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PHORBOL ESTER INDUCED PROSTAGLANDIN SYNTHESIS AND [^{3}H]-TPA METABOLISM BY TPA-SENSITIVE AND TPA-RESISTANT FRIEND ERYTHROLEUKEMIA CELLS

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SUMMARY: The potent mouse skin tumour promoter, 12-O-tetra-decanoyl phorbol-13-acetate (TPA), causes a release of arachidonic acid and prostaglandins E_2 and $F_{2\alpha}$ from Friend erythroleukemia cells (FELC). This effect is not seen with a subclone which is resistant to both TPA-induced inhibition of differentiation and TPA-induced adhesion. TPA-sensitive and TPA-resistant clones metabolize [^{3}H]-TPA at the same rate to phorbol-13-monoacetate (P(13)A). P(13)A has no effect on differentiation or adhesion of FELC. Together with previous findings, these results suggest that TPA-induced changes in membrane lipid metabolism are closely linked to its effects on growth, differentiation and adhesion. The resistance of a subclone of FELC to TPA appears to be at the level of membrane action rather than the metabolism of TPA.

A potent mouse skin tumour promoter, 12-O-tetra-decanoyl phorbol-13-acetate (TPA), inhibits differentiation in a variety of cell culture systems (1-8). This finding has reinforced the hypothesis that tumours may arise from aberrant differentiation and the speculations by Berenblum that the tumour promotion step in mouse skin is a result of retardation of epidermal cell maturation caused by tumour promoters (9).

Friend erythroleukemia cells (FELC) provide a well defined system for studies on the mechanism of the reversible inhibitory effect of TPA on terminal cell differentiation (1,2,4). FELC clones resistant to TPA-mediated inhibition of differentiation have recently been isolated and compared with respect to several properties to TPA-sensitive FELC clones (2,10). Whereas TPA-sensitive

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clones exposed to TPA showed transient growth inhibition, induction of cell adhesion to the culture dishes, induction of plasminogen activator and reversible inhibition of differentiation (1,2,10,11), none of these effects were found with TPA-resistant clones (2,10,11). Since the existence of TPA-sensitive and TPA-resistant clones of FELC provides a valuable system for elucidating the mechanism of TPA-mediated inhibition of cell differentiation, further characterization of these clones is in progress. In the present communication, we report the effect of TPA on the release of [^{3}H]-arachidonic acid and [^{3}H]-prostaglandin synthesis and compare the metabolism of [^{3}H]-TPA in these clones.

MATERIALS AND METHODS

[20-^{3}H]-TPA (8 Ci/mmole), phorbol-13-monoacetate and 12-O-tetra-decanoyl phorbol were obtained from Dr P. Borchert, University of Minnesota, Minneapolis, Minn. TPA and phorbol were purchased from Consolidated Midland Corp., Brewster, N.Y. [^{3}H]-Arachidonic acid (60-100 Ci/mmole) was purchased from the New England Nuclear Company, Boston, Mass. Arachidonic acid and prostaglandins (PG) E_2 and $F_{2\alpha}$ for thin layer chromatography (TLC) standards were purchased from Sigma Chemical Co., St Louis, Mo.

Two subclones (TS19-10 and TR19-9) isolated from FELC clone DS19 were employed for the experiments. FELC clone DS19 was isolated from strain 745A (12), which was a generous gift from Dr C. Friend. TS19-10 is one of the most sensitive clones of FELC in response to the action of TPA and TR19-9 is resistant to TPA (2,10). Cells were maintained in suspension culture in Eagle's basal medium, supplemented with 10% fetal calf serum, and were transferred twice weekly by diluting the cells to approximately 10^5/ml of fresh medium. For experiments, one-day old cultures of FELC were used.

Labelling of the cells with [^{3}H]-arachidonic acid and the analysis of subsequently released arachidonic acid metabolites by thin layer chromatography were carried out as previously described (13).

