Phospholipid sources of metabolically elongated gammalinolenic acid: Conversion to prostaglandin E_1 in stimulated mouse macrophages

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*We have previously demonstrated that macrophages possess an active long chain polyunsaturated fatty acid elongase capable of converting (>85%) gammalinolenic acid (18:3n-6, GLA) to dihomogammalinolenic acid (20:3n-6, DGLA), which, following cell stimulation, is converted to prostaglandin E_1 (PGE_1) (Chapkin, R.S. and Coble, K.J. (1991). Biochimica et Biophysica Acta **1085**, 365–370). This is noteworthy because PGE_1 is an eicosanoid with anti-aggregatory and anti-inflammatory properties. In the present study, mouse peritoneal macrophages were incubated with [^{14}C]GLA and [^{3}H]-glycerol for 20 hr and subsequently stimulated with calcium ionophore A23187 (phospholipase A_2 activator), phorbol ester (PMA, protein kinase C activator), PMA + A23187, merthiolate (lysophosphatide acyltransferase inhibitor) + A23187, or nothing. Following stimulation, PMA + A23187 and merthiolate + A23187 treated cells had significantly ($P < 0.05$) increased levels of [^{14}C]-(PGE_1) and [^{14}C]-prostaglandin E_2 (PGE_2) biosynthesis compared with A23187, PMA, and nonstimulated treatments. [^{14}C]-fatty acid (primarily DGLA) was primarily incorporated into phosphatidylcholine (PC) (64.8 ± 1.3%, in nonstimulated cells). A23187, PMA, PMA + A23187, and merthiolate + A23187 treatments had significantly ($P < 0.05$) decreased levels of [^{14}C]-PC and increased ($P < 0.05$) levels of [^{3}H]-lyso-PC relative to nonstimulated cells. Therefore, in vitro activation of phospholipase A_2 and inhibition of [^{14}C]DGLA (derived from [^{14}C]GLA) reacylation can significantly ($P < 0.05$) enhance [^{14}C]-(PGE_1) biosynthesis. These data indicate the regulatory importance of [^{14}C]DGLA reacylation relative to phospholipase A_2 activity in mouse peritoneal macrophage PGE_1 biosynthesis.* (J. Nutr. Biochem. **4**:602–607, 1993.)

Keywords: gammalinolenic acid, dihomogammalinolenic acid, prostaglandin E_1, deacylation-reacylation cycle, phospholipase A_2, lysophosphatide acyltransferase.

Introduction

Macrophages play a central role in the immune system and are capable of modulating several important biological functions.[1] In a previous study,[2] we determined that macrophages possess an active long chain polyunsaturated fatty acid elongase capable of converting gammalinolenic acid (18:3n-6, GLA) to dihomogammalinolenic acid (20:3n-6, DGLA), which, upon stimulation, is converted to prostaglandin E_1 (PGE_1).[3] In addition, we have shown that macrophage PGE_1 biosynthesis is enhanced by feeding GLA-rich dietary oils.[3] This has biological relevance because of the well-documented anti-aggregatory and anti-inflammatory properties of PGE_1.[4,5]

In general, arachidonic acid (20:4n-6, AA) is the primary substrate for prostaglandin synthase (cyclooxygenase, EC 1.14.99.1).[6–8] DGLA, relative to AA, is normally present at a much lower concentration in membrane phospholipids.[6–8] Interestingly, supplementation with dietary oils containing GLA can significantly enrich tissues in DGLA.[3,9,10] This is the result of the presence of an active long chain polyunsaturated fatty acid (PUFA) elongase and modest Δ5 desaturase activity.[2] Therefore, following GLA alimentation, membrane phospholipid

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DGLA can be enhanced, thereby augmenting DGLA-dependent eicosanoid synthesis.[3,11,12]

