Induction of lysosomal phospholipase A_2 through the retinoid X receptor in THP-1 cells

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Abstract **An acidic phospholipase A_2 ($LPLA_2$) was recently purified and cloned. THP-1 cells were used to characterize the gene induction of $LPLA_2$. THP-1 cells were stimulated with several differentiation agents. The $LPLA_2$ mRNA and activity increased in cells treated with phorbol ester but not with vitamin D3, interferon-γ, or granulocyte macrophage colony-stimulating factor. All-*trans*-retinoic acid enhanced mRNA expression and enzyme activity in a dose- and time-dependent manner. The natural 9-*cis* and 13-*cis* isomers of retinoic acid enhanced transcription and activity. Two classes of nuclear receptors, the retinoic acid receptor (RAR) and the retinoid X receptor (RXR), mediate retinoic acid signaling. Specific RAR and RXR agonists were used to identify the nuclear receptor responsible for $LPLA_2$ induction by retinoic acid. Treatment with the RAR agonist 4-[E-2-tetrahydro-5,5,8,8-tetra-methyl-2-naphthalenyl]1-propenyl benzoic acid (TTNPB) resulted in a small and statistically significant increase of the mRNA expression and activity of $LPLA_2$. The RXR agonist methoprene acid worked as well as all-*trans*-retinoic acid at increasing both mRNA and enzyme activity. The methoprene acid and TTNPB effects were not synergistic. The peroxisome proliferator-activated receptor γ agonists 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and troglitazone failed to induce $LPLA_2$ activity and mRNA. Thus, an RXR-dependent pathway controls $LPLA_2$ gene activation by retinoic acid in THP-1 cells.**—Abe, A., H. K. Poucher, M. Hiraoka, and J. A. Shayman. **Induction of lysosomal phospholipase A_2 through the retinoid X receptor in THP-1 cells. *J. Lipid Res.* 2004.** 45: **667–673.**

Supplementary key words retinoic acid • lysosome • phospholipase • phorbol ester

Recently, while investigating short-chain ceramide metabolism in MDCK cells, a novel enzyme was discovered that catalyzes the esterification of the ceramide molecule at the C-1 carbon (1). The esterification results from the transfer of the acyl group at the *sn*-2 position of a glycerophospholipid such as phosphatidylcholine or phosphatidylethanolamine to ceramide. The enzyme has both transacylase and phospholipase A_2 (PLA_2) activities that are Ca^{2+}-independent and favored under acidic conditions. The enzyme was subsequently purified from bovine brain (2). Based on the partial amino acid sequences obtained from the purified enzyme, the gene encoding the enzyme was cloned and the bovine, mouse, and human sequences were obtained (3). The enzyme is a water-soluble glycoprotein with a molecular mass of 45 kDa and consists of a single polypeptide chain. The enzyme has a pH optimum at 4.5. The cellular location of the enzyme is primarily lysosomal. Therefore, the enzyme was named lysosomal phospholipase A_2 ($LPLA_2$) (3). These data suggested that there is a new pathway for phospholipid and ceramide metabolism in lysosomes. However, the biological function of $LPLA_2$ still remains unknown.

The $LPLA_2$ gene was first reported in a study designed to identify lipid metabolism-related genes differentially expressed in foam cells. The foam cells were derived from PMA-treated THP-1 cells (4). THP-1 is a cell line derived from a patient with acute monocytic leukemia. These cells are of monocytic origin and are known to acquire increased phagocytic ability upon treatment with PMA. These cells can be stimulated and differentiated by a variety of agents, such as PMA, retinoic acid, vitamin D3, interferon-γ, and granulocyte macrophage colony-stimulating factor (GM-CSF) (5–10). Thus, agonists other than PMA could affect $LPLA_2$ gene expression in THP-1 cells. To better understand the biological function of $LPLA_2$, a search was initiated for a cellular mechanism of $LPLA_2$ gene expression using these agonists.

In this study, it is reported that all-*trans*-retinoic acid (ATRA) is able to induce $LPLA_2$ mRNA and enzyme activity in THP-1 cells and that the induction is through a retinoid X receptor (RXR)-dependent pathway, which is independent of retinoic acid receptor (RAR) or peroxisome proliferator-activated receptor γ (PPARγ).