[^{3}H]-TPA metabolism was measured by incubating one-day old cultures of FELC at a concentration of 2 x 10^5 cells/ml with [^{3}H]-TPA (100 ng/ml). Aliquots of the cell suspension were taken at the indicated time intervals and TPA and the metabolites were extracted with chloroform:methanol:water (14). The chloroform phase was evaporated to dryness under N_2 gas and redissolved in a small volume of acetone. This material was then analysed by thin layer chromatography (TLC) and by high pressure liquid chromatography (HPLC). TLC plates were developed in ethyl ether three times in order to obtain good separation. They were then cut and counted in a liquid scintillation counter. Authentic TPA, phorbol, 12-O-tetra-decanoyl phorbol and phorbol-13-monoacetate were used as reference compounds and were visualized with a UV lamp. HPLC was done on a Zorbax ODS column and a methanol solvent at 50 ^{o}C on a Dupont model 830 instrument.

Differentiation induced by hexamethylene bisacetamide (HMBA) and adhesion of FELC to tissue culture dishes in the presence or absence of TPA and its derivatives were measured as previously described (1,11). All assays were done in duplicate and all experiments were repeated at least twice, with variations of less than 10% between duplicates.

RESULTS AND DISCUSSION

[^{3}H]-Arachidonic acid release

The effect of TPA on the release into the medium of radioactivity from cells previously labelled with [^{3}H]-arachidonic acid by TPA-sensitive (TS19-10) and -resistant (TR19-9) FELC clones is shown in Fig. 1. In the absence of TPA, there was a gradual and similar rate of release in TS19-10 and TR19-9. In the presence of TPA there was a 2-3 fold stimulation of release of radioactive

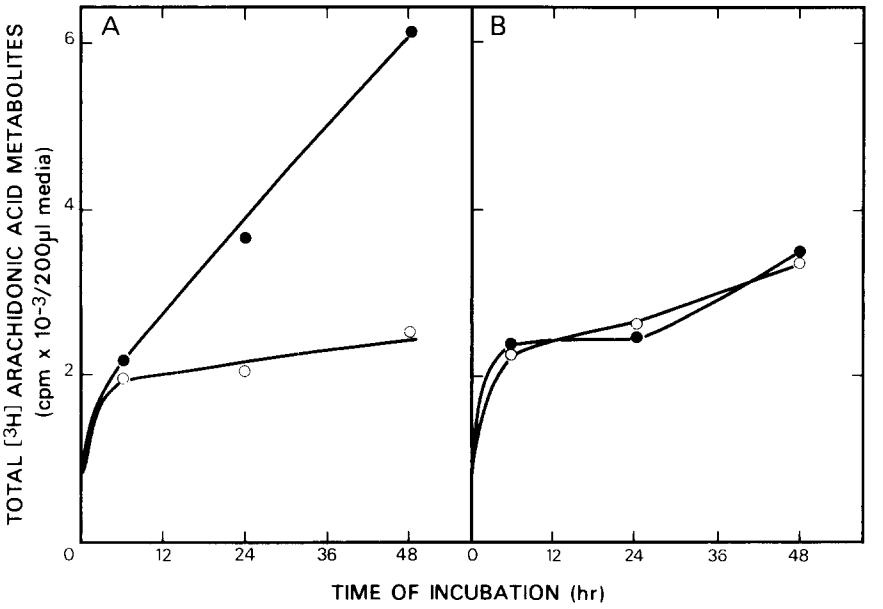

Fig. 1

Release of [^{3}H]-arachidonic acid metabolites by TPA-sensitive and TPA-resistant FELC. One-day old cultures of FELC (2 x 10^5 cells/ml) were incubated with [^{3}H]-arachidonic acid (5 µCi/ml) for 24 hr and were then washed three times with culture medium. These prelabelled cells were then resuspended in culture medium at 2 x 10^5 cells/ml and TPA was added at a concentration of 30 ng/ml or .01% DMSO. At the indicated time, an aliquot of the cell suspension was taken and centrifuged. Two hundred µl of the supernatant were subjected to liquid scintillation counting. A, TS19-10 cells; B, TR19-9 cells; O, control culture; ●, TPA-treated culture.

