Induction of Plasminogen Activator and Prostaglandin Biosynthesis in HeLa Cells by 12-O-Tetradecanoylphorbol-13-acetate

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ABSTRACT

Recent reports suggest that many of the biological effects of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate are mediated via intracellular prostaglandin biosynthesis. We have investigated whether the induction of plasminogen activator by 12-O-tetradecanoylphorbol-13-acetate in cultured HeLa cells is similarly mediated. 12-O-Tetradecanoylphorbol-13-acetate (0.5 to 50 nm) increased intra- and extracellular plasminogen activators and stimulated E- and F-type prostaglandin production. Changes in prostaglandin biosynthesis preceded those in plasminogen activator by several hr. Indomethacin (0.5 μM) abolished prostaglandin production but had no effect on either the magnitude or the time course of induction of plasminogen activator. Similar results were obtained with human skin fibroblasts and MDCK cells. Prostaglandins E1, E2, F2α, and I2 had no direct effect on plasminogen activator in HeLa cells or skin fibroblasts. We conclude that in these cells, phorbol ester independently induces plasminogen activator and prostaglandin biosynthesis.

INTRODUCTION

Tumor-promoting phorbol esters induce in cultured cells several changes resembling those seen in cells transformed by chemical carcinogens or viruses. Although the mechanisms underlying tumor promotion are not fully understood, recent studies have suggested that PGs3 may participate in this phenomenon. The most potent phorbol ester, TPA, in low concentrations stimulates PG biosynthesis in a canine kidney cell line (10). Increased PG biosynthesis apparently mediates the effects of TPA on ornithine decarboxylase, epidermal cell proliferation, and bone resorption (2, 14, 16). PG production is often increased in transformed cells and tumor tissues (12, 17), and PG’s can act directly as tumor promoters (7). On the other hand, TPA can induce morphology changes in a canine kidney cell line independently of PG production (10). In several cell lines including HeLa, TPA stimulates the synthesis of plasminogen activator, a protease which may contribute to tumor invasiveness by facilitating hydrolysis of surrounding tissue (5, 6, 18). No data are available on the effect of TPA on PG levels in these cells. PG’s of the E series have been reported to stimulate plasminogen activator in cells obtained from mouse neuroblastoma (4), rat ovarian granulosa (13), and human embryonic lung (11). We therefore tested the hypothesis that the induction of plasminogen activator by TPA is mediated via PG’s. Our results show that although TPA does indeed stimulate PG synthesis in the same doses which induce plasminogen activator, the 2 processes occur independently since TPA still induces plasminogen activator under conditions where PG biosynthesis is inhibited.

MATERIALS AND METHODS

HeLa and MDCK cells were obtained from the American Type Culture Collection, Rockville, Md. Human skin fibroblasts were derived from minced human foreskins by digestion with 0.2% collagenase for 2 hr at 37°. Cells were seeded at 10⁵ cells/35-mm dish and grown to at least 85% confluence by using Eagle’s minimal essential medium (HeLa and MDCK) or Medium 199 (fibroblasts). Media were supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, streptomycin (50 μg/ml), and penicillin (50 units/ml). Incubations were performed in triplicate or quadruplicate. The growth medium was removed and replaced by 3 ml of serum-free medium containing TPA in 0.1% dimethyl sulfoxide. In some experiments, cells were preincubated for 1 hr with indomethacin in serum-free medium containing 0.1% ethanol. The medium was removed and replaced with fresh serum-free medium containing the same concentration of indomethacin plus TPA. Neither dimethyl sulfoxide nor ethanol at the concentration used affected the cells. After incubation with TPA, the medium was removed and centrifuged for 5 min at 1000 × g to sediment detached cells. The supernatant was stored at −20° until assayed. Cells were washed twice with ice-cold Saline A (Grand Island Biological Co., Grand Island, N. Y.) containing 20 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid, pH 7.4, scraped into the same buffer and sedimented by centrifugation. The pellet was extracted with 2 ml 0.1 M Tris buffer (pH 8.1) containing 0.1% Triton X-100. Cell extracts were usually assayed immediately for fibrinolytic activity. Protein was estimated by a dye-binding assay (Bio-Rad Laboratories, Richmond, Calif.).

