Protein kinase C promotes arachidonate mobilization through enhancement of CoA-independent transacylase activity in platelets

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A role for protein kinase C in arachidonate mobilization was demonstrated. Treatment of rat platelets with phorbol myristate acetate (PMA) or the diacylglycerol 1-oleoyl-2-acetyl-glycerol increased the transfer rate of arachidonate (AA) from phosphatidylcholine to phosphatidylethanolamine and stimulated AA release. The transfer dose-dependently induced by PMA was inhibited by staurosporine. Ether phospholipids were the acceptors of AA in these stimulated transfer reactions. Membrane-bound protein kinase C activity was enhanced by PMA, and this increase was inhibited by staurosporine. AA transfer between phospholipids is due to the action of polyunsaturated-fatty-acid-specific transacylases. For this purpose, transacylase activities were assayed in cell-free systems from PMA-treated platelets. We observed that the CoA-independent transacylase activity was modulated in parallel to AA transfer as a function of PMA concentration. Taken together, the data show that protein kinase C activation might promote the mobilization of AA in platelets through the enhancement of CoA-independent transacylase activity.

INTRODUCTION

Activation of arachidonate (AA) release is currently considered as the principal mechanism for stimulation of eicosanoid synthesis. Platelets respond to a variety of agonists by rapidly releasing AA from cellular phospholipids, where it is predominantly esterified. However, the mechanisms which control the AA release are not completely understood. Receptor occupancy induces phosphoinositide hydrolysis by phospholipase C, promoting the formation of the intracellular messengers inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, which are responsible for the release of intracellular Ca^{2+} stores and the activation of protein kinase C respectively [1,2]. These early events could be responsible for AA mobilization from the major classes of phospholipids. Previous studies have shown that, in platelets [3–6], this mobilization involves AA transfer from diacyl-*sn*-glycero-3-phosphocholine (diacyl-GPC) to diacyl and ether phospholipids, together with AA release. Moreover, AA-specific transacylation reactions were demonstrated in various cell membranes [7–12].

Since phospholipase A_2 is a Ca^{2+} -dependent enzyme and Ca^{2+} ionophores are effective releasers of AA, phospholipase A_2 has been thought to play a major role in controlling AA release. Halenda *et al.* [13,14] have shown that the active phorbol esters potentiate AA release induced by the Ca^{2+} ionophore A23187 in platelets, and this potentiation has been interpreted in terms of activating phospholipase A_2 by the protein kinase C system. However, phorbol 12-myristate 13-acetate (PMA) alone, which does not mobilize Ca^{2+} [15,16], is able to release AA [17]. Thus factors other than phospholipase A_2 activation, such as inhibition of AA re-incorporation [18] or stimulation of transacylase reactions, might be involved in the protein kinase C-dependent AA release. Here, we showed that PMA alone was able to induce AA mobilization in rat platelets and that this mobilization might be achieved through modulation of CoA-independent transacylase activity.

MATERIALS AND METHODS

Materials

1,2-Dioleoyl-*sn*-glycero-3-phospho[2- ^{14}C]ethanolamine (1,2-di-

oleoyl-[^{14}C]GPE) (49 mCi/mmol) and [γ - ^{32}P]ATP (3000 Ci/mmol) were from Amersham International, U.K. [1- ^{14}C]AA (58 mCi/mmol) was purchased from CEA, France. 1-[1,2- ^3H]Alkyl-2-lyso-GPC was from Du Pont–New England Nuclear. 1-Acyl-2-lyso-[ethanolamine-2- ^{14}C]GPE was prepared from 1,2-dioleoyl-[^{14}C]GPE (49 mCi/mmol): 500 nmol of 1,2-dioleoyl-GPE was incubated with 5 units of bee venom phospholipase A_2 in 10 mM-Tris/HCl, pH 8.0, and 5 mM- Ca^{2+} for 60 min. Specific radioactivity of the lyso-compound purified on a silicic acid column was 7 mCi/mmol.

The following chemicals were purchased from the companies indicated in parentheses: 1-oleoyl-2-acetyl-glycerol (OAG) and 1-alkyl-2-lyso-GPC (Serdary, Ontario, Canada), staurosporine and bee venom phospholipase A_2 (Boehringer, Mannheim, Germany), gelatin powder (Merck, Darmstadt, Germany). CoA, ATP, histone type III, egg phosphatidylethanolamine, phosphatidylserine, 1,2-diolein, PMA, 4-phorbol 12,13-didecanoate and fatty-acid-free BSA were from Sigma.

