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Minireview

Lysophosphatidic acid as a phospholipid mediator: pathways of synthesis

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Abstract From very recent studies, including molecular cloning of cDNA coding for membrane receptors, lysophosphatidic acid (LPA) reached the status of a novel phospholipid mediator with various biological activities. Another strong argument supporting this view was the discovery that LPA is secreted from activated platelets, resulting in its appearance in serum upon blood coagulation. The metabolic pathways as well as the enzymes responsible for LPA production are poorly characterized. However, a survey of literature data indicates some interesting issues which might be used as the basis for further molecular characterization of phospholipases A able to degrade phosphatidic acid.

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Key words: Lysophosphatidic acid; Phospholipase A_2; Phospholipase A_1; Diacylglycerol lipase; Platelets

1. Introduction

Lysophosphatidic acid (LPA) is the simplest phospholipid found in nature and for a while was considered only as the transient first intermediate in the de novo synthesis of glycerophospholipids [1]. However, a number of studies starting in the 1960s described several biological activities displayed by LPA, including smooth muscle contraction [2], vasoactive effects [3] or platelet aggregation [4–6]. However, especially when considering platelet aggregation, LPA still appeared as a poor effector compared to the unique phospholipid mediator described so far, i.e. platelet activating factor or 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine [7]. More recently, Moolenaar and colleagues in Amsterdam observed that LPA possesses all the properties of an authentic growth factor [8]. This stimulated a renewed interest for LPA, which now appears to be a widespread mediator active not only on mammalian cells but also on more ancestral cells such as *Xenopus laevis* oocytes or *Dictyostelium dicoideum*. At the present time, an impressive number of biological activities have been described and include, in addition to properties mentioned above, stimulation of chemotaxis, of tumour invasion, dedifferentiating effects on neuronal cells in culture, antiproliferative effects (depending on cell type and concentration), cytoskeletal rearrangement, calcium mobilization as well as modulation of chloride channels. All these data have been recently compiled in a number of excellent reviews [9–15].

Confirming previous predictions based on the study of signal transduction mechanisms, two cDNA encoding putative LPA receptors have recently been cloned, indicating that the phospholipid acts via G-protein-coupled receptors displaying the characteristic features of seven transmembrane proteins [16–18]. This provided definitive evidence for LPA being an extracellular mediator, a concept which has only recently emerged from the observation that it is present in serum at micromolar concentrations and might be secreted by platelets [19,20]. Based on its effects on fibroblast proliferation, this has led to the suggestion that LPA might be involved in wound healing [9–15]. However, platelets might not be the only source of LPA. The aim of this short review is to focus on the available information dealing with LPA biosynthesis.

2. Role of platelets in LPA biosynthesis

By incubating platelets with phospholipase C (PLC) from *Clostridium welchii*, we obtained the first evidence that these cells are able to synthesize LPA, as shown by ^{32}P labelling (Fig. 1) [21]. The same was observed with thrombin, although at a much lower rate, probably because diacylglycerol (DAG) generated from phosphoinositides by endogenous PLC was much less abundant than DAG derived from hydrolysis of phosphatidylcholine by the bacterial enzyme. In both cases, it was evident that DAG was converted into phosphatidic acid by platelet DAG kinase. As illustrated in Fig. 2, one obvious possibility to generate LPA under these conditions would have been the hydrolysis of PA by a phospholipase A_2 (PLA_2). However, LPA formed in thrombin-stimulated platelets contains arachidonic acid, together with palmitic and stearic acids, suggesting the possible involvement of a phospholipase A_1 (PLA_1) [22]. In platelets treated with *C. welchii* PLC, the fatty acid composition of LPA is very similar to that of fatty acids occupying the *sn*-2 position of platelet phosphatidylcholine [21]. However, we were unable to detect any PLA activity in human platelet lysates, whereas PLA_1 and PLA_2 activities specific for PA were described in pig and horse platelets, respectively [23,24]. Such a discrepancy might be due to species differences or to conditions of incubation. In any case, this led us to suggest that DAG could be deacylated by a specific lipase, the intermediate monoacylglycerol (MAG) being either further deacylated or phosphorylated into LPA, as illustrated in Fig. 2. This was based on the identification in platelet lysates of a DAG lipase activity [21], which was later

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Abbreviations: LPA, lysophosphatidic acid; PA, phosphatidic acid; DAG, diacylglycerol; MAG, monoacylglycerol; PLA_1, phospholipase A_1; PLA_2, phospholipase A_2; $snpPLA_2$, secretory non-pancreatic phospholipase A_2; PLC, phospholipase C

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separated into a DAG and a MAG lipase [25,26]. At that time, a major interest arose as to the mechanisms of arachidonic acid release so that we and others suggested that this was the main role of platelet DAG lipase [21,27]. Since none of these enzymes has been isolated so far, it is still difficult to conclude how LPA is synthesized in activated platelets. Moreover, although phospholipase D seems to play a minor role in the production of platelet PA [28,29], its possible contribution to the metabolic pathway leading to LPA would deserve further consideration. This hypothesis is also indicated in Fig. 2.