Fibrinolytic activity was measured as described (11, 18) by using 35-mm Falcon dishes coated with 125I-labeled fibrin, 100,000 cpm/dish. Assays contained 2 μg human plasminogen purified by affinity chromatography (1), suitable aliquots of cell extract or media, and 0.1 M Tris buffer (pH 8.1) in a final volume of 0.5 ml. Each sample was assayed at 2 dilutions and at 3 incubation times. Solubilized 125I-labeled fibrin degradation products from the incubation mixture were measured by liquid scintillation techniques. Fibrinolytic activity was calculated by comparison with a urokinase standard curve and expressed as Ploug units of urokinase per mg of cell protein. Omission of plasminogen abolished fibrinolytic activity. None of the compounds used in the study directly affected the fibrinolytic assay.

PG’s in the cell media were assayed serologically without further extraction using commercial radioimmunoassay kits.

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2The abbreviations used are: PG, prostaglandin; TPA, 12-O-tetradecanoylphorbol-13-acetate; PGF2α, prostaglandin F2α; PGE1, prostaglandin E1; PGE2, prostaglandin E2; PGE3, prostaglandin E3; PGF, prostaglandin F; PGH2, prostaglandin H2; PGE1, prostaglandin I2; PGE2, prostaglandin I3; PGE3, prostaglandin I4.

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(Clinical Assays, Cambridge, Mass.). The kits permit direct assay of PGF$_{2\alpha}$ using anti-PGF$_{2\alpha}$ antiserum and assay of PGE$_2$ after prior conversion to prostaglandin B$_2$ by alkaline treatment (17) using an anti-prostaglandin B$_1$ antiserum. The anti-PGF$_{2\alpha}$ antiserum cross-reacts less than 0.01% with PGF$_{2\alpha}$, antiserum cross-reacts 0.02% with PGE$_2$, and the anti-prostaglandin B$_1$ antiserum cross-reacts less than 0.01% with PGF$_{2\alpha}$, but the antisera do not identify the PG's as monoenoic or dienoic. Results are therefore expressed as ng/ml PGE or PGF. Each sample was assayed in at least 2 dilutions. Neither TPA nor indomethacin in the concentrations used directly affected the binding of PG's to their appropriate antisera.

All materials for preparation of cell culture media were obtained from Grand Island Biological Co. TPA, indomethacin, and urokinase were obtained from Sigma Chemical Co., St. Louis, Mo. Collagenase, type CLS, was obtained from Worthington Biochemical Corp., Freehold, N. J.

RESULTS AND DISCUSSION

TPA in concentrations of 0.5 to 50.0 nM produced dose-dependent stimulation of plasminogen activator in HeLa cells (Chart 1). Approximately equal fibrinolytic activity was detected in the cell media, and changes in media activity generally followed those seen in cell lysates (data not shown). Serological examination of the cell media revealed that resting cells produced PGE and lesser amounts of PGF. Although the antisera used do not identify PG's as monoenoic or dienoic, the relative scarcity of eicosatetraenoic acid, the fatty acid precursor of monoenoic PG's, makes it likely that the PG's detected were PGE$_2$ and PGF$_{2\alpha}$. TPA stimulated PG biosynthesis in the same concentrations at which induction of plasminogen activator occurred (Chart 1). Plasminogen activator after 18 hr was linearly related to PGE ($r = 0.919$) and PGF ($r = 0.884$) over these concentrations of TPA. These results are consistent with, but of course do not prove, the hypothesis that plasminogen activator induction is mediated via PG's.

Examination of the time course of the responses to TPA revealed that increases in both PGE and PGF could be detected 1 hr after the addition of TPA. In contrast, increases in plasminogen activator were not detected until 6 hr after the addition of TPA (Chart 2). This again is consistent with the hypothesis that PG biosynthesis precedes and may mediate the induction of plasminogen activator.