AA transfer and lipid analysis

Platelets from 200–250 g Wistar rats were isolated by the method of Ardlie *et al.* [19] in Tyrode buffer, pH 6.5, containing 0.25% gelatin as previously described [4]. [1- ^{14}C]AA (0.25 $\mu\text{Ci}/10^9$ platelets) complexed to fatty-acid-free BSA was added to the platelet suspension (10^9 platelets/ml) and incubated at 37 °C for 30 min. Platelets were then washed twice and resuspended in the same Tyrode buffer, pH 6.5. AA mobilization was observed in platelet suspensions incubated at 37 °C for various periods of time and in the absence or presence of pharmacological agents. OAG was sonicated in Tris buffer, pH 6.5, just before use. PMA, 4-phorbol 12,13-didecanoate and staurosporine were dissolved in dimethyl sulphoxide. The dimethyl sulphoxide concentration in platelet incubations never exceeded 1%. Incubations were stopped by adding chloroform/methanol (1:2, v/v) and the lipids were extracted as described by Bligh & Dyer [20]. Solvents were removed under N_2 , and phospholipids and neutral lipids were separated by double migration in a single direction on silica-gel G thin-layer plates, first in chloroform/methanol/water (30:20:3, by vol.) and, after drying the plate, in light petroleum (b.p. 40–

Abbreviations used: AA, arachidonate; GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine; PMA, 4-phorbol 12-myristate 13-acetate; OAG, 1-oleoyl-2-acetyl-glycerol.

Fig. 1. Time course of OAG- and PMA-induced mobilization of AA

Platelets prelabelled with [^{14} C]AA for 30 min were incubated in Tyrode buffer (◆, control platelets), or in Tyrode buffer containing 20 nM-PMA (▲) or 30 μM-OAG (●) for the indicated time intervals. The reaction was terminated by the addition of chloroform/methanol (1:2, v/v), and distribution of AA labelling in non-esterified fatty acid (a), phosphatidylcholine (b), phosphatidylethanolamine (c) and triacylglycerol (d) fractions was analysed as described in the Materials and methods section. Results, expressed as percentage of total lipid labelling, are shown as mean values of three experiments, $\pm$ S.E.M.

65 °C)/diethyl ether/acetic acid, (90:30:1, by vol.). The alkyl, alkenyl and diacyl classes of phosphatidylethanolamine and phosphatidylcholine were resolved after conversion into glycerobenzoate derivatives as described by Blank *et al.* [21] and previously [6].

Assay of enzymes

Platelets treated with phorbol esters for 2 or 30 min without or with staurosporine and control platelets were, immediately centrifuged, washed and resuspended (4×10^9 platelets/ml) in Tris buffer, pH 6.5, containing 0.2 mM-EDTA. The platelets were then sonicated twice for 20 s each using a microprobe. These broken platelets were assayed for transacylase and protein kinase C activities.

Protein kinase C. The sonicated platelets were centrifuged at 100 000 *g* for 60 min at 4 °C, and protein kinase C was assayed as in [22]. Briefly, the membrane-bound protein kinase C was solubilized by treating the particulate fraction with 0.3 % Triton X-100 in 20 mM-Tris/HCl buffer, pH 7.5, containing 2 mM-EGTA, 2 mM-EDTA, 50 mM-mercaptoethanol and 2 mM-phenylmethanesulphonyl fluoride for 60 min at 4 °C. The non-solubilized material was removed by centrifugation at 100 000 *g* for 60 min at 4 °C. The supernatant from this step was purified by DEAE-cellulose column chromatography performed at 4 °C, and the membrane-bound protein kinase C fraction was eluted with the previous buffer containing 120 mM-NaCl. Protein kinase C activity was immediately assayed in a total volume of 0.1 ml of 40 mM-Hepes buffer, pH 7.4, containing 8 mM-MgCl₂, 0.5 mM-Ca²⁺, 25 μg of histone, 5 mg of phosphatidylserine, 1 mg of 1,2-diolein and 3 μg of sample protein. The lipids were dispersed in the reaction buffer by sonication. The reaction was started by addition of 20 μM- γ -³²P]ATP (10^6 c.p.m./assay). After 3 min at

30 °C, the reaction was stopped by retaining histone on ion-exchange filters (Whatman P81). The filters were washed successively with 1 % H₃PO₄, water, acetone and diethyl ether. The filters were dried and the radioactivity was counted. Protein kinase C activity was determined as the incorporation of ³²P (nmol/min per mg of protein) into histone in the presence of Ca²⁺, phosphatidylserine and diolein minus the incorporation in the absence of these activators.