Table 1: Analysis of radioactivity released in cultures pretreated with [^{3}H]-arachidonic acid.

Cells	Additions	[^{3}H] cpm/200 µl		
		Arachidonic acid	PGE_2	$PGF_{2\alpha}$
TS19-10	Control	778 ± 55	41 ± 5	60 ± 4
	TPA	2062 ± 82	90 ± 5	149 ± 11
TR19-9	Control	1110 ± 60	39 ± 5	68 ± 5
	TPA	1268 ± 99	52 ± 2	98 ± 5

The culture media were collected after a 48 hr inoculation in the absence or presence of TPA (30 ng/ml) or .01% DMSO, as described in Fig. 1, and were analysed by TLC as previously described (13).

material in TS19-10 cells but no stimulation was seen with TR19-9 cells. The stimulation was detectable as from 24 hr after the addition of TPA and continued for at least another 24 hr. The kinetics of the TPA-induced release in FELC are quite different from those in other cell lines, where TPA-induced arachidonic acid release is detectable within 60 min and maximal at 3-6 hr (13, 15-17)

When released radioactivity was analysed by TLC, most (> 86%) of it was free arachidonic acid. Low, but significant, amounts of PGE_2 and $PGF_{2\alpha}$ were detected (Table 1). TPA stimulated not only the release of free arachidonic acid, but also caused 2-3 fold stimulation in the synthesis of PGE_2 and $PGF_{2\alpha}$ in TS19-10. However, TR19-9 showed little or no stimulation of arachidonic acid release or prostaglandin synthesis in the presence of TPA (Table 1). The induction of arachidonic acid release and prostaglandin synthesis by TPA was originally reported in a dog kidney cell line (15) and subsequently extended to mouse peritoneal macrophages (17), chick embryo fibroblasts (13), the $10T\frac{1}{2}$ mouse fibroblast cell line (18) and now to FELC. Thus this effect on lipid metabolism by TPA appears to be a very general one, occurring in diverse cell types.

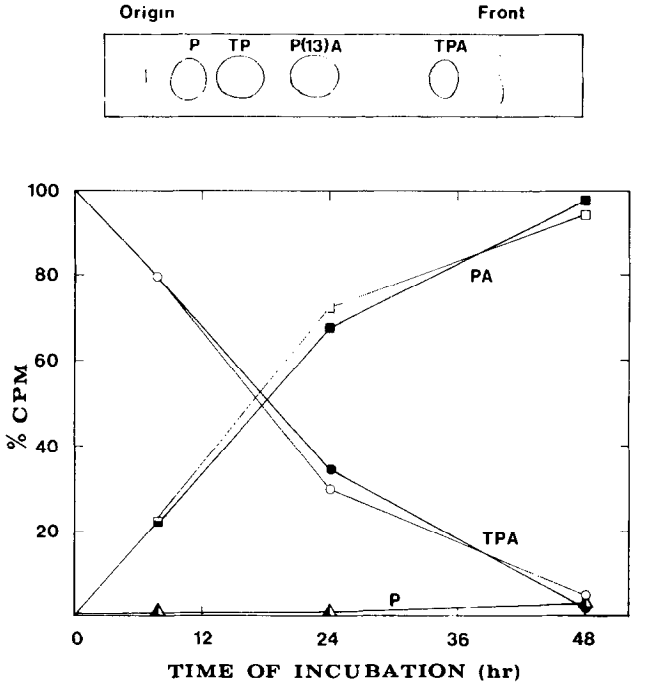

Fig. 2

Metabolism of [^{3}H]-TPA by TPA-sensitive and TPA-resistant FELC. Cells were incubated with [^{3}H]-TPA for the indicated period of time and the metabolites were extracted and analysed as described in Methods. The upper figure shows the relative mobility of authentic phorbol (P), 12-O-tetra-decanoyl phorbol (TP), phorbol-13-monoacetate (P(13)A) and TPA on TLC. The lower figure illustrates the time course of disappearance of TPA (O,●), appearance of PA (□,■) and P (△,▲). Open symbols are for TS19-10 and closed ones for TR19-9 cells.