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MATERIALS AND METHODS

Reagents

PMA, 8-bromo-cAMP, and ATRA were obtained from Sigma (St. Louis, MO). 9-*Cis*- and 13-*cis*-retinoic acids, 4-[E-2-tetrahydro-5,5,8,8-tetra-methyl-2-naphthalenyl]1-propenyl benzoic acid (TTNPB), methoprene acid, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (PGJ_2), and troglitazone were purchased from Biomol (Plymouth Meeting, PA). Vitamin D3 was from Calbiochem (La Jolla, CA), and interferon-γ was from Roche Applied Science (Indianapolis, IN). GM-CSF was obtained from BD Biosciences (San Jose, CA).

$LPLA_2$ induction study

One million THP-1 cells were seeded into a 10 cm-style dish containing 10 ml of RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 4.5 g/l D-glucose, 1 mM sodium pyruvate, and 55 μM 2-mercaptoethanol. The cells were maintained in an incubator with 5% CO_2 at 37°C. In $LPLA_2$ induction studies, 1,000,000 THP-1 cells were seeded into a 10 cm-style dish containing 10 ml of RPMI 1640 supplemented medium as described above and incubated for 24 h. Then, the cells were treated with or without stimulant as indicated in the figure legends.

RT-PCR

After the treatment, total RNA was prepared from the THP-1 cells using the RNeasy kit (Qiagen, Valencia, CA). Five micrograms of total RNA was used to synthesize cDNA with oligo(dT) using the Super Script First-Stand synthesis system (Invitrogen, Carlsbad, CA) in a total volume of 20 μl. One microliter of the cDNA mixture synthesized was used for PCR. The sequences of PCR primers were 5′-CCATGGAGAAGGCTGGGG-3′ and 5′-CCAAAGTTGTCATGGATGACC-3′ for sense and antisense, respectively, of GAPDH and 5′-GGGACTACCGCAAGTTCTT-3′ and 5′-AGGTTCCAGAAGCACACGTTT-3′ for sense and antisense, respectively, of $LPLA_2$. The PCR amplifications of GAPDH and $LPLA_2$ were performed separately using *Taq* polymerase. The samples were denatured at 94°C for 2 min followed by cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The subsequent extension incubation was at 72°C for 5 min. To analyze PCR products semiquantitatively, 19–23 cycles were performed. Amplified products were separated by agarose gel (1.5%) electrophoresis, stained with ethidium bromide, and visualized under an ultraviolet transilluminator.

Transacylase activity in soluble fraction

The treated cells were washed twice with cold phosphate-buffered saline and once with cold 0.25 M sucrose, 10 mM Hepes (pH 7.4). The resultant cell pellet was dispersed in 0.25 M sucrose, 10 mM Hepes (pH 7.4) and disrupted five times for 10 s by a probe-type sonicator at 0°C. The suspension was centrifuged for 1 h at 100,000 *g* at 4°C. The resultant supernatant was passed through a 0.2 μm filter and used for the transacylase assay. *N*-Acetylsphingosine (NAS) was used as an acyl group acceptor. The reaction mixture consisted of 40 mM sodium citrate (pH 4.5), 10 μg/ml BSA, and 40 μM NAS incorporated into phospholipid liposomes [phosphatidylcholine/phosphatidylethanolamine/dicetyl phosphate/NAS (5:2:1:2 in molar ratio)] and enzyme fraction (7 μg) in a total volume of 500 μl. The reaction was initiated by adding the enzyme fraction, kept for 30, 60, or 120 min at 37°C, and terminated by adding 3 ml of chloroform-methanol (2:1) plus 0.3 ml of 0.9% (w/v) NaCl. The mixture was centrifuged for 5 min at room temperature. The resultant lower layer was transferred into another glass tube and dried down under a stream of nitrogen gas. The dried lipid was dissolved in 40 μl of chloroform-methanol (2:1), half of which was applied on an HPTLC plate and developed in a solvent system consisting of chloroform-acetic acid (9:1). The plated was dried down and soaked in 8% (w/v) $CuSO_4$, $5H_2O$, 6.8% (v/v) H_3PO_4, and 32% (v/v) methanol. The plate was briefly dried with a hair dryer and charred for 15 min in a 150°C oven. The plate was scanned, and the product content (1-*O*-acyl-*N*-acetylsphingosine) was measured with NIH Image 1.62 software. The data were compared between experimental groups as the specific activity of the transacylase (micrograms of 1-*O*-acyl-*N*-acetylsphingosine per minute per milligram of protein), and statistical significance was determined by a paired Student's *t*-test.