As stated above, a main advance in the field of LPA was the demonstration of its release from platelets [20]. Since it was identified in serum but not in plasma, an obvious conclusion was that platelets represented the main source of LPA formed during blood coagulation [19,20]. This point deserves some discussion, based on quantitative data.

3. Are platelets the only source of LPA present in serum?

As shown in Table 1, the amounts of LPA synthesized by PLC-treated platelets reached 26 nmol/10^9 platelets within 15 min [21]. Starting from these data, it was possible to convert LPA radioactivity determined under other conditions into mass estimations. The value of 36 nmol/10^9 platelets upon incubation with PLC for 30 min could be considered as the maximal capacity of platelets to synthesize LPA. In vivo, this would result into a final concentration of LPA in serum of almost 20 μM. In contrast, when thrombin is used as a stimulant, the expected final concentration of serum LPA would be 0.6–0.9 μM when taking into account data from [20,21]. The latter values are higher than the mass determination made by Gerrard and Robinson [22] using gas-liquid chromatography. However, one can notice that the shorter incubation time used by these authors (2 min vs. 10–15 min) also resulted into a poorer labelling of LPA (Table 1). Thus, we think that the values of 0.6–0.9 μM extrapolated from radioactivity determination would better fit with the reality. These numbers are in the same range as those determined in serum by phosphorus measurement (1–5 μM) [20], although they appear significantly lower. In fact, LPA concentrations attaining 28 μM were reported in fetal calf serum [30]. This comparison leads to additional suggestions, which might be the basis of further investigations: either other blood cells (neutrophils, monocytes, ...) contribute to the production of LPA or thrombin stimulation of platelets does not reflect exactly what occurs in vivo.

At the same time, one should keep in mind that rat plasma contains a lysophospholipase D responsible for the accumulation of LPA upon prolonged incubation [31]. According to the latter study, plasma concentrations of LPA as high as 66 μM can be obtained after 48 h incubations. Whether this still poorly characterized enzyme is activated during coagulation should also be checked before reaching a definitive conclusion.

Finally, Tokumura et al. [31] found that plasma lysophospholipase D can also degrade the lyso derivative of platelet activating factor, allowing the formation of alkyl-lysophosphatidic acid. Since this analogue shares some biological properties with LPA and is even 30-fold more active towards platelets [7,32,33], this possible metabolic pathway should not be neglected.

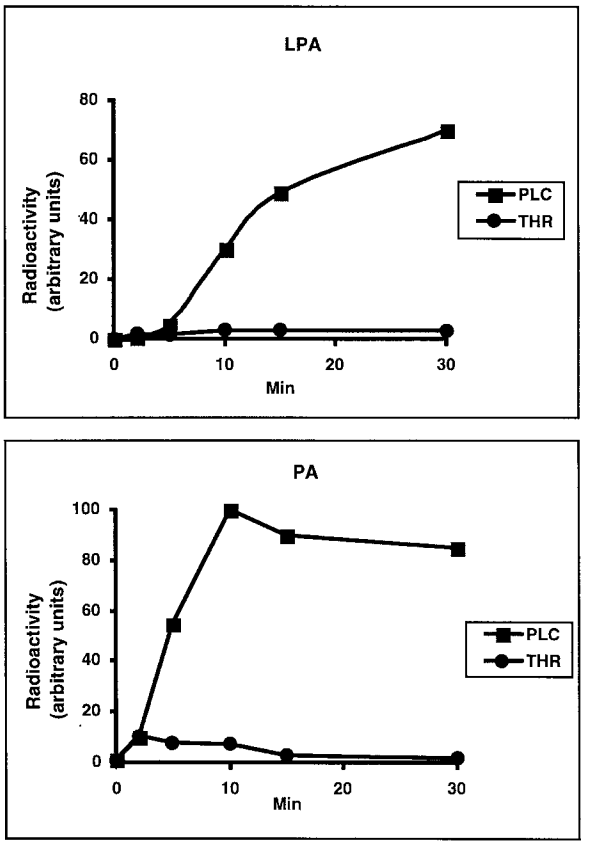

Fig. 1. Production of ^{32}P-labelled LPA and PA in human platelets stimulated with PLC or thrombin. Human platelets were incubated with [^{32}P]*o*-phosphate, washed and incubated for the indicated times with either *C. welchii* PLC (0.1 IU/ml) or thrombin (THR, 5 NIH U/ml). Lipids were then extracted, separated by thin-layer chromatography and determined for radioactivity. Data are from Figs. 1 and 2 in [21].