The hypothesis was then tested directly in studies with indomethacin, which blocks PG synthesis by inhibiting cyclooxygenase (15). Pretreatment of cells with 0.5 µM indomethacin abolished the biosynthesis of PGE and PGF in response to TPA (Chart 2). However, indomethacin had no effect on either the magnitude or the kinetics of the induction of plasminogen activator during 12 hr of incubation with TPA. Inhibition of cyclooxygenase by indomethacin appeared to be complete at the time of the addition of TPA since medium added to indomethacin-treated cells and then immediately withdrawn (Chart 2, 0 point) contained no detectable PG's, whereas corresponding medium from untreated cells had low but detectable levels of both PGE and PGF. Moreover, at no time during the 12-hr incubation period with TPA was PG biosynthesis detected in indomethacin-treated cells. Indomethacin in concentrations of up to 1 µM also had no effect on the induction of plasminogen activator by longer (18 hr) incubation with TPA (Chart 3). In contrast, PG biosynthesis was significantly inhibited by 10 nM indomethacin, and virtually complete inhibition occurred at 100 nM.

Our studies with PGE and PGF do not exclude the possible participation of other more active cyclooxygenase derivatives such as thromboxane A$_2$ or PGI$_2$. However, the measurement of PGE$_2$ would seem to be a good indication of cyclooxygenase activity.
Plasminogen Activator and PG’s in HeLa Cells

Effects of indomethacin on TPA-induced plasminogen activator and PG biosynthesis in human skin fibroblasts and canine kidney (MDCK) cells. Cells were incubated for 1 hr with 0.5 μM indomethacin (fibroblasts) or 1 μM indomethacin (MDCK) and then incubated for 18 hr with indomethacin plus 5 nM TPA. After incubation, cell medium was assayed for PG’s and cell lysates were assayed for fibrinolytic activity.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Addition</th>
<th>Plasminogen activator (Pn. units/mg protein)</th>
<th>PGE (ng/ml)</th>
<th>PGF (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast</td>
<td>None</td>
<td>1.6 ± 0.2a</td>
<td>0.10 ± 0.03</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>TPA</td>
<td>3.0 ± 0.4</td>
<td>8.80 ± 0.99</td>
<td>4.42 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>TPA + indomethacin</td>
<td>2.8 ± 0.4</td>
<td>0.07 ± 0.01</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>MDCK</td>
<td>None</td>
<td>1.0 ± 0.4</td>
<td>&lt;0.06c</td>
<td>NTd</td>
</tr>
<tr>
<td></td>
<td>TPA</td>
<td>13.7 ± 1.1</td>
<td>20.40 ± 1.00</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>TPA + indomethacin</td>
<td>12.3 ± 1.8</td>
<td>&lt;0.06c</td>
<td>NT</td>
</tr>
</tbody>
</table>

a Mean ± S.E. of triplicate determinations.
b Significantly different from WA treatment, p < 0.01 as determined by Student’s t test.
c Lower limit of detection.
d NT, not tested.

Table 1

Chart 3. Effects of indomethacin on plasminogen activator and PG biosynthesis following 18 hr of incubation with TPA. HeLa cells were preincubated for 1 hr with various concentrations of indomethacin or vehicle and then incubated for 1 hr with the same concentration of indomethacin plus 5 nM TPA. O, plasminogen activator; □, PGE; △, PGF. Each point, mean ± S.E. of quadruplicate incubations.

activity since PGE2 production is widespread in many tissues and PGE2 can be derived both enzymically and nonenzymically from the intermediate PG endoperoxides (3, 9). The virtually complete inhibition of PGE2 production by indomethacin therefore suggests that the production of other cyclooxygenase derivatives was similarly inhibited.

Our results indicate that although HeLa cells do produce PG’s in response to TPA, such production is not essential to the induction of plasminogen activator by the phorbol ester. This conclusion does not seem to pertain solely to the transformed HeLa cell line since a similar conclusion can be drawn from plasminogen activator studies performed with a normal (human skin fibroblast) and an established (canine kidney MDCK) cell line (Table 1). Moreover, during the completion of these studies, Mufson et al. (8) have reported that PG’s do not mediate TPA-induced changes in plasminogen activator in chick embryo fibroblasts.

Finally, an experiment to test the direct effect of PG’s on plasminogen activator production was performed. No changes in plasminogen activator were observed following 18-hr incubation of HeLa cells or human skin fibroblasts with PG’s E1, E2, F2α (0.3 μM), or following 3- and 18-hr incubation with PGFs (1 μM) (data not shown).

In summary, although reports in the literature suggest that several of the biological effects of tumor-promoting phorbol ester are mediated via PG biosynthesis, this does not seem to be an obligatory pathway whereby phorbol esters induce plasminogen activator.

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