RESULTS AND DISCUSSION

Effects of agonists on $LPLA_2$ induction in THP-1 cells

In a preliminary study, THP-1 cells were treated with different agonists for 3 days. During the treatment period, PMA-treated THP-1 cells became adherent to the plastic surface of the culture dish and developed a macrophage-like appearance, but ATRA-, interferon-γ-, and GM-CSF-

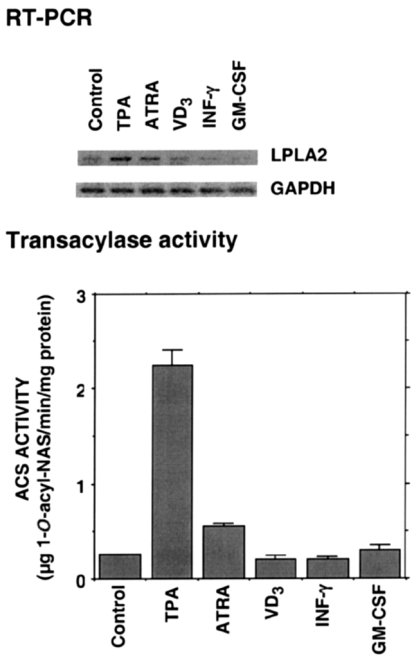

Fig. 1. Effect of PMA (TPA), *all-trans*-retinoic acid (ATRA), vitamin D3 (VD_3), interferon-γ, or granulocyte macrophage colony-stimulating factor (GM-CSF) on lysosomal phospholipase A_2 ($LPLA_2$) expression in THP-1 cells. One million THP-1 cells were seeded and treated with or without 10 nM PMA, 10 μM ATRA, 100 nM vitamin D3, 100 U/ml interferon-γ, or 100 pg/ml GM-CSF for 3 days. After treatment, RT-PCR amplification of total RNA extracted from the treated cells was carried out and the 1-*O*-acylceramide synthase activity of the soluble fraction obtained from the treated cells was determined as described in Materials and Methods. The transacylase activity (ACS) values represent means ± SD of three independent measurements. The specific activities and *P* values of the agonist-stimulated groups versus the control group were as follows: control, 0.254 ± 0.008; PMA, 2.249 ± 0.160 (7.72×10^{-5}); ATRA, 0.554 ± 0.030 (2.72×10^{-5}); vitamin D3, 0.198 ± 0.045 (0.01); INF-γ, 0.209 ± 0.023 (0.031); and GM-CSF, 0.301 ± 0.055 (0.222). NAS, *N*-acetylsphingosine.

treated cells remained as aggregated, floating cells. Although half of the vitamin D3-treated THP-1 cells became adherent, they did not acquire a macrophage shape. After the treatment period, $LPLA_2$ and GAPDH mRNAs were detected by RT-PCR, and the enzyme activity in the soluble fraction was determined by its transacylase activity. As expected, both $LPLA_2$ messenger level and enzyme activity were increased in the PMA-treated cells (**Fig. 1**). However, vitamin D3, interferon-γ, and GM-CSF did not have any significant effect on $LPLA_2$ gene expression. Interest-

