Effect of Retinoic Acid on Phorbol Ester-stimulated Differentiation and Protein Kinase C-dependent Phosphorylation in the U937 Human Monoblastoid Cell

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INTRODUCTION

Retinoic acid stimulates differentiation of epithelial cells and other cell types (1-5). Retinoic acid stimulates the human monoblastoid cell line, U937, and the promyelocytic cell line, HL-60, to express phenotypic characteristics of more terminally differentiated cells (6-9). In addition to this direct effect, retinoic acid synergistically enhances U937 and HL-60 cell differentiation in response to dibutyryl cyclic AMP and lymphokines (7, 8).

Phorbol esters are another group of agents which induce differentiation of human leukemic cells (10-13). In the mouse epidermis tumor promotion model, retinoic acid inhibits phorbol ester induced increases in ornithine decarboxylase activity and inhibits tumor promotion (14, 15). The inability of retinoic acid to inhibit tumor formation in response to non-phorbol ester tumor promoters suggests that retinoic acid interacts in a specific manner with the mechanisms responsible for phorbol ester-stimulated tumor production (16, 17). Retinoic acid also inhibits the effects of phorbol esters in other cell types (18, 19). In certain cell types, rather than inhibiting, retinoic acid enhances phorbol ester-stimulated cellular events (20, 21). Thus, in a variety of cells, retinoic acid modulates the effects of phorbol esters.

In response to treatment with phorbol esters, the U937 cell differentiates into a monocyte-macrophage-like cell (22, 23). One possible mechanism by which phorbol esters exert an effect on cellular function is through activation of the calcium-phospholipid-dependent protein kinase, protein kinase C. By substituting for diacylglycerol, an endogenous protein kinase C activator, phorbol esters activate protein kinase C (24). As evidenced by the ability of exogenously added diacylglycerol analogues to mimic certain effects of phorbol esters, protein kinase C activation may mediate at least a portion of the effects of phorbol esters on cellular function (25-31). However, controversy exists as to whether activation of protein kinase C is a sufficient stimulus to induce leukemic cell differentiation. Most studies (32-35), but not all (36), have demonstrated that the sole addition of diacylglycerol analogues does not stimulate leukemic cell differentiation. The inability of diacylglycerol analogues to mimic all of the effects of phorbol esters has also been demonstrated in other cell types (37-43).

To gain insight into the role of protein kinase C activation in mediating phorbol ester-induced leukemic cell differentiation, we examined the effects of retinoic acid on protein kinase C-dependent phosphorylation and phorbol ester-induced differentiation in the U937 leukemic cell line.

MATERIALS AND METHODS

Materials. DMEM: Ham's F-12, Eagle's minimal essential medium without glucose and phosphates, fetal bovine serum, penicillin, and streptomycin were purchased from Grand Island Biological Co. [γ-32P]ATP and [32P]P, were purchased from New England Nuclear. P-81 papers and DEAE-cellulose were obtained from Whatman. Scintiverse and molecular weight standards for gel electrophoresis were purchased from Fisher and Bio-Rad, respectively. 1-L-Tosylamido-2-phenyltetrahydrochloromethyl ketone-treated trypsin was obtained from Worthington. Trans-Retinoic acid and all other chemicals were purchased from Sigma.

Cell Culture Conditions. Characteristics of the U937 cell, derived from a patient with histiocytic lymphoma, have been described previously (44). Cells were passaged every 2 to 3 days in medium consisting of DMEM: Ham's F-12 medium (1:1) supplemented with 5% fetal bovine serum, 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 100 µM/mL penicillin, and 100 µg/mL streptomycin. Cells were

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The abbreviations used are: DMEM, Dulbecco's modified Eagle's minimal essential medium; EGTA, ethyleneglycol bis(β-aminoethyl ether)N,N,N',N'-tetraacetic acid; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; TPA, 12-O-tetradecanoylphorbol-13-acetate; MeSO4, dimethyl sulfoxide; ED50, 50% effective dose.
incubated at 37°C in humidified 5% CO2:95% air. Viability was deter-
mixed by trypsin blue exclusion. Cells were greater than 90% viable
portant to all experimental treatments. When added, retinoic acid
solved in ethanol, while TPA was dissolved in Me2SO. The maximal
concentrations of ethanol and Me2SO exposed to the cells were <0.01%
and <0.001%, respectively. These solvent concentrations did not affect