It is well known the PGE_1 biosynthesis is primarily dependent on the abundance of nonesterified DGLA (derived from GLA), which is released from membrane phospholipids.[6-8] However, the mechanisms regulating DGLA release from membrane phospholipid pools following GLA supplementation has not been fully elucidated. For example, although PUFA can be released from macrophage membrane phospholipids primarily by phospholipase A_2 (PLA_2),[13] the significance of lysophosphatide acyltransferase (LAT)-dependent reacylation of liberated DGLA remains to be determined. Calcium ionophore (A23187) can activate PLA_2 via Ca^{2+} translocation and promote the formation of prostaglandins in macrophages.[14] In addition, the activation of protein kinase C (PKC) is involved in the regulation of prostaglandin synthesis in resident macrophages.[15,16] PKC can enhance PLA_2 activity[17] and directly increase the release of PUFA from membrane phospholipids. PKC may also indirectly increase the concentration of nonesterified PUFA by inhibiting LAT activity.[15] To determine the metabolic turnover of GLA-derived DGLA in mouse peritoneal macrophages, we labeled macrophages with [^{14}C]-GLA to mimic in vivo DGLA formation and examined the effect of phorbol-12-myristate-13-acetate (PMA), a potent and specific PKC activator,[18] and ethylmercurisalicylate (merthiolate), an LAT inhibitor,[19] on the macrophage phospholipid deacylation-reacylation cycle and GLA-dependent prostaglandin biosynthesis.

Methods and materials

Materials

[1-^{14}C]-6c, 9c, 12c-gammalinolenic acid (GLA, 55 mCi/mmole, 99% radiochemical purity) was purchased from American Radiolabeled Chemicals (St. Louis, MO USA). [1, 2, 3-^{3}H]-glycerol (55.7 Ci/mmole, 99% radiochemical purity) was purchased from New England Nuclear (Boston, MA USA). All tissue culture media was from Whitaker M. A. Bioproducts (Walkersville, MO USA). Fetal bovine serum (FBS) was from Hyclone (Logan, UT USA). Gentamicin and penicillin-streptomycin were from Gibco Laboratories (Grand Island, NY USA). Divalent cation ionophore (A23187) was from Calbiochem (San Diego, CA USA). PMA was from LC Services Corporation (Woburn, MA USA). Merthiolate and α-naphthyl acetate esterase staining kit were from Sigma Chemical (St. Louis, MO USA). Phospholipid and lyso-phospholipid standards were from Avanti Polar Lipids, Inc. (Birmingham, AL USA). Prostaglandin standards were from Cayman Chemicals (Ann Arbor, MI USA). Silica gel 60 G plates were from E. Merck (Darmstadt, Germany). All solvents were of Optima grade (Fisher Scientific, Fair Lawn, NJ USA). C57BL/6 female mice were from Charles River (Wilmington, MA USA).

Methods

Macrophage cultures. Mouse peritoneal macrophages were isolated by adherence from resident cells as previously described.[20] Briefly, peritoneal exudate cells were obtained by lavage using Hanks' balanced salt solution (HBSS) supplemented with 10 U/mL heparin. Cells were pooled from several mice, centrifuged for 5 min at 500*g*, rinsed with HBSS and plated in 35 mm culture dishes using 2 mL media A (alpha-MEM containing 2 mM glutamine, 20 mM HEPES, 5 μg/mL gentamicin and 100 U/mL penicillin-streptomycin) supplemented with 5% heat-inactived FBS. After 2 hr at 37° C in 5% CO_2, nonadherent cells were removed by vigorous rinsing with HBSS. Greater than 95% of the adherent cells were found to be macrophages as quantified by α-naphthyl acetate esterase staining.

Macrophage labeling and stimulation. Cells prepared as described above were incubated in media A supplemented with 1% FBS and 0.6 μCi [^{14}C]GLA (5.4 μM) and 125 μCi [^{3}H]-glycerol (1.1 μM) for 20 hr at 37° C in 5% CO_2. A 20 hr labeling period was chosen because a maximum of >85% of [^{14}C]GLA is elongated to [^{14}C]DGLA during this period.[2] Cells were subsequently washed with HBSS and incubated in media A containing one of five different treatments: (1) NS (no stimulation); (2) A23187 (5 μM, 30 min); (3) PMA (100 nM, 2 hr); (4) PMA + A23187 (preincubation with 100 nM PMA 2 hr, followed by 5 μM A23187, 30 min); and (5) MER + A23187 (50 μM merthiolate preincubated for 5 min, followed by 5 μM A23187, 30 min). [^{3}H]/[^{14}C] uptake and release was determined by scintillation counting of cellular and media extracts.[20] Selected monolayers were solubilized in 0.1 M sodium hydroxide for protein determination using a modified Lowry method.[21]

Macrophage harvesting and lipid extraction. Following incubation, cells and media were collected separately. The cells were washed twice with phosphate-buffered saline (PBS) containing 0.1% fatty acid-free bovine serum albumin (BSA). Monolayers were then scraped into chloroform/methanol (2:1, vol/vol) and extracted by the method of Bligh and Dyer.[22] The organic layer was withdrawn and used for thin layer chromatography (TLC).[23] Incubation media were centrifuged at 800*g* for 5 min, supernatants were acidified to pH 3 with 1 M citric acid, and subsequently Folch extracted.[24] The supernatant extracts were analyzed using high performance liquid chromatography (HPLC) as described below.