Transacylases. CoA-independent and CoA-dependent transacylases were evaluated by the acylation rate of labelled 1-ether and 1-acyl lyso-derivatives in the absence and in the presence of 50 μM-CoA. The incubation mixture contained the lyso-derivatives, 0.16 μM-1-[1,2-³H]hexadecyl-2-lyso-GPC or 1.6 μM-1-acyl-2-lysophosphatidyl[2-¹⁴C]ethanolamine, dispersed in Tyrode buffer, added to platelet suspensions. After 10 min at 37 °C, the reaction was stopped by adding chloroform/methanol (1:2, v/v). The lipids were extracted as described above, and the acylated compound was separated from the lyso-derivative by t.l.c. in chloroform/methanol/water (30:20:3, by vol.). Radioactive lipid spots were scraped from the plate and counted for radioactivity.

RESULTS

Effect of protein kinase C activators on AA mobilization

Phorbol esters such as PMA can substitute for diacylglycerol in directly activating protein kinase C. The diacylglycerol OAG and PMA were added to [^{14} C]AA-prelabelled platelets in order to observe the effect of protein kinase C activation on AA mobilization. Platelet lipids were labelled for 30 min. Then free [^{14} C]AA was chased and platelets were reincubated. In resting

Table 1. [^{14} C]AA transfer in the presence of excess of unlabelled AA, BSA or BW 755C

Platelets prelabelled as in Fig. 1 were incubated for 2 h in Tyrode buffer (control platelets), or in Tyrode buffer containing 20 nM-PMA, 10 μ M-AA, 0.25% fatty-acid-free BSA or 100 μ M-BW 755C as indicated. The distribution of labelled AA in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions was analysed and expressed as in Fig. 1. Data are means $\pm$ S.E.M. of triplicates.

	[^{14} C]AA distribution (%)	
	PC	PE
None	48.9 $\pm$ 0.5	23.2 $\pm$ 0.3
PMA	30.6 $\pm$ 0.2	35.4 $\pm$ 0.5
PMA + AA	30.0 $\pm$ 0.7	36.5 $\pm$ 0.6
PMA + AA + BSA	29.6 $\pm$ 0.9	33.8 $\pm$ 1.3
PMA + BW 755C	31.7 $\pm$ 1.1	34.2 $\pm$ 0.8

Fig. 2. Effect of increasing concentrations of PMA on AA mobilization

Prelabelled platelets were incubated for 2 h in Tyrode buffer containing increasing PMA concentrations. Distribution of labelled AA in the phosphatidylcholine (●), phosphatidylethanolamine (▲), phosphatidylinositol (○) and non-esterified fatty acid (◆) fractions was analysed and expressed as in Fig. 1.

platelets, newly incorporated AA was slowly transferred from phosphatidylcholine to phosphatidylethanolamine, as previously observed [23]. Part of the labelled AA was also released from phospholipids. PMA and OAG treatment increased the rate of AA transfer and release (Fig. 1). The [^{14} C]AA transfer from phosphatidylcholine to phosphatidylethanolamine was significantly increased when platelets were treated for 15 min with the protein kinase C activators. The AA release together with the decrease in phosphatidylcholine and increase in phosphatidylethanolamine labelling were twice as high in PMA-treated as in control platelets after re-incubation for 2 h.

The rate of AA release from OAG-treated platelets was lower than in PMA-challenged cells. This should be compared with a higher rate of AA transfer to phosphatidylethanolamine in OAG-treated platelets. However, a considerably higher concentration of OAG than of PMA was necessary to observe an identical decrease in phosphatidylcholine labelling. For this purpose, we used only PMA to investigate further the involvement of protein kinase C in AA mobilization.