4. Loss of membrane phospholipid asymmetry regulating phosphatidic acid hydrolysis by secretory non-pancreatic phospholipase A_2

In a more recent study, we have focused our attention on the possible involvement of secretory non-pancreatic PLA_2 (snpPLA_2 or type II PLA_2) in the generation of lipid mediators like LPA [34]. This enzyme is produced by a number of cells stimulated by pro-inflammatory cytokines such as interleukin-1β or tumor necrosis factor-α and it accumulates in inflammatory fluids as well as in the plasma of patients suffering from septic shock [35–37]. In these situations, the amounts of snpPLA_2 secreted in these extracellular media reflect the severity of the inflammatory reaction [35–37]. A classical view is to consider that snpPLA_2 might be involved as a distal effector of inflammation participating in the release of arachidonic acid or lysophospholipids, thus allowing the synthesis of various lipid mediators [36]. A difficulty with snpPLA_2 is to understand how it could participate in this process as an extracellular enzyme, owing to its failure to degrade phospholipids of intact cells. We provided evidence that snpPLA_2 acquires the ability to hydrolyse its substrates in cell membranes after loss of phospholipid asymmetry [34]. Indeed, type II PLA_2 remains almost inactive against choline-containing phospholipids forming the external layer of plasma membranes but hydrolyses much more efficiently the aminophos-

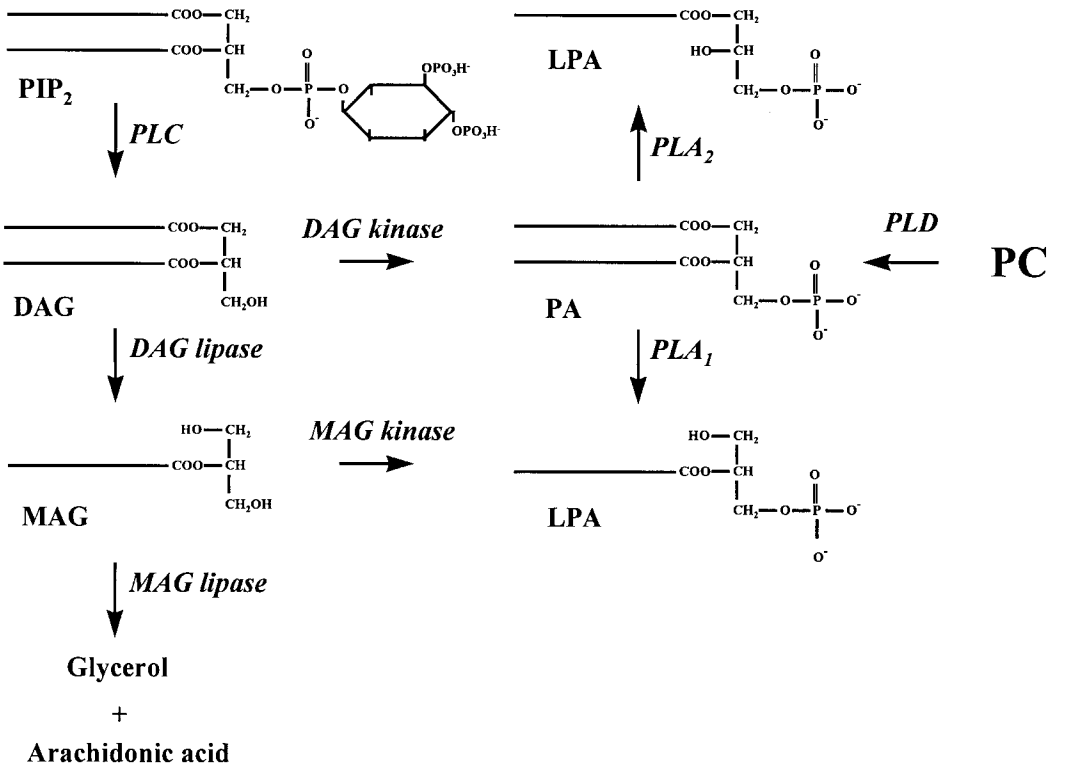

Fig. 2. Possible pathways of LPA production in activated platelets. Abbreviations not defined in the text are: PIP_2, phosphatidylinositol(4,5)*bis*-phosphate; PC, phosphatidylcholine; PLD, phospholipase D.

pholipids confined to the inner leaflet of cell membranes. Such a behaviour was demonstrated using microvesicles shed from red cells treated with a calcium ionophore, which have been shown to expose on their surface phosphatidylethanolamine and phosphatidylserine translocated from the internal leaflet. This model actually reflects what occurs under physiological and pathophysiological conditions such as platelet activation with thrombin *plus* collagen [38], cell damage with perforins [39,40], or apoptosis [41].