calcium chloride, and 20 mM Tris-HCl, pH 7.4, in a final volume of
the elution was terminated by adding 25 μl of a 10% (w/v) SDS stop
solution followed by heating to 110°C for 3 min. One-dimensional
polyacrylamide electrophoresis was performed as described by Laemmli
(50). The stacking and running gels were 3 and 10% acrylamide, respec-
tively. Autoradiography was done utilizing Kodak XAR film at
−70°C. Concurrent electrophoresis of molecular weight standards allowed
assignment of approximate molecular weights to the phosphorylated
substrates. In certain experiments, 32P incorporation into endo-
genous substrates was quantitated by densitometric analysis of the
autoradiogram. The autoradiograms were scanned at 600 nm using a
Gilford spectrophotometer. Autoradiograms, when used for densito-
metric analysis, were derived from gels exposed to X-ray film for a
shorter period of time than the autoradiograms used as figures.

Isolated Cell Phosphorylation. Cells were suspended in Eagle's minimal
essential medium without glucose and phosphates for 30 min. 20 μCi/3 ×
10^6 cells, was added for 2 h, after which the cells were centrifuged and the supernatant was removed. The cells were resus-

tained in fresh phosphate-free medium at a concentration of 3 × 10^6/
ml.

After a 60-min incubation of the 32P-labeled cells with 0.001% Me2SO or TPA at the indicated concentration, the reaction was termin-
ated by adding one-third volume of a 10% (w/v) SDS stop solution followed by heating to 110°C for 3 min. Electrophoresis, using a 12% polyacrylamide running gel, was performed. After staining and destain-
ing, the gels were treated with 1 N potassium hydroxide for 1 h at 56°C. The KOH treatment was followed by exposure to the destaining solu-
tion for 1 h. The gels were dried and autoradiography was performed. Alkali treatment was used to facilitate visualization of TPA-dependent
substrate phosphorylation.

Tryptic Phosphopeptide Mapping. Protein kinase C-dependent phos-
phorylation was stimulated in 32P-labeled intact cells and cellular ex-
tracts. The reactions were terminated by adding one-third volume of a 10% (w/v) SDS stop solution followed by heating to 110°C for 3 min. After electrophoresis, using a 0.75-mm slab gel, autoradiography was performed on the wet gel. Using the autoradiogram as a template, the phosphorylated M, 48,000 substrate was excised from the gel. Gel slices were dehydrated with 4 changes of acetone (15 min each) and reswollen in 50 mM ammonium bicarbonate, pH 7.0, for 10 min. After two quick washes with ammonium bicarbonate, the gel piece was incubated overnight with tumbler in 0.5 ml of 50 mM ammonium bicarbonate containing 1 mg/ml 1,1-tosylamido-2-phenylethyl chloromethyl
ke-tone-treated trypsin. Supernatants were collected and gel pieces were
washed once with additional ammonium bicarbonate. The combined
supernatants contained greater than 85% of the 32P. Tryptic phospho-
phosphate fragments were separated using a Car-bondadapak column (Waters). The samples were eluted with a linear 0–60% acetonitrile
gradient in 0.1% trifluoroacetic acid, pH 2.0. The protein pattern of the
dayed trypsin was monitored at 214 nm. One hundred 0.5-mI fractions were collected at a flow rate of 1 ml/min. 32P associated with the phosphopeptides was detected on line using a Radiometric Flo-one with a BD flow cell (500 μl volume). Data were plotted using Dysc-

RESULTS

At micromolar concentrations retinoic acid stimulates U937
differentiation as assessed by increases in the number of cells
adhering to plastic surfaces and decreases in cellular prolifera-
(Fig. 1). However, when added at concentrations less than
100 nM, retinoic acid did not significantly stimulate U937 differentiation as evidenced by a lack of an effect on adherence to plastic or cellular growth (Fig. 1). At concentrations of 0.1 to 10 nM, TPA stimulated U937 differentiation as demonstrated by an increased number of cells adherent to plastic (Fig. 2). Exposure to 1 nM retinoic acid for 24 h prior to and during the 72-h incubation with TPA enhanced the phorbol ester-induced increases in cellular adherence (Fig. 2). While retinoic acid increased the maximal response approximately 4-fold, the approximate ED50 for TPA-stimulated adherence to plastic was similar, 0.3 nM, in both vehicle- and retinoic acid-treated cells. Increases in TPA-stimulated adherence to plastic were observed at retinoic acid concentrations of 0.03 to 1 nM (Fig. 3). Maximal effects on TPA-stimulated differentiation were observed after a 24-h preexposure followed by coincubation of retinoic acid with the phorbol ester. However, continuous exposure to retinoic acid was not required to enhance phorbol ester-induced differentiation since preincubation alone significantly enhanced TPA-stimulated differentiation.