To eliminate the possible sequential action of phospholipase A_2 and acyl-CoA acyltransferase in the observed AA changes

Fig. 3. Effect of staurosporine on the mobilization of AA induced by PMA

Prelabelled platelets were incubated in Tyrode buffer containing 20 nM-PMA (▲), 20 nM-PMA plus 1 μ M-staurosporine (●), or no addition (◆), for the indicated time intervals. Distribution of labelled AA was analysed and expressed as described in Fig. 1: non-esterified fatty acid (a), phosphatidylethanolamine (b) and phosphatidylcholine (c) fractions. Results are shown as means of two experiments.

Table 2. Membrane-bound protein kinase C activity

Platelets were treated with 20 nM-PMA or 20 nM-PMA + 1 μ M-staurosporine for 2 min and immediately centrifuged. Plasma membranes and cytosol were separated and a Triton X-100 extract from the membrane fraction was prepared as described in the Materials and methods section. These samples were then chromatographed on DEAE-cellulose, and protein kinase C was eluted with 120 mM-NaCl buffer. Protein kinase C was assayed in the absence and in the presence of Ca^{2+} , phosphatidylserine and diolein and expressed as nmol of ^{32}P transferred to histone. Abbreviation: α -PDD, 4 α -phorbol 12,13-didecanoate.

Addition	Protein kinase C activity (nmol/min per mg)
None	6.4 $\pm$ 1.3 (n = 4)
Staurosporine	4.8 $\pm$ 0.9 (n = 2)
PMA	10.5 $\pm$ 1.6 (n = 4)
PMA + staurosporine	6.9 $\pm$ 0.7 (n = 2)
α -PDD	7.1 $\pm$ 0.5 (n = 2)

induced by protein kinase C activators, the transacylation experiments were performed in the presence of 10 μ M unlabelled AA. This excess of unlabelled AA, added in ethanol or complexed to fatty-acid-free BSA, did not modify PMA-induced AA redistribution in the phospholipids (Table 1). Thus neither an excess of free AA nor trapping by BSA of free AA or oxygenated derivatives of AA prevented AA transfer between phospholipids. This lack of effect of BSA is in accordance with our previous observation showing that the compound BW 755C, an inhibitor of both cyclo-oxygenase and lipoxygenase, had no effect on AA transfer induced by thrombin in platelets [6]. This compound was

Table 3. Stimulation of CoA-independent transacylase by PMA

Sonicates from control, PMA- and PMA + staurosporine-treated platelets were prepared and incubated with hexadecyl-lyso-GPC or acyl-lyso-GPE added as a sonicated dispersion as described in the Materials and methods section. Transacylase activities were assayed by measuring the acylation rate of the lyso-derivatives in the absence or the presence of 50 μ M-CoA (CoA-independent and CoA-dependent transacylase respectively).

	Acylation (%)			
	CoA-independent transacylase		CoA-dependent transacylase	
	Hexadecyl-lyso-GPC	Acyl-lyso-GPE	Hexadecyl-lyso-GPC	Acyl-lyso-GPE
Control	32.7 $\pm$ 2.8 (n = 6)	11.5 $\pm$ 0.8 (n = 5)	11.6 $\pm$ 2.1 (n = 3)	23.6 $\pm$ 3 (n = 3)
PMA (30 min)	44.2 $\pm$ 4.3 (n = 6)	16.2 $\pm$ 1.3 (n = 5)	14 $\pm$ 2 (n = 3)	26.5 $\pm$ 4.8 (n = 3)
PMA + staurosporine (30 min)	36.2 $\pm$ 3.4 (n = 6)	13.3 $\pm$ 0.8 (n = 2)	10.9 $\pm$ 3.5 (n = 3)	23 $\pm$ 3 (n = 2)

Fig. 4. CoA-independent transacylase activity as a function of PMA

CoA-independent transacylase activity was assayed with hexadecyl-lyso-GPC as substrate as in Table 3. The data are means $\pm$ S.D. of one experiment performed in duplicate.

also assayed in PMA-treated platelets and did not prevent AA redistribution from phosphatidylcholine to phosphatidylethanolamine (Table 1).