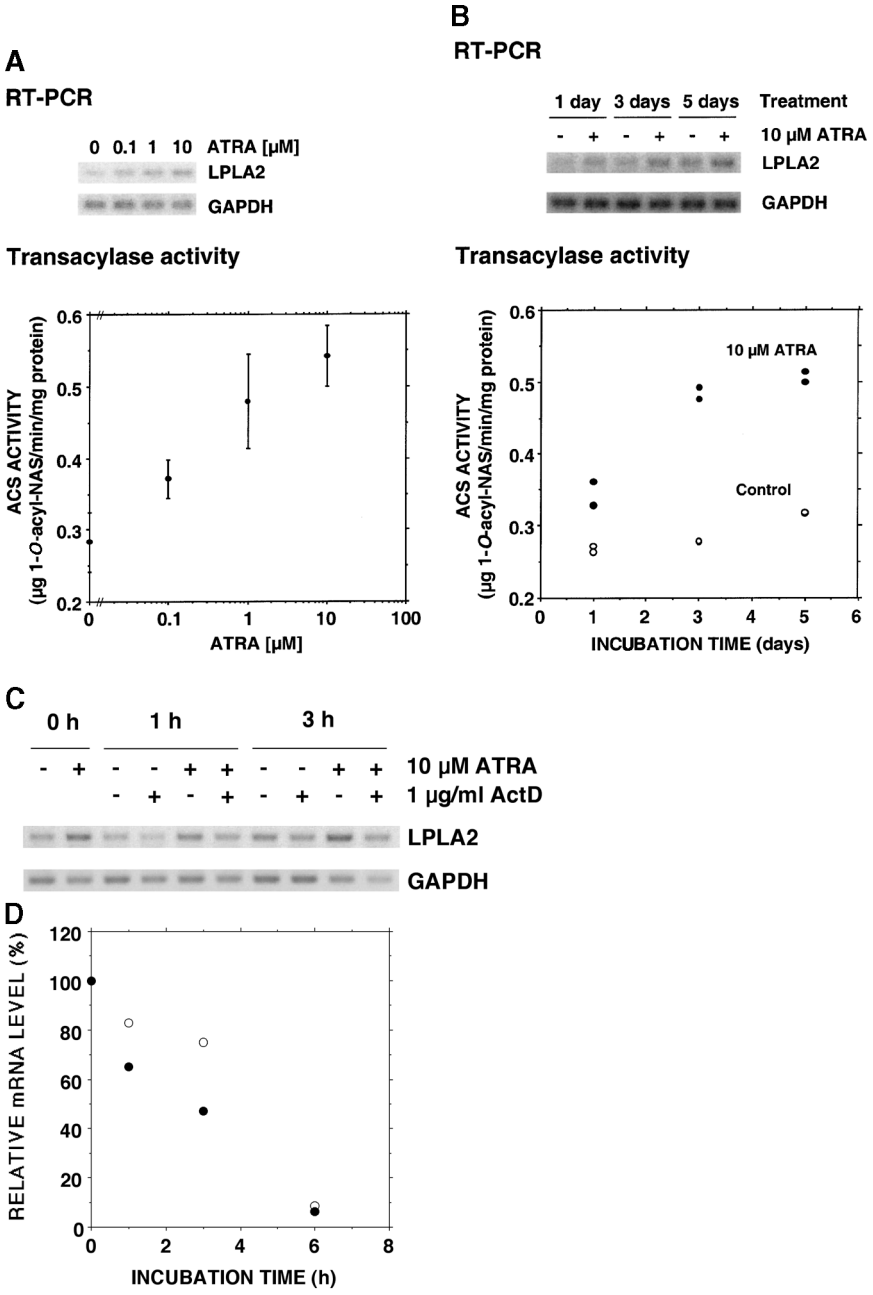

Fig. 2. ATRA induces $LPLA_2$ expression in a dose- and time-dependent manner in THP-1 cells. One million THP-1 cells were seeded and treated with different concentrations of ATRA for 3 days (A) or with 10 μM ATRA for the indicated times (B). After treatment, $LPLA_2$ messenger level and $LPLA_2$ activity in the treated cells were measured as described in Materials and Methods. The ACS values represent means ± SD of three independent measurements. For studies of mRNA stability (C and D), 1,000,000 THP-1 cells were seeded and treated with or without 10 μM ATRA for 4 days and then exposed to actinomycin D for 0, 1, 3, and 6 h. The relative mRNA levels of $LPLA_2$ were defined as follows: relative mRNA level (%) = 100 × (band intensity of $LPLA_2$ mRNA of actinomycin D-treated cells)/(band intensity of $LPLA_2$ mRNA of untreated cells). The mRNA bands were scanned and quantified by NIH Image 1.62 software. The closed and open circles represent ATRA-treated and ATRA-untreated cells, respectively. In B, the specific activities and *P* values of the ATRA-stimulated cells versus the control group were as follows: control, 0.283 ± 0.041; 0.1 μM, 0.371 ± 0.027 (0.036); 1.0 μM, 0.479 ± 0.066 (0.012); 10 μM, 0.542 ± 0.042 (0.002).