Cellular lipid separation. The phospholipid and lyso-phospholipid classes were separated by two-dimensional TLC using the method of Thomas and Holub.[23] Briefly, samples were separated on silica gel 60 G plates using chloroform/methanol/ammonium hydroxide (65:35:5.5, vol/vol) as the first developing solvent and using chloroform/methanol/88% formic acid/water (55:28:5:1, vol/vol) as the second developing solvent. Following chromatography, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA), sphingomyelin (SPH), cardiolipin (CLP), lyso-phosphatidylcholine (lyso-PC), lyso-phosphatidylethanolamine (lyso-PE), lyso-phosphatidylinositol (lyso-PI), lyso-phosphatidylserine (lyso-PS), lyso-phosphatidic acid (lyso-PA), and neutral lipids (NL) were scraped from the TLC plate into vials for liquid scintillation counting.[25]

Identification of radiolabeled metabolites. The supernatant extracts were chromatographed using two reverse-phase HPLC systems. 1- and 2-series cyclooxygenase products were separated using an acetonitrile/1.9 mM *o*-phosphoric acid gradient as previously described.[2,26] Cyclooxygenase and lipoxygenase products were separated using a methanol/water gradient as previously described.[2,27] Radiolabeled metabolites were quan-

titated using an on-line radioactivity detector (Flo-one, Radiomatic, Tampa, FL USA).[2,26,27]

Statistical analysis. Data were analyzed using one-way analysis of variance (ANOVA) and Fisher's least significant difference procedure for comparing treatment means.[28] A value of $P < 0.05$ was considered statistically significant.

Results

The percent of radiolabel uptake at 20 hr was 29.9 ± 2.8 for [^{14}C]GLA and 7.2 ± 0.9 for [^{3}H]-glycerol (*Table 1*). The effect of stimulation on macrophage [^{14}C] fatty acid release is also shown in *Table 1*. MER + A23187 treatment had a significantly ($P < 0.05$) higher level of [^{14}C] release compared with the other treatments.

The distribution of [^{14}C]-fatty acid (primarily DGLA)[2] in the individual phospholipids and neutral lipids is shown in *Table 2*. The radiolabel was primarily incorporated into PC. The level of [^{14}C]-PC in NS cells was significantly ($P < 0.05$) higher relative to stimulated macrophages (except PMA treatment). The percent [^{14}C]-distribution in PC relative to cellular lipids was: NS, 64.8 ± 1.3%; A23187, 61.6 ± 1.2%; PMA, 66.7 ± 1.4%; PMA + A23187, 59.7 ± 1.1%; MER + A23187, 60.0 ± 0.5%, indicating a maximum liberation of ^{14}C-fatty acid from the A23187, PMA + A23187 and MER + A23187 groups. In general, A23187, A23187 + PMA, and MER + A23187 treatments had the lowest levels of [^{14}C]-PI. In addition, A23187 treatment had a significantly ($P < 0.05$) increased level of [^{14}C]-PA compared with the other treatments.

Table 3 shows the macrophage lysophospholipid composition following [^{3}H]-glycerol metabolic labeling. All stimulated cultures had increased formation of lyso-PC. PMA significantly ($P < 0.05$) increased the formation of [^{3}H]-lyso-PE compared with the other treatments. No significant differences ($P > 0.05$) were found