The effect of PMA on AA mobilization was dose-dependent (Fig. 2). A maximum transfer rate between phosphatidylcholine and phosphatidylethanolamine was observed between 4 nm- and 8 nm-PMA. To ensure the maximum effect, 20 nm-PMA was routinely used in all experiments. We have previously observed that AA was not transferred from or to phosphatidylinositol in resting platelets [23]. In our incubation conditions, PMA did not induce any change in the labelling of phosphatidylinositol (Fig. 2). Thus PMA did not promote phosphatidylinositol biphosphate hydrolysis, as previously described [24,25], nor did it elicit AA redistribution from or to phosphatidylinositol.

Effect of staurosporine on PMA-induced AA mobilization

Staurosporine has been shown to be a potent protein kinase C inhibitor in human platelets [26]. When added to PMA-treated rat platelets, staurosporine inhibited by 40–60% the PMA-induced AA transfer and almost abolished the AA release induced by PMA (Fig. 3). Staurosporine had no effect on AA transfer and release observed in resting platelets.

Activation of protein kinase C from rat platelets

Protein kinase C activity was measured in the cytosol and in a Triton X-100 extract from the particulate fraction partially purified by DEAE-cellulose ion-exchange column chromatography. In PMA-treated platelets, protein kinase C activity

Table 4. PMA-stimulated transfer of AA to ether phospholipids

[^{14} C]AA-prelabelled platelets were incubated as in Fig. 3 for 2 h. Subclasses of choline- and ethanolamine-containing phospholipids were separated as indicated in the Materials and methods section. The data, expressed as percentage labelling relative to total lipid labelling, are means of two experiments.

	AA labelling (%)		
	Control	PMA	PMA + staurosporine
Acyl-AA-GPC	41.0	36.7	38.4
Alkyl-AA-GPC	3.6	5.0	4.4
Acyl-AA-GPE	14.8	14.4	15.7
Alkyl-AA-GPE	4.8	5.2	5.3
Alkenyl-AA-GPE	5.1	8.1	6.2

found in the particulate fraction increased by 64% as compared with control platelets, and this increase was inhibited by staurosporine (Table 2).

PMA-induced stimulation of CoA-independent transacylase activities

To examine the regulation of transacylase activities by PMA, sonicated platelets prepared from control, PMA- and PMA + staurosporine-treated platelets were assayed for transacylase activities by measuring the acylation rate of lyso-derivatives. The acylation rates of ether and acyl lyso-derivatives in the absence and in the presence of CoA were compared (Table 3). Hexadecyl-lyso-GPC was a better substrate than acyl-lyso-GPE in the CoA-independent reaction. The opposite was observed in the CoA-dependent reaction. After 30 min treatment with 20 nm-PMA, the CoA-independent acylations of hexadecyl-lyso-GPC and acyl-lyso-GPE were increased by 35 $\pm$ 11% and 40 $\pm$ 9% respectively, and this increase was considerably decreased by 1 μ M-staurosporine. CoA-dependent acylation of either lyso-compound was not modified by PMA treatment of the platelets.

These experiments demonstrate that PMA stimulates specifically CoA-independent transacylases. The acylation rate of hexadecyl-lyso-GPC dose-dependently increased as a function of PMA and was maximum around 10 nm-PMA (Fig. 4). Since CoA-independent transacylases are involved in AA transfer to ether phospholipids, we investigated whether PMA-stimulated AA transfer was directed to ether phospholipids. AA transfer experiments were performed for 2 h in control, PMA- and

PMA + staurosporine-treated platelets, and then choline- and ethanolamine-containing phospholipids were separated, as well as their diacyl and ether subclasses. PMA increased AA transfer from diacyl-GPC to alkylacyl-GPC and to alkenylacyl-GPE. The PMA-induced AA transfer to ether phospholipids was inhibited by staurosporine (Table 4).

DISCUSSION

Transfer reactions have been demonstrated in a number of resting cells [27–29], including platelets [23]. In receptor-dependent platelet activation, the liberation of AA from esterified pools is correlated with an increase in AA transfer between phospholipids, mainly from diacyl-GPC to ether phospholipids [3,4]. Furthermore, we have shown that AA was not released directly from diacyl-GPC; it was first transferred to other phospholipids, to be released subsequently [6]. Since protein kinase C activation has been involved in AA release, it was decided to investigate whether protein kinase C activation also promoted an increase in AA transfer between phospholipids.