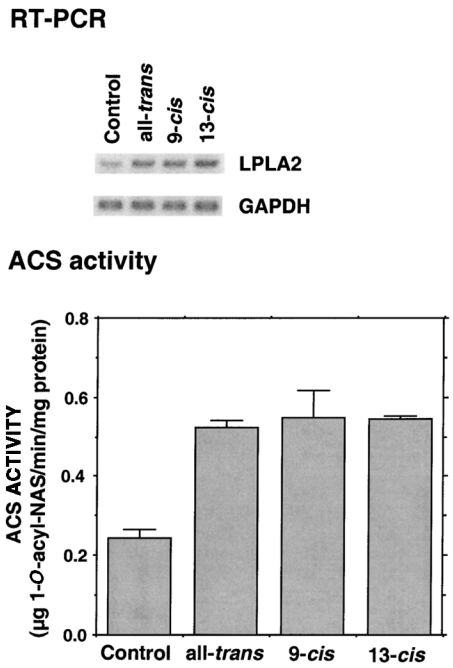

Fig. 3. Effect of retinoic acid isomers on $LPLA_2$ induction in THP-1 cells. One million THP-1 cells were seeded and treated in the presence of 10 μM ATRA, 10 μM 9-*cis*-retinoic acid, or 10 μM 13-*cis*-retinoic acid for 3 days. After treatment, $LPLA_2$ messenger level and $LPLA_2$ activity in the treated cells were measured as described in Materials and Methods. The ACS values represent means ± SD of three independent measurements. The specific activities and *P* values of the agonist-stimulated groups versus the control group were as follows: control, 0.245 ± 0.022; ATRA, 0.522 ± 0.020 (0.0001); 9-*cis*-retinoic acid, 0.548 ± 0.067 (0.002); 13-*cis*-retinoic acid, 0.545 ± 0.008 (2.36×10^{-5}).

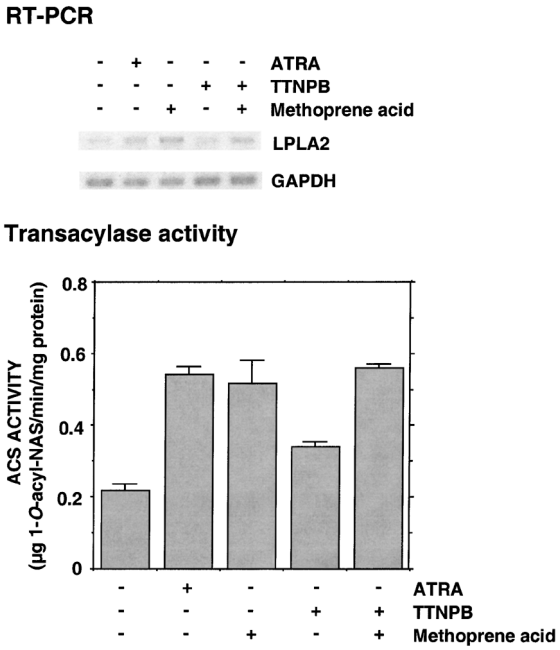

Fig. 4. Effect of retinoic acid receptor or retinoid X receptor (RXR) agonist on $LPLA_2$ induction in THP-1 cells. One million THP-1 cells were seeded and treated in the presence of 10 μM ATRA, 10 μM 4-[E-2-tetrahydro-5,5,8,8-tetra-methyl-2-naphthalenyl]1-propenyl benzoic acid (TTNPB), 100 μM methoprene acid, or 10 μM TTNPB plus 100 μM methoprene acid for 3 days. After treatment, $LPLA_2$ messenger level and $LPLA_2$ activity in the treated cells were measured as described in Materials and Methods. The ACS values represent means ± SD of three independent measurements. The specific activities and *P* values of the agonist-treated groups versus the control group were as follows: control, 0.219 ± 0.020; ATRA, 0.541 ± 0.024 (5.46×10^{-5}); methoprene acid, 0.516 ± 0.066 (0.0017); TTNPB, 0.341 ± 0.016 (0.011); TTNPB plus methoprene acid, 0.561 ± 0.008 (9.89×10^{-6}).

ingly, ATRA was able to induce both the $LPLA_2$ mRNA and enzyme activity.

