

# Differential changes in lipid metabolism of myeloid and lymphoid cell lines induced by treatment with 12-*O*-tetradecanoylphorbol-13-acetate (TPA)

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Received 1 August 1990; revised version received 9 October 1990

Treatment of the myeloid cell lines, U-937 or HL-60, with 10 nM of the phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), for 24 h increased the rate of incorporation of [ $^3\text{H}$ ]glycerol into total chloroform extracts. A proportionally greater labeling of the non-polar lipid (NL) fraction compared to the polar, phospholipid (PL), fraction was observed. Chromatographic analysis showed a 6-fold increase in the labeling of triacylglycerols (TAG), a 2-fold increase in diacylglycerols, and no changes in monoacylglycerols. PL labeling showed a 3-fold increase in phosphatidylcholine (PC). The effect of TPA on TAG labeling was selectively observed in myeloid cell lines. No such a change was found in the lymphoid cell line, MOLT-3, which did respond to TPA with increased PC labeling. Incorporation of [ $^3\text{H}$ ]arachidonic acid (AA) into TAG by U-937 cells was selectively increased (2.5-fold) after treatment with TPA for 24 h. Treatment of U-937 cells with TPA in serum-free medium resulted in no increased labeling of TAG. These studies suggest that changes in TAG metabolism may be characteristic of myeloid differentiation and depend on the presence of serum factor(s).

Phorbol ester; Lipid metabolism; Cell differentiation

## 1. INTRODUCTION

12-*O*-Tetradecanoylphorbol-13-acetate (TPA), is an active tumor promoter and a potent inducer of monocyte-macrophage differentiation of myeloid cell lines [1–5]. A calcium and phospholipid (PL)-dependent protein kinase, protein kinase C, has been identified as a receptor for phorbol esters in a variety of cells [6], although the role of this kinase in myeloid cell differentiation is controversial [7–10]. Changes in lipid metabolism are amongst the metabolic effects of TPA described in several cellular models [11–15]. In an attempt to delineate metabolic changes associated with the myeloid differentiation process, we have studied the effect of TPA on lipid metabolism of two human myeloid cell lines and compared it with that of a human lymphoid cell line.

## 2. MATERIALS AND METHODS

### 2.1. Materials

All tissue culture media and fetal bovine serum (FBS) were obtained from Gibco (Grand Island, NY). TPA was obtained from CCR

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*Abbreviations:* AA, arachidonic acid; NL, non-polar lipids; PC, phosphatidylcholine; PL, phospholipids; TAG, triacylglycerols; TPA, 12-*O*-tetradecanoylphorbol-13-acetate

Research (Eden Prairie, MN), and stored at  $-20^\circ\text{C}$  in acetone at 1 mg/ml. [ $^3\text{H}$ ]Glycerol (spec. act. 58.2 Ci/mmol) and [ $^3\text{H}$ ]arachidonic acid (AA) (spec. act. 83.6 Ci/mmol) were from NEN Inc. (Boston, MA).

### 2.2. Cells

Human U-937 [16], HL-60 [17] and MOLT-3 [18] cell lines were grown in suspension culture in RPMI-1640 supplemented with glutamine (2 mM), penicillin (50 IU/ml), streptomycin (50  $\mu\text{g}/\text{ml}$ ), and 10% FBS (complete medium) at  $37^\circ\text{C}$  in a humidified, 5%  $\text{CO}_2$  incubator. TPA treatment was performed under this culture conditions.

### 2.3. Labeling and extraction procedures

TPA-treated or untreated cells were labeled with [ $^3\text{H}$ ]glycerol (40  $\mu\text{Ci}/\text{ml}$ ) or [ $^3\text{H}$ ]AA (5  $\mu\text{Ci}/\text{ml}$ ) for 1 h at  $37^\circ\text{C}$  in a shaking water bath. The reaction was stopped by adding 3 ml of chloroform/methanol (1:2 v/v) to the cell suspension. Cells labeled with [ $^3\text{H}$ ]AA were washed twice before the addition of the organic solvents. Partition into two phases was obtained by the addition of 0.5 ml of chloroform and 0.5 ml of 2.4 N HCl [19], and aliquots of the extracted lipids were counted for radioactivity. Non-polar lipids (NL) were further purified from total chloroform extracts by acetone precipitation of phosphatides and heptane/methanol partition [20,21].