Retinol, a vitamin A analogue having 1/1000th the potency of retinoic acid in other biologic systems (9), was analyzed for effects on TPA-stimulated differentiation. While ineffective at concentrations up to 1 µM when evaluated alone (data not shown), retinol enhanced TPA-stimulated differentiation over a concentration range of 10 to 1000 nM. Retinol potentiated TPA-stimulated differentiation to the same maximal extent as did retinoic acid. The approximate ED50 for potentiating TPA-induced differentiation was 100 nM for retinol as compared to 0.1 nM for retinoic acid. Thus, retinol was approximately 1/1000th as potent as retinoic acid in enhancing TPA-stimulated differentiation.

Under conditions that maximally increased TPA-stimulated adherence to plastic, retinoic acid also enhanced TPA-induced increases in expression of another indicator of differentiation, nonspecific esterase activity (Table 1).

Unlike the synergistic enhancement of other parameters of TPA-induced differentiation, retinoic acid did not potentiate the TPA-induced decrease in cellular proliferation. In four separate experiments total cell number was 12.5 x 10^4 ± 2.7 (SEM) in cells exposed for 72 h to 10 nM TPA and 18.0 x 10^4 ± 2.0 in cells exposed to 1 nM retinoic acid for 24 h prior to and during a 72-h incubation with 10 nM TPA (0.05 < P < 0.1, Student’s t test). The slight increase in total cell number in retinoic acid-treated cells was due to an increase in the adherent cell number (control, 1.2 x 10^4 ± 0.3; retinoic acid, 4.7 x 10^4 ± 1.6). The nonadherent cell numbers were 11.3 x 10^4 ± 2.9 and 13.3 x 10^4 ± 3.4 in control and retinoic acid-treated cells, respectively (P > 0.1, Student’s t test).

Since activation of protein kinase C and subsequent protein phosphorylation may mediate the cellular responses stimulated by phorbol esters (24), the effect of retinoic acid on TPA-stimulated phosphorylation was examined. Exposure of intact 32P-labeled cells to 10 nM TPA for 60 min stimulated phosphorylation of a M, 48,000 substrate (Fig. 4A). Prior exposure to 1
48,000 substrate was increased by a 48-h exposure to 1 nM TPA: N = 3 determinations pooled from three separate experiments. Methods.

Following this preincubation the cells were resuspended in fresh medium containing the additives listed in Column 2. At the end of this incubation nonspecific esterase activity was determined in duplicate as described in "Materials and Methods." The activity was determined by the Sibley and Lardy (1978) method. The tryptic digestes were analyzed by reverse phase chromatography and the tryptic phosphopeptide maps were analyzed by autoradiography, excised from the gel, and digested with trypsin. The approximate ED_{50} for TPA-stimulated phosphorylation of the M_{48k} substrate was similar, 2 nM, in vehicle- and retinoic acid-treated cells. Following a 48-h preincubation with retinoic acid, an enhancement in TPA-stimulated phosphorylation of the M_{48k} substrate was clearly discernible at an retinoic acid concentration of 0.3 nM and maximal by a concentration of 1 nM (data not shown).

The effect of retinoic acid on protein kinase C-dependent endogenous substrate phosphorylation in cellular extracts was examined. Addition of protein kinase C activators (calcium, phosphatidylserine, and diolein) to a cellular extract containing cytosolic and solubilized particulate fractions stimulated phosphorylation of several substrates, the most prominent being a substrate with a molecular weight of 48,000 (Fig. 5). Substrates with molecular weights of 80,000, 140,000, and 170,000 were also phosphorylated in response to protein kinase C activation. Pretreatment for 48 h with 1 nM retinoic acid enhanced the protein kinase C-dependent phosphorylation of the M_{48k} and M_{80k} substrates but did not alter the protein kinase C-dependent phosphorylation of the M_{140k} and M_{170k} substrates (Fig. 5). The direct addition of 0.1 μM retinoic acid to cellular homogenates did not alter the protein kinase C-dependent phosphorylation of endogenous substrates (data not shown).