Table 1 Percentage of radiolabel uptake and release

20-hrs					
			[^{3}H]		[^{14}C]
dpm added to media (a)			(2.3 ± 0.0) × 10^{8}		(1.1 + 0.1) × 10^{6}
dpm remaining in media after 20 hr (b)			(2.1 ± 0.3) × 10^{8}		(7.7 ± 0.9) × 10^{5}
dpm incorporated after 20 hr (a-b)			(1.6 ± 0.2) × 10^{7}		(3.3 ± 0.4) × 10^{5}
% Uptake* (a-b)/a			7.2 ± 0.9%		29.9 ± 2.8%
After stimulation					
	NS	A23187	PMA	PMA + A23187	MER + A23187
[^{14}C] % release†	2.0 ± 0.3^{a}	5.4 ± 0.8^{b}	5.3 ± 0.9^{b}	7.4 ± 1.1^{b}	11.4 ± 1.2^{c}

$$*\%\ \text{uptake} = \frac{\text{initially added dpm} - \text{dpm remaining in media after 20 hr incubation}}{\text{initially added dpm}} \times 100$$

$$\dagger\%\ \text{release} = \frac{\text{dpm in media following stimulation}}{\text{dpm incorporated by cells during the metabolic labeling period (20 hr)}} \times 100$$

Macrophages were incubated with [^{3}H]-glycerol and [^{14}C]GLA for 20 hr, after which the nonadherent cells and radiolabel remaining in the media were removed. Fresh radiolabel-free media was subsequently added and cells stimulated with different treatments as described in the Methods and materials. The percentage of [^{3}H] and [^{14}C] incorporated at 20 hr and [^{14}C] released after stimulation was determined.
Results are expressed as means ± SEM (n = 30 for 20-hours, n = 6 for after stimulation) from two separate experiments. Row values with the same or no superscript are not significantly different ($P > 0.05$).
NS, no stimulation; A23187, stimulated with A23187 alone; PMA, stimulated with PMA alone; PMA + A23187, stimulated with PMA and A23187; MER + A23187, stimulated with merthiolate and A23187.

Table 2 Distribution of [^{14}C] radiolabel in macrophage individual phospholipids and neutral lipids

	NS	A23187	PMA	PMA + A23187	MER + A23187
PC	30160 ± 2157^{c}	22205 ± 1181^{ab}	28451 ± 2959^{b}	22007 ± 1869^{a}	20571 ± 1677^{a}
PE	1503 ± 401	1554 ± 93	656 ± 108	1486 ± 296	1410 ± 228
PS	1973 ± 196	1827 ± 188	926 ± 121	1837 ± 257	1878 ± 287
PI	4686 ± 296^{bc}	3912 ± 272^{ab}	5245 ± 475^{c}	3470 ± 384^{a}	3790 ± 207^{a}
PA	415 ± 63^{a}	950 ± 96^{c}	380 ± 68^{a}	620 ± 53^{b}	422 ± 19^{a}
SPH	516 ± 36	572 ± 42	631 ± 105	603 ± 44	537 ± 53
CLP	1013 ± 91	883 ± 55	1116 ± 112	970 ± 70	831 ± 36
NL	5722 ± 577^{b}	3616 ± 151^{a}	4738 ± 613^{ab}	5408 ± 262^{b}	4393 ± 286^{a}

Macrophages were incubated with [^{14}C]GLA for 20 hr. Cellular lipids were extracted and separated by two-dimensional TLC as described in the Methods and materials. Data are shown as dpm/100 μg protein.
Results are expressed as means ± SEM (n = 6) from two separate experiments. Row values with the same or no superscript are not significantly different ($P > 0.05$).
PC, phosphatidylycholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; SPH, sphingomyelin; CLP, cardiolipin; NL, neutral lipids.

Table 3 Distribution of [^{3}H]-glycerol in macrophage individual lsyo-phospholipids

	NS	A23187	PMA	PMA + A23187	MER + A23187
lyso-PC	6593 ± 778^a	12275 ± 1724^b	9368 ± 1091^{ab}	12346 ± 1207^b	11913 ± 913^b
lyso-PE	1729 ± 352^a	2855 ± 434^b	5519 ± 91^c	2318 ± 461^{ab}	2418 ± 273^{ab}
lyso-PS	1122 ± 148	965 ± 136	880 ± 62	1012 ± 126	827 ± 95
lyso-PI	1281 ± 142	1363 ± 134	1390 ± 363	1529 ± 300	1400 ± 147
lyso-PA	284 ± 40	240 ± 33	207 ± 48	257 ± 47	190 ± 30

Macrophages were incubated with [^{14}C]GLA for 20 hr. Cellular lipids were extracted and separated by two-dimensional TLC as described in the Methods and materials. Data are shown as dpm/100 μg protein.
Results are expressed as means ± SEM (n = 6) from two separate experiments. Row values with the same or no superscript are not significantly different ($P > 0.05$).
PC, phosphatidylycholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; SPH, sphingomyelin; CLP, cardiolipin; NL, neutral lipids.

in [^{3}H]-lyso-PS, [^{3}H]-lyso-PI, and [^{3}H]-lyso-PA among the five different treatments.