When microvesicles are released from calcium-loaded erythrocytes, phosphoinositide-specific PLC is activated [42]. This is followed by accumulation of PA, which is redistributed between the tow leaflets of the membrane, part of it becoming accessible to snpPLA$_2$ attack. LPA generated under these conditions can be bound to serum albumin and was shown to display the same biological properties as LPA [34].

This study thus unravelled a novel possible pathway for LPA synthesis. Studies are still in progress to define pathophysiological conditions where activated cells would associate accumulation of PA in their membrane with profound changes of phospholipid transverse distribution. Although some possibilities exist, particularly in the field of inflammation, evidence is still lacking that LPA accumulates in inflammatory fluids. However, this study opens the view that the generation of LPA on the outer surface of cell membranes might represent a key event allowing its availability to albumin and secondarily to target cells. Although we did not yet obtain definitive proof for this, it is our conviction that only extracellularly generated LPA could become a mediator, whereas intracellular LPA would remain sequestered by fatty acid binding protein [43–45]. But this is only a speculative view, and one should also remember that other lipid mediators are synthesized intracellularly, for instance in nuclear or endoplasmic reticulum membrane [46–48], and are secreted to the surrounding medium by a still undefined mechanism [49].

In any case, snpPLA$_2$ was the first reported example of a well-characterized phospholipase able to generate LPA directly from PA. There are presently an increasing number of

Table 1
Estimation of the amounts of LPA produced by platelets

Conditions of stimulation	Radioactivity[a] (arbitrary units)	Mass (nmol/10^9 platelets)	Expected serum concentration[c] (μM)	Ref.
PLC (15 min)	50	26	14	[21]
PLC (30 min)	70	36[b]	19.6	[21]
Thrombin (15 min)	3.0	1.6[b]	0.9	[21]
Thrombin (10 min)	1.9	1.0[b]	0.6	[20]
Thrombin (2 min)	0.13	0.014	0.008	[22]

[a]Arbitrary units are defined as the ratio between LPA radioactivity in stimulated platelets and PA radioactivity in resting platelets.
[b]These are values extrapolated from the comparison between radioactivity and mass determination of LPA in platelets treated for 15 min with PLC.
[c]Calculated with a platelet concentration of 3×10^9 cells/ml and an haematocrit of 45%.

purified phospholipases A displaying either an absolute specificity or a high selectivity for PA. These are discussed below.

5. Calcium-independent phospholipases A displaying selectivity for phosphatidic acid

Thomson and Clark [50] recently purified from rat brain a 58-kDa PLA_2 absolutely specific for PA and acting in the absence of calcium. This enzyme would certainly deserve further characterization, including a precise cellular and subcellular localization, since LPA receptors appear to be rather abundant in cerebral tissue [16,18]. Bovine brain also contains a PA-preferring PLA_1, which is expressed in testis as well [51]. The purified enzyme appears to be a homotetramer of 110-kDa subunits. A similar tetrameric structure has been observed for a calcium-independent PLA_2 whose cDNA was recently cloned and which displays a 20-fold preference for PA compared to phosphatidylcholine [52–54]. Of course, none of these enzymes can yet be considered as a direct effector of LPA production, but their molecular characterization could lead to interesting conclusions in a near future, allowing for instance to manipulate cells by transfection of cDNA coding for active or inactive enzymes.

6. Conclusions

At the end of this short review, we are far from providing a clear picture of the main metabolic pathway(s) responsible for LPA generation. Even in platelets, a rather high number of possibilities (and uncertainties) still exist and will probably be clarified in a near future. However, platelets are certainly not the only source of LPA, which has been found for instance at relatively high concentrations in normal follicular [55] and pathological cerebrospinal fluids [56]. Very surprisingly, LPA has been also detected in various tissues from rat and guinea pig at levels comprised between 50 and 140 nmol/g [57]. In these cases, it was found highly enriched in stearic acid, suggesting another source than phospholipid de novo synthesis. This kind of investigation might appear obsolete now that a great deal of effort will be devoted to the functional and structural characterization of recently cloned LPA receptors [15–17]. We are convinced that the metabolic pathways of LPA biosynthesis are of prime importance for a better biological definition of this novel mediator. They might also offer some clues to find pharmacological compounds able to modulate not only the activity but also the availability of LPA.

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