Increases in the protein kinase C-dependent phosphorylation of the M_{48k}, M_{80k} and M_{140k} substrates were discernible at a retinoic acid concentration of 0.01 nM and maximal by a concentration of 0.3 nM (Fig. 6A). Increases in the protein kinase C-dependent phosphorylation of the M_{48k}, M_{80k} and M_{140k} substrates were observed after a 24-h incubation with 1 nM retinoic acid and were maximal after 48- to 72-h exposure (Fig. 6B).

While the basal phosphorylation of the M_{48k}, M_{80k} and M_{140k} substrates was not affected, retinoic acid did enhance the basal protein kinase C-independent phosphorylation of M_{56k}, M_{64k} and M_{64k} substrates (Fig. 5).

Retinoic acid-induced increases in protein kinase C-dependent and independent phosphorylations were predominantly localized to the cytosolic fraction (data not shown).

A decrease in phosphatase activity responsible for dephosphorylating the M_{48k}, M_{80k} and M_{140k} substrates could produce an apparent increase in the protein kinase C-dependent phosphorylation of these substrates. To examine whether retinoic acid decreased the rate of substrate dephosphorylation, the following experiment was done. 32P incorporation into the substrates was stimulated by adding protein kinase C activators to the cytosolic extracts. Following a 1-min period of 32P incorporation 100-fold excess of unlabeled ATP, 0.1 mM, was added. Sixty s after the addition of unlabeled ATP, the decline in 32P associated with the M_{48k} and M_{80k} substrates was minimal and similar between vehicle- and retinoic acid-treated cells (Table 2).

To determine if an identical M_{48k} substrate in intact cells and cellular extracts was phosphorylated in response to protein kinase C activation, tryptic phosphopeptide maps of the M_{48k} substrates were compared. Phosphorylation of the M_{48k} substrate in cellular extracts was stimulated by adding calcium, phosphatidylserine, and diolein. The M_{48k} substrate phosphorylation was stimulated in the 32P-labeled, intact cells by a 60-min exposure to 10 nM TPA. Following termination of the phosphorylation reactions, the substrates were separated by polyacrylamide gel electrophoresis. The phosphorylated M_{48k} substrates were identified by autoradiography, excised from the gel, and digested with trypsin. The tryptic digests were analyzed by reverse phase chromatography.
Preincubation with: Vehicle Vehicle Retinoic Acid
Exposure to 100 nM TPA: — + +

RETINOIC ACID ALTERS PROTEIN KINASE C

- p48 - p48

Fig. 4. Effect of retinoic acid on phorbol ester-stimulated phosphorylation in intact 32P-labeled U937 cells. In A, cells were treated with 0.01% ethanol or 1 nM retinoic acid as shown above the autoradiogram. After a 48-h incubation, cells from each treatment group were labeled with 32P. As indicated above the autoradiogram, 3 x 10^5 32P-cells were exposed to 0.001% Me2SO or 10 nM TPA. After 60 min the reaction was terminated and the samples were subjected to polyacrylamide gel electrophoresis followed by autoradiography. The TPA-dependent phosphorylation of a M, 48,000 substrate is indicated to the side of the autoradiogram. In B, cells were incubated with 0.01% ethanol (O) or 1 nM retinoic acid (A). After a 48-h incubation the cells were labeled with 32P. 32P-labeled cells (3 x 10^5) were incubated with vehicle or varying concentrations of TPA as shown on the abscissa. After a 60-min incubation the reaction was terminated and the samples were analyzed using polyacrylamide gel electrophoresis followed by autoradiography. Quantitation of M, 48,000 substrate (p 48) phosphorylation was determined by densitometric analysis of the autoradiogram. This experiment was repeated with similar results.

In both cellular extracts and intact cells, tryptic digestion of the phosphorylated M, 48,000 substrate yielded three peaks of radioactivity with nearly identical retention times (Fig. 7).

The retinoic acid-induced increases in protein kinase C-dependent phosphorylation could have been due to an increase in the amount of protein kinase C available for activation or an increase in the amount of endogenous substrate phosphorylated by protein kinase C. Using exogenous histone as a phosphate acceptor, protein kinase C activity was directly examined. Since the most pronounced alterations in protein kinase C-dependent endogenous substrate phosphorylation occurred in the cytosol, protein kinase C activity in this fraction was initially evaluated. Prior to measurement of activity, protein kinase C was extracted by DEAE chromatography. Retinoic acid treatment increased cytosolic protein kinase C activity eluting from a DEAE resin (Fig. 8). Kinase activity specifically stimulated by calcium, phosphatidylserine, and diolein was measured in cytosolic fractions which had been previously batch extracted using DEAE resin (see "Materials and Methods"). Protein kinase C activity contained in DEAE-extracted cytosol derived from retinoic acid-treated cells was 236 ± 19% of vehicle-treated cells (P < 0.01 versus control, Student's t test, N = 6). Retinoic acid treatment did not alter protein kinase C activity in DEAE-extracted solubilized particulate fraction (90 ± 12% of vehicle-treated cells, P > 0.1, Student's t test, N = 6).