Although $LPLA_2$ has some possible phosphorylation sites, intracellular $LPLA_2$ is localized to the lysosomal lumen. Therefore, $LPLA_2$ should not be available as a substrate for the wide variety of cytosolic kinases that regulate many enzyme activities and intracellular signals. As previously reported, the sugar moieties of the glycosylation sites of $LPLA_2$ do not have any effect on the enzyme activity (3). Other posttranslational modification sites of $LPLA_2$ that may affect enzyme activity were not found during analysis of the primary sequence. Therefore, an enhancement of $LPLA_2$ activity in PMA- and ATRA-treated THP-1 cells is thought to be attributable to an increase in $LPLA_2$ protein levels.

$LPLA_2$ induction by retinoic acids in THP-1 cells

ATRA is known to activate retinoic acid response genes (11). Also, retinoic acid induces the differentiation of many types of cells, including THP-1 cells (5–7, 9). We next focused on $LPLA_2$ induction by retinoic acid in THP-1 cells.

$LPLA_2$ mRNA and activity were induced in a concentration-dependent manner (**Fig. 2A**). THP-1 cells were then treated with 10 μM ATRA for 1, 3, and 5 days. Both $LPLA_2$ mRNA and activity were increased in a time-dependent manner (Fig. 2B). To understand the mechanism underlying the enhancement of $LPLA_2$ mRNA and activity levels by ATRA, THP-1 cells were treated with 10 μM ATRA for 4 days and then exposed to 1 μg/ml actinomycin D, a potent inhibitor of RNA polymerase II. $LPLA_2$ mRNA levels were significantly higher in the ATRA-treated cells (Fig. 2C). When cells were incubated with actinomycin D for 1, 3, and 6 h, a significant reduction in $LPLA_2$ mRNA was observed at 1 and 3 h after treatment in both ATRA-treated and untreated cells. The relative reduction was slightly greater in ATRA-treated cells compared with cells not exposed to ATRA. Thus, the increase in $LPLA_2$ mRNA observed in the presence of ATRA was not attributable to an increase in mRNA stability. By 6 h, the mRNA levels were less than 10% of those observed in the absence of actinomycin D (Fig. 2D).

There are several natural retinoic acid isomers that display biological activity in a number of cell lines and tissues (12). THP-1 cells were treated with 10 μM ATRA, 9-*cis*-retinoic acid, or 13-*cis*-retinoic acid for 3 days. 9-*Cis*- and 13-*cis*-retinoic acids showed the same effect as ATRA on $LPLA_2$ induction (**Fig. 3**). These retinoic acids can be interconvertible within the cell (13–15).

Effect of TTNPB or methoprene acid on $LPLA_2$ induction in THP-1 cells

There are two types of nuclear receptors involved in retinoic acid signaling. One is the RAR that binds ATRA and 9-*cis*-retinoic acid (16, 17). The other is the RXR that binds 9-*cis*-retinoic acid specifically (18–20). Ligand binding results in the activation of each receptor. The activated receptor monomer forms a homodimer or a heterodimer with another activated nuclear receptor and then activates a target gene (21). To identify the nuclear receptor that responds to $LPLA_2$ induction by retinoic acid in THP-1 cells, RAR and RXR agonists were used.

TTNPB was used as an RAR agonist (22), and methoprene acid was used as an RXR agonist (23). THP-1 cells were treated with TTNPB and/or methoprene acid for 3 days. TTNPB treatment resulted in a slight increase of the mRNA expression and activity of $LPLA_2$ (**Fig. 4**). By contrast, the RXR agonist methoprene acid enhanced $LPLA_2$ gene expression, as did ATRA (Fig. 4). However, methoprene acid did not work with TTNPB additively or synergistically, suggesting that the formation of an RAR-RXR dimer is not related to the upregulation of $LPLA_2$ gene expression by retinoic acid in THP-1 cells. These results indicate that RXR is primarily involved in $LPLA_2$ gene activation by retinoic acid in THP-1 cells and also suggest that 9-*cis*-retinoic acid converted from other retinoic acids in the THP-1 cells acts as an active ligand in $LPLA_2$ gene activation by retinoic acid.