### 2.4. Chromatographic separations

PL were analyzed by thin-layer chromatography (TLC) on silica-gel LK5DF plates (Whatman Chemical Separation Inc., Clifton, NY) impregnated with 2% boric acid, activated at  $110^\circ\text{C}$  for 1 h [22] and developed with chloroform/methanol/4 M  $\text{NH}_4\text{OH}$  (75:58:17 v/v/v). NL were separated on silica-gel LK6DF plates (Whatman), developed with hexane/diethylether/acetic acid (89:9.5:1.5 v/v/v) [20]. Authentic standards were included with the samples. Lipid bands were visualized with iodine vapors. Radiolabeled compounds were autoradiographed and analyzed by densitometry scanning. Alternatively, spots corresponding to the location of authentic standards were scraped and counted for radioactivity.

## 3. RESULTS

Upon treatment with TPA (10 nM) at 37°C for 3–5 days, the human promonocytic cell line, U-937, and the human promyelocytic cell line, HL-60, stained strongly for  $\alpha$ -naphthyl acetate esterase (data not shown), a marker of monocytic differentiation [23]. Treatment of U-937 cells with TPA for 24 h resulted in an increased rate of incorporation of [ $^3$ H]glycerol into cellular lipids as compared to untreated cells. Maximal stimulation was observed at 10 nM TPA; higher concentrations of TPA (up to 200 nM) did not further affect the response.

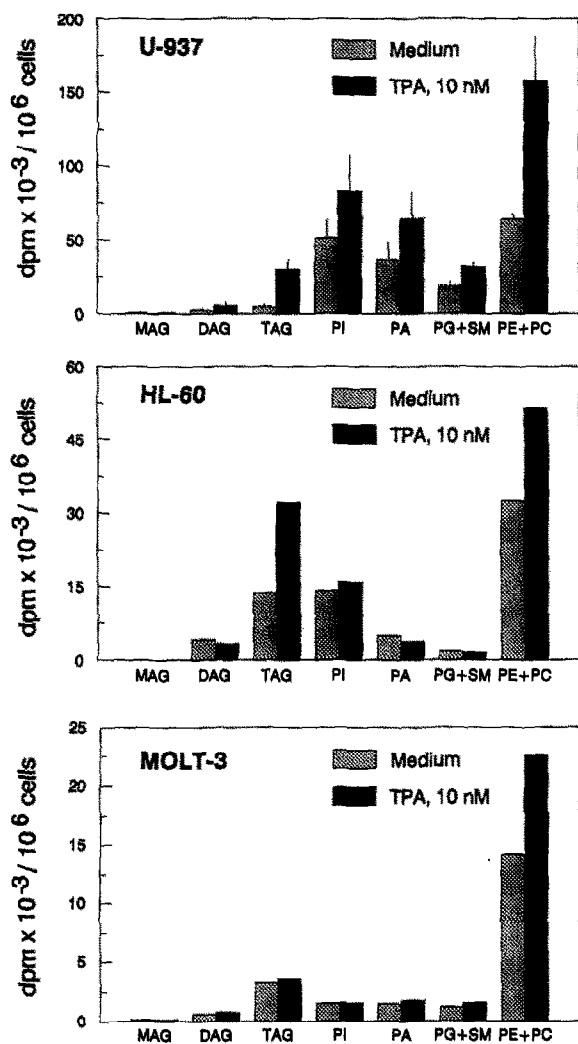


Fig. 1. Chromatographic analysis of [ $^3$ H]glycerol-labeled lipids from U-937, HL-60 and MOLT-3 cells. Cells were treated with or without 10 nM TPA for 24 h and labeled with [ $^3$ H]glycerol (40  $\mu$ Ci/ml) for 1 h at 37°C. Lipids were extracted and separated by TLC. The data represented are the mean  $\pm$  1 SD of 3 separate experiments for U-937 cells (upper panel), or the mean of two experiments for HL-60 cells (center panel) and MOLT-3 cells (lower panel). Individual experiments were performed in duplicate. Abbreviations: MAG, monoacylglycerols; DAG, diacylglycerols; TAG, triacylglycerols; PI, phosphatidylinositol; PA, phosphatidic acid; PG, phosphatidylglycerol; SM, sphingomyelin; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