The major cyclooxygenase products in the supernatant were [^{14}C]-PGE_1 and [^{14}C]-PGE_2. The conversion of [^{14}C]GLA (via [^{14}C]DGLA) into [^{14}C]-PGE_1 and [^{14}C]-PGE_2 is shown in *Table 4*. The levels of [^{14}C]-PGE_1 were much higher than [^{14}C]-PGE_2 in the four stimulated treatments ($E_1/E_2 \approx 4$). PMA + A23187 treatment had the highest ratio of [^{14}C]-PGE_1:[^{14}C]-PGE_2 (≈ 5.3). In addition, PMA + A23187 and MER + A23187 treatments had the highest levels of [^{14}C]-PGE_1 and [^{14}C]-PGE_2 relative to the other treatments. There was also a significant difference between A23187 and PMA treatments with regard to the levels of [^{14}C]-PGE_1 and [^{14}C]-PGE_2. In general, except for A23187 treatment, the summed dpm for [^{14}C]-PGE_1 and [^{14}C]-PGE_2 was proportional (≈ 90%) to the loss of [^{14}C] from phospholipid (*Table 2*). Consistent with previous work,[2,3] there was no detectable [^{14}C]-PGE_1 and [^{14}C]-PGE_2 in NS incubations. In addition, there were no detectable [^{14}C]GLA-derived lipoxygenase products in incubation supernatants after stimulation, as determined by reverse phase and normal phase HPLC analysis (data not shown).

Discussion

It is well established that prostaglandin biosynthesis is dependent on the pool size of nonesterified fatty acids derived from membrane phospholipids.[6–8] The hydrolysis of fatty acyl bonds is catalyzed by phospholipase A_2,[29] and has generally been regarded as the rate-limiting step in prostaglandin biosynthesis.[13] It was recently demonstrated, however, that the kinetics of fatty acid reincorporation of released fatty acids by acyltransferases may have been underestimated.[15,30,31] In the present study, we monitored GLA incorporation and cyclooxygenase metabolism of DGLA-derived from GLA in the mouse peritoneal macrophage to determine phospholipid pool source(s) of PGE_1.

Mouse peritoneal macrophages were metabolically labeled with [^{3}H]-glycerol and [^{14}C]GLA to clarify the relationship between cellular membrane phospholipids and lysophospholipids. Examination of the radiolabel levels in individual phospholipids and lysophospholipids (*Tables 2 and 3*) shows that [^{14}C] was primarily incorporated into PC, whereas with regard to lysophospholipids, [^{3}H] was largely associated with lysophosphatidylcholine (lyso-PC). Macrophages stimulated with A23187, PMA, PMA + A23187, and MER + A23187 had significantly ($P < 0.05$) decreased [^{14}C]-PC levels relative to NS treatment. In contrast, A23187, PMA + A23187, and MER + A23187 had significantly ($P < 0.05$) increased [^{3}H]-lyso-PC levels compared with NS treatment. These results indicate that [^{14}C]GLA (extensively elongated to [^{14}C]DGLA) was primarily incorporated into PC and, upon stimulation, liberated from PC, thereby increasing the formation of lyso-PC. Therefore, PC is the predominant source of DGLA and AA (derived from GLA) for prostaglandin synthesis in the macrophage. Interestingly, the levels of [^{14}C]-PA in A23187 and PMA + A23187 treatments were elevated relative to NS treatment. Although the levels of [^{14}C]-

Table 4 Macrophage conversion of [^{14}C]-GLA (via [^{14}C]-DGLA) into [^{14}C]-prostaglandin E_1 and [^{14}C]-prostaglandin E_2

