, 10, 28). Injections of PGE2 and PGF2\textsubscript{\alpha} appeared to enhance formation of squamous cell carcinoma in mice treated with 3-methylcholanthrene (18). Elevated levels of prostaglandins are found in various tumors (13). Addition of carcinogens or tumor-promoting phorbol diesters to cultured dog kidney cells (MDCK) stimulates the release of prostaglandins in the medium (11, 12, 15, 16), and a correlation has been shown to exist between irritant and promoting activity in mouse skin and PGE2 release by macrophages by a series of tumor promoters (3). Furthermore, tumor promoters cause an enhanced synthesis of epidermal phospholipids (29) and accumulation of PGE and PGF levels, and the activities of various phorbol esters for increasing PGE levels paralleled their tumor-promoting activity (1). Now, we report that prostaglandins may play a crucial role in TPA-induced ODC activity, an enzyme involved in mouse skin tumor promotion (2, 22, 38).

The results presented indicate that mouse skin treated with indomethacin, naproxen, flufenamic acid, or acetylsalicylic acid (prostaglandin synthesis inhibitors) before TPA treatment inhibited the induction of epidermal ODC activity (Table 1). It is highly unlikely that the inhibitory effect of these agents on enzyme induction is the result of general cytotoxicity. Application of indomethacin affected neither normal epidermal protein nor DNA synthesis (Table 2), nor did it inhibit the induction of S-adenosylmethionine decarboxylase (Table 3) or cyclic AMP phosphodiesterase (Table 4), the other enzymes known to be induced by TPA treatment to mouse skin (21, 23, 35). It is more probable that the suppression of enzyme induction by the nonsteroidal antiinflammatory agents is the result of either a direct inhibition of prostaglandin synthetase or the release of prostaglandins (6, 33). Interestingly, the ability of prostaglandin synthetase inhibitors to inhibit the induction of ODC activity correlates with their ability to inhibit prostaglandin synthetase in vitro systems (6). However, we did not measure the activity of prostaglandin synthetase in mouse epidermis.

Convincing evidence strengthening the role of prostaglandins in ODC induction is the observation that the inhibition of the induction of ODC activity by the inhibitors of prostaglandin synthesis was overcome by application of prostaglandins concurrently with TPA. PGE\textsubscript{1}, PGE\textsubscript{2}, PGD\textsubscript{2}, and 6,9-thio-PG\textsubscript{2} were effective whereas PGF\textsubscript{1\alpha}, PGF\textsubscript{2\alpha}, 6-keto-PGF\textsubscript{1\alpha}, and arachidonic acid were inactive in their ability to counteract the inhibition of the induction of ODC activity by indomethacin (Table 7; Ref. 37). These results suggest that indomethacin may inhibit the production of those prostaglandins that modulate ODC induction by TPA. This possibility was strengthened by the finding that TPA treatment led to an enhanced accumulation of PGE levels as early as 4 hr following TPA treatment (Table 9). Accumulation of PGE by TPA could be blocked by pretreatment with indomethacin, suggesting that inhibition of the induction of ODC activity by indomethacin may involve the inhibition of an increase in PGE levels by TPA that may play a role in ODC induction. However, a detailed analysis of accumulation of all prostaglandins (Chart 5) following TPA treatment was not obtained. Furthermore, it remains to be determined in mouse epidermis whether enhanced PGE levels are specific for promoters (1) or can be increased following nonpromoting hyperplastic agents and whether the antipromoting activity of retinoic acid (38) and dexamethasone (39) involve their ability to affect TPA-enhanced accumulation of prostaglandins.

Although the data suggest that prostaglandins may mediate ODC induction by TPA in mouse epidermis, application of those prostaglandins alone, which counteracted indomethacin inhibition, did not induce epidermal ODC activity. However, in some experiments, application of certain prostaglandins with TPA potentiated the induction of ODC activity by TPA (Chart 4). These findings suggest that prostaglandins may be necessary but are not sufficient for ODC induction by TPA. TPA must release some other factors in addition to prostaglandins which are important for induction of ODC activity by TPA. However, addition of PGE\textsubscript{2} alone has been shown to induce ODC activity in cultured cells (26).

Application of PGE\textsubscript{2} alone could not promote skin tumor formation in an initiation/promotion experiment (data not shown). In contrast, Lupulescu (18) reported that PGE\textsubscript{2} when given i.m. concomitantly with 3-methylcholanthrene 3 times a week enhanced formation of skin carcinomas in male albino Swiss mice.

The data showing that prostaglandins alone can neither induce ODC activity nor promote skin tumor formation imply that the relationship between prostaglandins and promotion is complex. However, the findings that indomethacin treatment before application of TPA inhibits the accumulation of prostaglandins, the induction of ODC activity, and the formation of skin papillomas strongly suggest that prostaglandins play a role in ODC induction as well as skin tumor promotion. The exact biological role of prostaglandins during skin tumor promotion remains speculative.

ACKNOWLEDGMENTS

We thank Hope Rice, Barbara Shapas, and Tim O’Neil for expert technical assistance as well as The Upjohn Company for a generous supply of prostaglandins.

REFERENCES

Prostaglandins and Epidermal ODC Induction


Inhibition by Prostaglandin Synthesis Inhibitors of the Induction of Epidermal Ornithine Decarboxylase Activity, the Accumulation of Prostaglandins, and Tumor Promotion Caused by 12-O-Tetradecanoylphorbol-13-acetate

Ajit K. Verma, Curtis L. Ashendel and R. K. Boutwell


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/40/2/308

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.