PMA and OAG, two well-known activators of protein kinase C [30,31], increased the transfer rate of AA from phosphatidylcholine to phosphatidylethanolamine as well as AA liberation from esterified pools. Reacylation as a possible control mechanism for the cellular levels of free AA has been shown in macrophages [18], in smooth-muscle cells [32] and in platelets [33]. However, the acyl-CoA acyltransferase is highly specific for lysophosphatidylcholine and lysophosphatidylinositol in platelets [34,35]. Therefore, the decrease in phosphatidylcholine labelling and the absence of change in labelling of phosphatidylinositol are not likely to account for the sequential action of phospholipase A₂ and acyl-CoA acyltransferase. Moreover, an excess of unlabelled AA did not modify AA transfer induced by PMA, clearly showing that AA was not released and re-incorporated, but was directly transferred from phosphatidylcholine to phosphatidylethanolamine. We have previously shown that inhibition of cyclo-oxygenase and lipoxygenase with compound BW 755C did not modify the AA transfer induced by thrombin [6]. This compound, or trapping of free AA by fatty-acid-free BSA, did not change the AA transfer elicited by PMA. These results demonstrate that oxygenated derivatives of AA or free AA had no effect on the transfer reactions.

Thus, in addition to potentiating the effect of Ca²⁺ ionophores on AA release [13,14], PMA by itself is able to induce AA mobilization in platelets. However, this process is slow as compared with AA release elicited by the Ca²⁺ ionophore A23187 plus PMA, and it was not detected previously in platelets [14], in contrast with cultured cells [17].

A PMA concentration of 10 nM elicited maximal AA mobilization, which corresponded to the dose inducing maximal protein kinase C activation in human platelets [30]. This PMA concentration effectively increased the activity of rat platelet membrane-associated protein kinase C. The phorbol-diester-induced AA transfer and release were inhibited by the protein kinase C inhibitor staurosporine [26]. The inhibition observed in the presence of staurosporine was between 70 and 90 % for both AA transfer and protein kinase C. Thus we can conclude that protein kinase C activation promotes an increase in AA transfer rate from phosphatidylcholine to phosphatidylethanolamine.

AA transfer between phospholipids is assumed to depend on polyunsaturated-fatty-acid-specific transacylases [8–11], which are induced in cell-free systems by addition of lyso-derivatives, which can be produced through the action of phospholipase A₂. Since phospholipase A₂ is a Ca²⁺-dependent enzyme and protein kinase C interacts with Ca²⁺ in a synergistic manner to increase AA release, protein kinase C has been thought to increase

phospholipase A₂. In PMA-activated mesangial cells, an increase in the phospholipase A₂ recovered in a cell-free extract was observed [36]. Here we show that the mobilization of AA induced by protein kinase C activators in platelets might be achieved through the enhancement of CoA-independent transacylase activity.

The acylation rate of alkyl-lyso-GPC by cell-free fractions was increased by platelet pretreatment with PMA. This increase correlated closely with the activation of AA transfer in intact cells as a function of PMA concentration. Transacylation of this ether phospholipid, which is the precursor of the bioactive lipid Paf-acether, is essentially CoA-independent [9–11], whereas the transacylation of acyl phospholipids is primarily CoA-dependent [12]. Therefore we used acyl-lyso-GPE to demonstrate that only CoA-independent transacylase was increased after PMA treatment. Staurosporine, which inhibited PMA-induced protein kinase C activation, also inhibited AA transfer and CoA-independent transacylase activity induced by PMA. Moreover, using prelabelled platelets, we showed that PMA induced the transfer of AA to the ether phospholipids, alkylacyl-GPC and alkenylacyl-GPE. Such transfers are involved in AA release, since AA is never directly released from diacyl-GPC during receptor-mediated platelet activation [6]. AA might be funnelled through alkylacyl-GPC and/or ethanolamine-containing phospholipids [6,37]. Thus, when platelets are stimulated with an agonist that increases the level of diacylglycerol, protein kinase C is in turn activated. This activation might play an important role in the mobilization of AA from major sources of AA, which are the ethanolamine- and choline-containing phospholipids, by stimulating CoA-independent transacylases. Taken together, our data demonstrate that CoA-independent transacylase might control both AA release and lysophospholipid formation, and thus participate in the synthesis of bioactive lipids such as Paf-acether and AA derivatives.

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