The mean rate of incorporation of [ $^3$ H]glycerol into chloroform extracts was 163 670 dpm/ $10^6$  cells/h ( $\pm$  36 359 SD) for medium-treated cells and 374 827 dpm/ $10^6$  cells/h ( $\pm$  94 794 SD) for TPA (10 nM)-treated cells ( $P < 0.01$ , 2-sided Student's *t*-test for unpaired groups, 7 separate experiments). The amount of protein recovered did not differ between the two groups, ruling out the possibility that changes in cell recovery and/or cell dimension may contribute to the difference observed (data not shown).

Incorporation of the labeled precursors into all major lipid classes was enhanced to some extent by TPA-treatment; however, labeling was most increased in those compounds co-migrating with TAG and the combination of phosphatidylethanolamine + phosphatidylcholine (PC) standards (Fig. 1, top panel). Less than 5% of the total radioactivity co-migrated with phosphatidylethanolamine when a TLC system which resolved this PL from PC [20] was used (data not shown). Therefore, the radioactivity co-migrating with the combination of phosphatidylethanolamine + PC standard in the TLC system routinely used was for most part incorporated into PC, and will thereafter be referred to as PC labeling. Treatment with TPA of HL-60 cells resulted in a 1.5- and 0.5-fold increase in [ $^3$ H]glycerol incorporated into TAG and PC, respectively (Fig. 1, center panel). No differences were observed in the other lipids. Treatment of the human lymphoid cell line, MOLT-3 [18], resulted exclusively in a 0.6-fold increased labeling of PC, with no changes in the other lipids, including TAG (Fig. 1, lower panel).

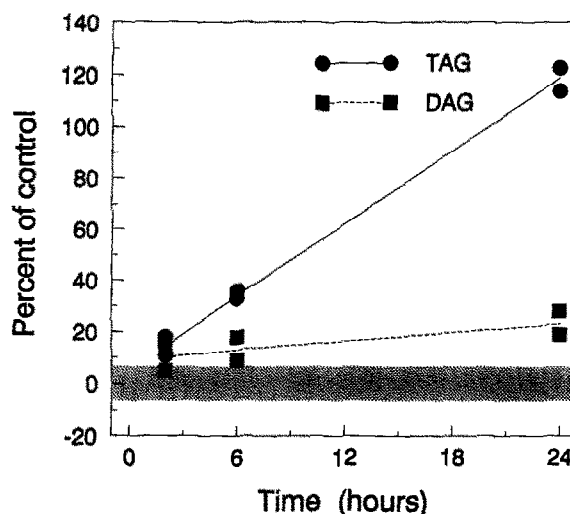


Fig. 2. Time dependence of the effect of TPA on the incorporation of [ $^3$ H]glycerol into NL from U-937 cells. Cells were treated with TPA (10 nM) for the indicated times and labeled with [ $^3$ H]glycerol (40  $\mu$ Ci/ml) for 1 h at 37°C. Diacylglycerols (DAG) and triacylglycerols (TAG) were separated by TLC. The results are represented as the mean percent variation of TPA-treated cells compared to medium-treated cells as control. The symbols represent individual values from two separate experiments; the lines connect the mean values. The shaded area represents the range of variation of the control values.