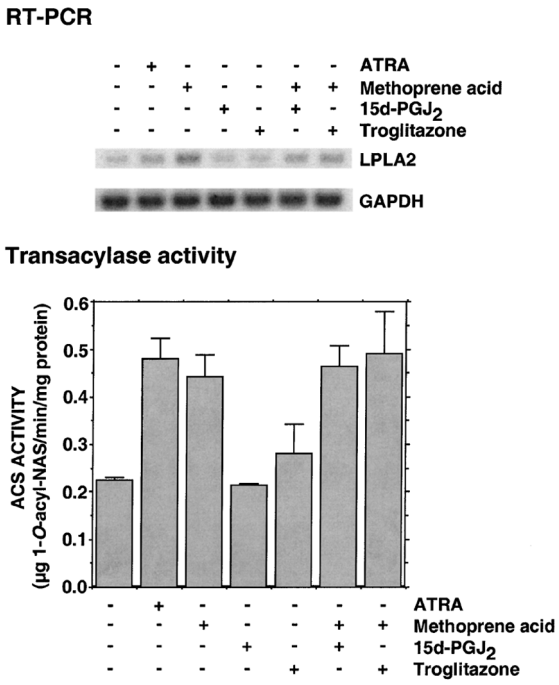

Fig. 5. Effect of peroxisome proliferator-activated receptor γ agonist on $LPLA_2$ induction by RXR agonist in THP-1 cells. One million THP-1 cells were seeded and treated in the presence of 10 μM ATRA, 100 μM methoprene acid, 3 μM 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), 15 μM troglitazone, 100 μM methoprene acid plus 3 μM PGJ_2, or 100 μM methoprene acid plus 15 μM troglitazone for 3 days. After treatment, $LPLA_2$ messenger level and $LPLA_2$ activity in the treated cells were measured as described in Materials and Methods. The ACS values represent means ± SD of three independent measurements. The specific activities and *P* values of the agonist-treated groups versus the control group were as follows: control, 0.225 ± 0.006; ATRA, 0.479 ± 0.043 (0.0005); methoprene acid, 0.442 ± 0.043 (0.009); 15d-PGJ_2, 0.215 ± 0.009 (0.039); troglitazone, 0.280 ± 0.061 (0.196); methoprene acid plus PGJ_2, 0.465 ± 0.040 (0.0005); methoprene acid plus troglitazone, 0.490 ± 0.085 (0.006).