The following experiments were done to determine whether the conditions of retinoic acid treatment inducing changes in protein kinase C activity were similar to those increasing protein kinase C-dependent phosphorylation of endogenous substrates and potentiating TPA-stimulated differentiation. The duration of exposure and concentration of retinoic acid altering protein kinase C activity were examined (Fig. 9). After exposure to 1 nM retinoic acid an effect was discernible by 12 h and maximal by 36 h. Following a 48-h exposure, the retinoic acid effect was evident at 0.03 nM and maximal at a concentration of 1 nM.

DISCUSSION

Retinoic acid potentiated TPA-stimulated differentiation of the human U937 monoblastoid cell. The more mature monocytic phenotype was manifested by increased adherence to plastic surfaces and the acquisition of nonspecific esterase activity. While retinoic acid potentiated the effects of TPA on certain parameters indicative of a more differentiated phenotype, cellular growth was not affected by retinoic acid treatment. In fact, total cell number was slightly, but not significantly, greater in cells treated with retinoic acid and TPA than in those treated with only TPA. The increase in total cell number following
Pretreatment with: Vehicle Retinoic Acid
Protein Kinase C Activators. - + - +

Fig. 5. Effect of retinoic acid on protein kinase C-dependent phosphorylation of endogenous U937 substrates. Cells (15 x 10^6/50 ml of medium) were incubated with 0.01% ethanol or 1 nM retinoic acid (indicated above the autoradiograms). After a 48-h incubation a cellular extract containing both cytosolic and solubilized particulate fractions was prepared. Endogenous substrate phosphorylation in the presence (+) and absence (−) of protein kinase C activators (shown above the autoradiograms) followed by polyacrylamide gel electrophoresis with autoradiography was performed as described in "Materials and Methods." Molecular weight estimates of substrates are indicated on the ordinate of the autoradiogram.

exposure to both agents was due to an increase in the number of more differentiated, adherent cells. The ability of agents inducing U937 differentiation to affect certain parameters of differentiation more so than others has been documented previously (51, 52).

The similar conditions of retinoic acid treatment increasing the protein kinase C-dependent phosphorylation of histone and endogenous substrates suggested that the increase in endogenous substrate phosphorylation could have been due either to an increase in the quantity of kinase available for activation or to a facilitation of preexisting kinase activation. In addition to the increase in protein kinase C-dependent phosphorylation in cellular homogenates, retinoic acid enhanced TPA-stimulated phosphorylation in intact 32P-labeled cells. The similar tryptic phosphopeptide maps of the M, 48,000 substrate suggested that a similar mechanism mediated the enhanced phosphorylation of this substrate in the intact cell and cellular homogenates. Retinoic acid selectively increased the protein kinase C-dependent phosphorylation of certain endogenous substrates (M, 48,000 and M, 80,000) but not in others (M, 140,000 and M, 170,000). Possible mechanisms explaining the preferential enhancement in phosphorylation of the M, 48,000 and M, 80,000 substrates would include increased synthesis of these substrates or a selective increase in expression of a specific protein kinase C isozyme. Protein kinase C is a member of a gene family (53).

Three protein kinase C isozymes have been isolated (54). Protein kinase C isozymes thus far characterized exhibit differences in substrate specificity (55). Thus, retinoic acid could potentiate phosphorylation of the M, 48,000 and M, 80,000 substrates by enhancing the expression of a protein kinase C isozyme which preferentially phosphorylates these substrates.