	NS	A23187	PMA	PMA + A23187	MER + A23187
PGE_1	n.d.^{a}	4550 ± 510^c	2676 ± 427^b	8126 ± 1780^d	8159 ± 1750^d
PGE_2	n.d.^{a}	1255 ± 146^c	741 ± 99^b	1524 ± 225^c	2087 ± 438^c
PGE_1/PGE_2	—	3.71	3.61	5.33	3.91

Macrophages were incubated with [^{14}C]GLA and [^{3}H]-glycerol for 20 hr. Cells were rinsed with HBSS and stimulated with different treatments. Refer to Table 1 for details. Media extracts were analyzed by reverse phase HPLC as described in the Methods and materials.
Data are shown as dpm/100 μg protein. Results are expressed as means ± SEM (n = 6) from two separate experiments. Row values with the same superscript are not significantly different ($P > 0.05$).
n.d., not detected.

PA (compared with [^{14}C]-PC) were low and are likely of minimal importance relative to prostaglandin synthesis, its formation likely indicates the enzymatic involvement of phospholipase D and/or combined phospholipase C-diglyceride kinase.

Macrophages incubated with [^{14}C]GLA, upon stimulation, rapidly synthesized [^{14}C]-PGE$_1$ and [^{14}C]-PGE$_2$ ([^{14}C]-PGE$_1$>>[^{14}C]-PGE$_2$)(*Table 4*). This is consistent with our previous observations and confirms that macrophages possess an extremely active PUFA elongase and modest Δ5 desaturase activity, capable of converting GLA primarily to DGLA.[2,20,32] Also, the inverse relationship between [^{14}C]-PGE$_1$ and [^{14}C]-PGE$_2$ formation (*Table 4*) and the total [^{14}C] loss from the lipid esters in stimulated cells (*Table 2*) is consistent with other studies,[6–8] indicating that prostaglandin synthesis is dependent on the pool size of nonesterified fatty acids derived from membrane phospholipids. It is noteworthy that PMA ± A23187 and MER + A23187 treatments had significantly ($P < 0.05$) increased levels of [^{14}C]-PGE$_1$ (8126 and 8159 dpm/100 μg protein, respectively) compared with A23187 and PMA treatments (4550 and 2676 dpm/100 μg protein, respectively). The enhancement of PGE$_1$ synthesis in MER + A23187 treatment can be attributed to the inhibition of lysophospholipid reacylation and thereby an increase in the size of the nonesterified fatty acid pool for prostaglandin synthesis.[33] With regard to PMA + A23187 incubations, intracellular calcium mobilization and PKC activation may both be involved in the activation of phospholipase A$_2$ (PLA$_2$).[14,34] For example, it has been demonstrated that PMA has a major effect on the activation of PLA$_2$ by increasing PKC activity, thereby enhancing the liberation of fatty acids from membrane phospholipids.[35] In addition, PKC activation may increase the concentration of nonesterified PUFA by inhibiting LAT activity.[15] Therefore, the enhancement of PGE$_1$ synthesis in PMA + A23187 treatment relative to A23187 or PMA treatments can be attributed to the additive effects of A23187 and PMA on PLA$_2$ and LAT activities. These results demonstrate that relative to PLA$_2$ activity, the inhibition of lysophospholipid reacylation has a proportionally significant effect on enhancing in vitro macrophage PGE$_1$ biosynthesis. Interestingly, PMA + A23187 treatment had the highest ratio of [^{14}C]-PGE$_1$:[^{14}C]-PGE$_2$ among treatments. The data suggest that PLA$_2$ fatty acid specificity can be influenced by agonist treatment. Further studies are required to clarify this observation.

In conclusion, both the inhibition of GLA-derived nonesterified fatty acid reacylation by the lysophosphatide acyltransferase inhibitor, merthiolate, and the activation of phospholipase A$_2$ by calcium ionophore (A23187) and the protein kinase C activator, PMA, can augment in vitro macrophage prostaglandin synthesis. In addition, with regard to [^{14}C]PGE$_1$ biosynthesis, the present data demonstrate the regulatory importance of [^{14}C]DGLA reacylation relative to phospholipase A$_2$ activity. Further studies are required to determine the ability of agents to selectively impair lysophosphatide acyltransferase and thereby enhance macrophage PGE$_1$ biosynthesis. This is significant, in view of the anti-inflammatory, anti-aggregatory properties of PGE$_1$.

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