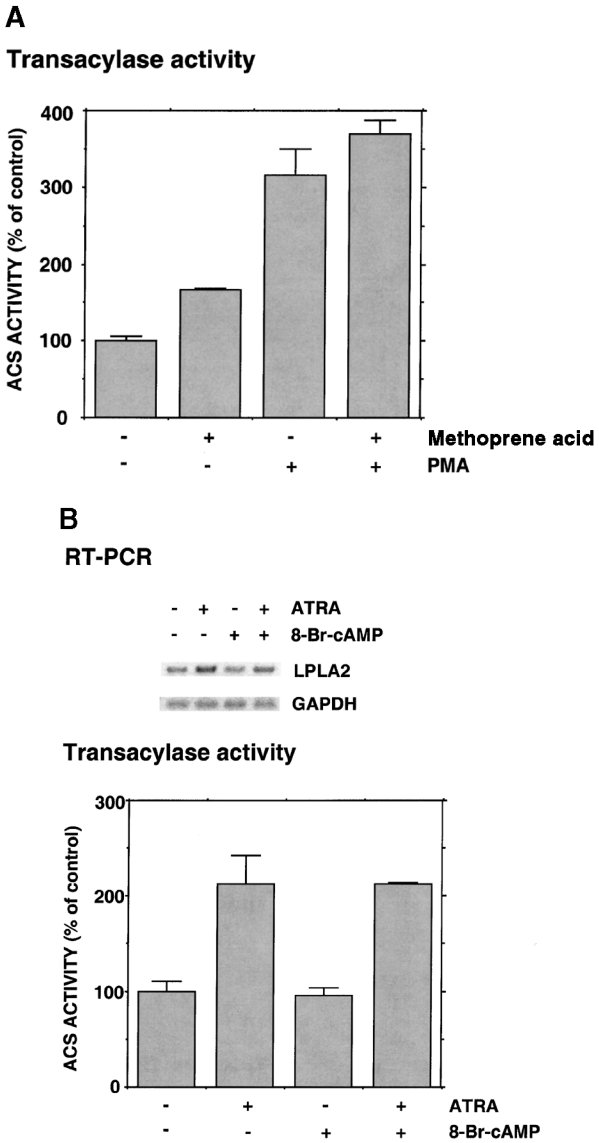

Fig. 6. Effect of PMA or 8-bromo-cAMP (8-Br-cAMP) on $LPLA_2$ induction by RXR agonist or ATRA in THP-1 cells. One million THP-1 cells were seeded and treated in the presence of 100 μM methoprene acid, 10 nM PMA, or 100 μM methoprene acid plus 10 nM PMA for 3 days (A) or 10 μM ATRA, 1 mM 8-bromo-cAMP, or 10 μM ATRA plus 1 mM 8-bromo-cAMP for 3 days (B). After treatment, $LPLA_2$ activity or $LPLA_2$ messenger level in the treated cells was measured as described in Materials and Methods. The ACS values represent means ± SD of three independent measurements. For panel A, the specific activities and *P* values of the agonist-treated groups versus the control group were as follows: control, 0.267 ± 0.014; methoprene acid, 0.443 ± 0.067 (4.28×10^{-5}); PMA, 0.843 ± 0.089 (0.004); methoprene acid plus PMA, 0.988 ± 0.043 (1.06×10^{-5}).

Effect of RXR or PPARγ agonists on $LPLA_2$ expression in THP-1 cells

Recently, RXR was found to form a heterodimer with PPARγ, which is one of the lipid-sensing nuclear receptors (24). The possibility that PPARγ is involved in $LPLA_2$ gene activation by retinoic acid was next considered. In this study, PGJ_2 and troglitazone were used as PPARγ agonists (25–28). The cells were treated for 3 days with these agents. Neither PGJ_2 nor troglitazone significantly enhanced $LPLA_2$ gene expression (**Fig. 5**). Also, these ligands did not work additively or synergistically with methoprene acid to increase $LPLA_2$ expression. These results indicate that $LPLA_2$ gene activation by retinoic acid in THP-1 cells is independent of PPARγ.

The effect of PMA or cAMP on $LPLA_2$ induction via RXR in THP-1 cells

Phorbol myristate acetate is able to activate $LPLA_2$ gene induction to a greater degree than ATRA, suggesting the possibility that PMA augments the induction pathway of $LPLA_2$ by retinoic acid (Fig. 1). Therefore, THP-1 cells were treated with methoprene acid and/or phorbol ester for 3 days. The increase of enzyme activity in the cells treated with both agonists was additive (**Fig. 6A**). cAMP is known to enhance the expression of some genes synergistically with ATRA (5, 29, 30).The cell-permeable cAMP analog, 8-bromo-cAMP, did not have any effect on $LPLA_2$ induction in the presence or absence of ATRA in THP-1 cells (Fig. 6B). These results suggest that the upregulation of $LPLA_2$ gene expression by ATRA is independent of a protein kinase C (PKC)- or PKA-related pathway.

In summary, three primary observations resulted from this study. First, the transcriptional expression and enzyme activity of $LPLA_2$ is increased as a function of its concentration and time after ATRA exposure. These changes do not appear to be the result of an increase in mRNA stability. Second, the upregulation of $LPLA_2$ gene expression by retinoic acid in THP-1 cells is primarily attained via an RXR-dependent pathway, which is independent of RAR or PPARγ. Third, the RXR-dependent pathway is not affected by the PKA or PKC pathway.

These results are consistent with a direct role for RXR in the transcriptional regulation of $LPLA_2$. However, the time course for the increase in $LPLA_2$ mRNA in response to ATRA and the RXR agonist is prolonged, lasting several days. Thus, more global, retinoid-dependent changes in THP-1 cells may be contributing to the increases in mRNA and enzyme activities. Future studies on the $LPLA_2$ promotor will be required to delineate this possibility.

THP-1 cells differentiate and acquire macrophage-like properties and phagocytose foreign substances such as cell fragments, microorganisms, and lipoproteins. The substances ingested into the cell are sorted within an endosome or phagosome by receptor- or nonreceptor-mediated endocytosis (31–34). The endosome or phagosome fuses with a lysosome that contains a variety of hydrolases. As a result, the foreign substances are degraded. Retinoic acid may stimulate the degradation or turnover of such foreign substances containing phospholipids in phagocytic THP-1 cells.

The observed upregulation of $LPLA_2$ gene expression by retinoic acid may be a cell- or tissue-specific event because retinoic acid has failed to induce $LPLA_2$ gene expression in COS-7, MDCK, or T293 cells (our unpublished data). A better understanding of the molecular mechanisms of $LPLA_2$ gene expression by retinoic acid will require the study of the retinoic acid response elements regulating the $LPLA_2$ gene.

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