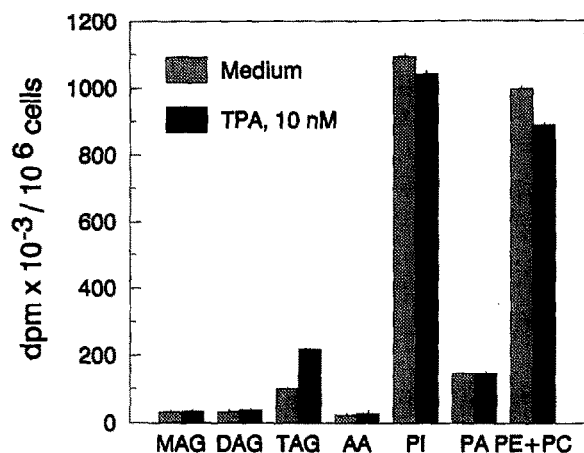


Fig. 3. Effect of TPA on incorporation of [<sup>3</sup>H]AA into cellular lipids from U-937 cells. Cells were treated with or without 10 nM TPA for 24 h and labeled with [<sup>3</sup>H]AA (5  $\mu$ Ci/ml) for 1 h at 37°C. Lipids were extracted and analyzed by TLC. The results represent the mean of two separate experiments. Lines above the bars represent the difference between the mean value and the value of the highest datum. Abbreviations as in Fig. 1.

The earliest metabolic change detected in U-937 cells was the incorporation of [<sup>3</sup>H]glycerol into TAG, which was increased 35% after 6 h of treatment of U-937 cells with TPA (Fig. 2). TAG labeling reached a 1.5-fold increase after 24 h of treatment. Limited changes were observed in diacylglycerols (Fig. 2) and no change was observed in the incorporation of [<sup>3</sup>H]glycerol into monoacylglycerols at any time point examined. The labeling of other lipids was not affected by TPA treatment for less than 24 h. The above experiments were duplicated in HL-60 cells with similar results (data not shown).

Using [<sup>3</sup>H]AA an alternative metabolic label, we observed a 50% increase in the rate of incorporation of

[<sup>3</sup>H]AA into TAG as early as 6 h after TPA treatment (data not shown). The results obtained after 24 h of TPA-treatment are shown in Fig. 3. A 1.5-fold increase in TAG labeling was observed, confirming our previous observation using [<sup>3</sup>H]glycerol as the label. Interestingly no change in PC labeling was observed when [<sup>3</sup>H]AA was employed as the label.

Since serum in the culture medium represents an exogenous supply of fatty acids, which are important substrates for lipid synthesis, NL and PL metabolism were investigated in U-937 cells treated for 24 h with TPA in the presence or absence of serum. The results showed no difference in the labeling of PC between cells treated in medium containing FBS compared to cells treated in serum-free medium (Table I). Increased TAG labeling in TPA-treated cells, however, was prevented by the omission of serum from the cultures.

#### 4. DISCUSSION

Stimulation of NL metabolism represented the most salient consequence of TPA treatment of myeloid cells. Treatment of HL-60 cells with TPA for 24–48 h has been previously reported to increase the NL content and the incorporation of acetate or fatty acids into this lipid fraction [12,13]. We have found that increased TAG metabolism may occur as early as 6 h after TPA treatment, preceding the effect on PC metabolism, as detected by [<sup>3</sup>H]glycerol incorporation. The two events may be unrelated, as indicated by the observation that stimulation of TAG metabolism by TPA required the presence of serum in the culture medium during treatment, while no such a requirement was observed for the effect on PC metabolism. Increased PC metabolism in response to TPA treatment of HeLa cells has been previously associated with the stimulation of CTP:phosphocholine cytidyltransferase activity, a

Table I  
Effect of serum on the incorporation of [<sup>3</sup>H]glycerol and [<sup>3</sup>H]AA into TAG and PC from U-937 cells treated with or without TPA