[^{3}H]-TPA metabolism

To determine whether the resistance of TR19-9 cells reflected an alteration in TPA metabolism or an altered response of cellular targets to TPA, the TPA-resistant clone (TR19-9) and the TPA-sensitive clone (TS19-10) were incubated with [^{3}H]-TPA for 48 hr and at various time points the metabolites were analysed by TLC. Fig. 2 shows the rate of disappearance of [^{3}H]-TPA and the appearance of [^{3}H]-phorbol-13-monoacetate and [^{3}H]-phorbol in TS19-10 and TR19-9 FELC. This result clearly shows that both TS19-10 and TR19-9 metabolize [^{3}H]-TPA at very similar rates and that with both clones the major metabolite is phorbol-13-monoacetate. The metabolites were further analysed by HPLC and compared to authentic markers. The major metabolite was confirmed to be phorbol-13-monoacetate; no other TPA metabolites were detected.

Table 2: Effect of TPA, its monoesters or phorbol on the differentiation and adhesion of FELC.

Compound (100 ng/ml)	% Inhibition of differentiation	% Cells attached to culture dish
TPA	94	88
Phorbol-13-monoacetate	< 1	< 1
12-O-Tetra-decanoyl phorbol	< 1	3
Phorbol	< 1	< 1

Cell differentiation was induced by 4 mM hexamethylene bisacetamide (1). Cell adhesion was determined quantitatively as previously described (2, 11). One-day old cultures of TS19-10 cells were used.

The biological activity of the major metabolite of TPA in FELC, phorbol-13-monoacetate, was also studied in the TS19-10 clone of FELC. As indicated in Table 2, phorbol-13-monoacetate (100 ng/ml) showed no inhibitory effect on HMBA induced erythroid differentiation and did not induce cell adhesion. Under the same conditions, TPA produced a 94% inhibition of differentiation and induced adhesion of 88% of the cells (Table 2). Two other known metabolites of TPA (19), phorbol and 12-O-tetradecanoyl phorbol, also failed to inhibit differentiation and did not induce adhesion of TS19-10 cells (Table 2). Therefore it is likely that in FELC, as in other cell types (19,20), TPA acts directly and does not require conversion to an active derivative. On the contrary, the metabolism of TPA leads to biologically inactive derivatives. We cannot, however, exclude the possibility that small amounts of "active" metabolites are produced, but escape detection because of their short life time and/or low concentration; however, this seems unlikely.

TPA-resistant clones of FELC were originally isolated for their resistance to TPA-mediated inhibition of cell differentiation (2,10). In subse-

quent studies, it was found that TPA-resistant cells are also resistant to certain other effects of TPA seen in TPA-sensitive FELC, including transient growth inhibition (2,10), induction of plasminogen activator (2) and induction of cell adhesion to tissue culture dishes (2,11). Our present results show that another rather general effect of TPA, induction of arachidonic acid release from membrane phospholipids and increased prostaglandin synthesis, is also absent in TPA-resistant FELC. The acquisition of TPA-resistance in these clones does not appear to be due to trivial mechanisms, such as decreased conversion of TPA to an active derivative, or increased conversion to inactive derivatives. A number of studies suggest that in various cell types the primary site of action of the phorbol esters is the cell membrane (for review see 21). These results, taken together with those described in the present study, suggest that the effect of TPA on growth and differentiation of FELC is closely coupled to the effects of TPA on membrane lipid metabolism.

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