Using the human promyelocytic HL-60 cell, retinoic acid treatment has also been shown to increase protein kinase C-dependent phosphorylation (56, 57). In this cell line, the increases in protein kinase C-dependent phosphorylation have been observed at retinoic acid concentrations which directly stimulate differentiation (58). Protein kinase C-dependent phosphorylation is also increased following treatment with
Table 2. Comparison of rates of dephosphorylation of the M, 48,000 and M, 80,000 substrates between vehicle- and retinoic acid-treated cells

<table>
<thead>
<tr>
<th>Time after addition of unlabeled ATP</th>
<th>M, 48,000</th>
<th>M, 80,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Retinoic acid Vehicle Retinoic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediately</td>
<td>8.7 17.2 5.8 9.4</td>
<td></td>
</tr>
<tr>
<td>60 s</td>
<td>8.4 16.2 5.0 8.9</td>
<td></td>
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Fig. 7. Tryptic phosphopeptide mapping of the M, 48,000 substrate. Cells (15 x 10^6/50 ml of medium) were treated with 1 nM retinoic acid. After a 48-h incubation a cytosolic extract was prepared and protein kinase C-dependent phosphorylation was stimulated. In addition, a portion of the cells were labeled with 32P and exposed to 10 nM TPA for 60 min. Phosphorylation in the cytosolic extract and the intact cell was terminated and the samples were subjected to polyacrylamide gel electrophoresis. A gel piece containing the M, 48,000 substrate, identified by autoradiography, was excised from the gel, treated with ammonium bicarbonate, and digested with trypsin. Equal cpm of the digests were analyzed by reverse phase chromatography. The retention time is shown on the abscissa and the radioactivity contained in the fractions is indicated on the ordinate. The tryptic phosphopeptide maps are derived from the M, 48,000 substrate phosphorylated in the cytosolic extract (A) and in the intact cell (B).

Fig. 8. DEAE-cellulose chromatography of cytosol derived from vehicle- or retinoic acid-treated cells. After a 48-h incubation with 0.01% ethanol or 1 nM retinoic acid, cytosolic fractions were prepared. Equal amounts of the extracts, 1 mg of protein, were applied to and eluted from the DEAE columns as described in “Materials and Methods.” Protein kinase C activity in the vehicle (O)- and retinoic acid (A)-treated cells was determined. ----, conductivity of the eluate. Similar elution profiles of protein kinase C activity were obtained in four separate experiments.

Fig. 9. Effects of the duration of exposure and concentration of retinoic acid on protein kinase C activity. Cells (30 x 10^6/50 ml of medium) were exposed to 1 nM retinoic acid for varying periods of time (A) or to varying concentrations of retinoic acid for 48 h (B). At the end of the treatment periods, cytosolic fractions were prepared and the extracts, 1 mg of protein, were applied to DEAE-cellulose resin. Protein kinase C activity was eluted from the resin in a batchwise fashion. Protein kinase C activity was determined in the DEAE eluates. Each point is the mean of two determinations within a single experiment. Each experiment was repeated on a separate occasion with similar results.

Other agents stimulating HL-60 differentiation (56). Thus, it is difficult to determine if the retinoic acid-induced increase in protein kinase C-dependent phosphorylation was due to a primary effect of retinoic acid or to the more differentiated phenotype induced by exposure to retinoic acid. In the U937 cell line the concentrations of retinoic acid increasing protein kinase C-dependent phosphorylation (0.01 to 1.0 nM) were at least 100-fold less than those concentrations which directly stimulated differentiation (≥100 nM). Although not definitive, these data suggest that potentiation of protein kinase C-dependent phosphorylation may be a primary effect of retinoic acid rather than secondary to induction of a more differentiated phenotype. If retinoic acid directly induces this response, then alterations of protein kinase C activity could mediate the ability of retinoic acid to modulate the effects of phorbol esters in other cell types.

One possible mechanism by which retinoic acid could enhance TPA-stimulated differentiation was through its effects on protein phosphorylation. In both TPA-stimulated differentiation and substrate phosphorylation in the intact 32P-labeled cell, retinoic acid increased the maximal response to TPA...
without significantly altering the ED50 for TPA. The similar concentrations of retinoic acid enhancing protein kinase C-dependent phosphorylation and potentiating TPA-stimulated differentiation were also consistent with a possible relationship between these events. Thus, by enhancing TPA-stimulated phosphorylation retinoic acid amplified phorbol ester signal transduction. Such an amplification could mediate the potentiation of phorbol ester-stimulated cellular events such as differentiation. However, byrostatin and diacylglycerol derivatives which like phorbol esters activate protein kinase C fail to stimulate leukemic cell differentiation (32, 34, 59). These findings suggest that the sole activation of protein kinase C may not directly stimulate differentiation. Despite the controversy as to whether protein kinase C activation solely mediates TPA-stimulated differentiation, the close correlation between the effects of retinoic acid on differentiation and protein kinase C-dependent phosphorylation suggests that activation of this kinase is involved in the signal transduction mediating TPA-stimulated differentiation.

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