Treatment	PC labeling		TAG labeling	
	[ <sup>3</sup> H]Glycerol	[ <sup>3</sup> H]AA (dpm $\times$ 10 <sup>3</sup> /10 <sup>6</sup> cells)	[ <sup>3</sup> H]Glycerol	[ <sup>3</sup> H]AA
Medium + FBS	68.9 (71.2–66.5)	995.2 (964.3–1026)	15.3 (14.1–16.4)	93.4 (88.1–98.6)
TPA + FBS	202.9 (187.2–218.6)	749.6 (633.5–865.7)	48.4 (43.9–52.8)	134.9 (114.1–155.6)
Medium + FAF-BSA	72.4 (71.5–73.2)	1183.5 (1136–1231)	9.3 (8.5–10.1)	59.5 (54.0–64.9)
TPA + FAF-BSA	144.0 (131.0–157.0)	840.1 (807.9–872.3)	11.2 (11.2–11.2)	60.5 (51.1–69.9)

Cells were treated with TPA (10 nM) for 24 h in the presence of 10% FBS or 1 mg/ml fatty acid-free bovine serum albumin (FAF-BSA). At the end of this treatment, cells were washed and allowed to incorporate [<sup>3</sup>H]glycerol or [<sup>3</sup>H]AA for one hour at 37°C. The reaction was terminated, and the lipids were extracted and analyzed by TLC. Shown is the amount of radioactivity recovered into PC or TAG. The results are from one of two experiments, each performed in duplicate. The values of the individual replicates are shown in parentheses.

limiting reaction in PC synthesis, by translocating the enzyme from the cytosol to a particulate fraction [24]. This effect appeared rapidly (within 1–3 h) after treatment with 100 nM TPA. Lockney et al. [25] have shown increased choline incorporation into PC within 3 h of treatment of human hairy cell leukemia cells with 10 nM TPA. An equally rapid effect was reported in HL-60 cells treated with 500 nM phorbol dibutyrate [26]. We failed to observe stimulation of PC metabolism before 24 h of treatment, as measured by [ $^3\text{H}$ ]glycerol incorporation. Our use of a different radiolabeled precursor may account for the difference. As glycerol constitutes the backbone structure common to all glycerophospholipids, changes in its incorporation levels in a particular subclass represent newly synthesized lipids. Choline incorporation, on the other hand, may be augmented as a result of increased phospholipid turnover, in the absence of net synthesis. Increased PC turnover may be an early consequence of TPA treatment, eventually followed by augmented PC synthesis. Moreover, the effect of TPA may be selective for a subclass of choline PL which do not contain AA in their acyl groups, as no response to TPA on PC metabolism was detected when the incorporation of [ $^3\text{H}$ ]AA was studied.

A conventional addition to cell culture media, serum is a source of factors and metabolites, including fatty acids essential for cell growth. Since phorbol esters may alter cell permeability [27], an increased uptake of fatty acids from serum-supplemented medium during TPA treatment may result in stimulation of TAG synthesis as a secondary effect. This was deemed unlikely, however, because the effect was selective for myeloid cells and incorporation of the fatty acid, AA, into TAG was not affected by TPA treatment when measured in the absence of serum. Therefore, serum factor(s) other than fatty acids is/are required for the effect of TPA on NL metabolism. Two different batches of FBS sustained the TPA-induced changes in NL metabolism in a similar fashion (data not shown), suggesting that the factor involved was not a feature of a particular serum lot. This role of serum may be analogous to that reported by Welsh et al. [28], who have shown an increased TPA requirement to differentiate HL-60 cells cultured under serum-free conditions.

The specific changes in NL metabolism induced by TPA treatment of myeloid cells susceptible to differentiation may be due to direct activation of the relevant metabolic pathways by the phorbol ester or represents the expression of a new phenotype by the cell population as a whole or by a subset of differentiating cells. Although discrimination between these two possibilities is difficult, its relatively early occurrence, before full cell differentiation has occurred, favors the former hypothesis. The existence of a common metabolic effect in PC metabolism triggered by TPA in cells of different histological origin further supports the idea that TPA-treatment directly affects the relevant metabolic

pathways. The increased NL metabolism associated with the induction of myeloid differentiation may contribute to changing the physical characteristic of the membrane of differentiated cells.

*Acknowledgements:* We wish to acknowledge Drs K. Prodouze and J. Fratanoti for critically reviewing the manuscript.

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