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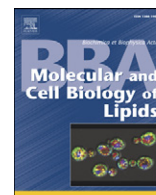

Review

Control of free arachidonic acid levels by phospholipases A_2 and lysophospholipid acyltransferases

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ABSTRACT

Arachidonic acid (AA) and its oxygenated derivatives, collectively known as the eicosanoids, are key mediators of a wide variety of physiological and pathophysiological states. AA, obtained from the diet or synthesized from linoleic acid, is rapidly incorporated into cellular phospholipids by the concerted action of arachidonoyl-CoA synthetase and lysophospholipid acyltransferases. Under the appropriate conditions, AA is liberated from its phospholipid storage sites by the action of one or various phospholipase A_2 enzymes. Thus, cellular availability of AA, and hence the amount of eicosanoids produced, depends on an exquisite balance between phospholipid reacylation and hydrolysis reactions. This review focuses on the enzyme families that are involved in these reactions in resting and stimulated cells.

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1. Introduction

Arachidonic acid (5,8,11,14-eicosatetraenoic acid, ω-6) (AA) is an essential fatty acid that is obtained directly from dietary sources or indirectly from conversion of linoleic acid. AA is the precursor of a large family of bioactive compounds called the eicosanoids, produced by oxygenation through cyclooxygenase and lipoxygenase pathways [1,2]. Because of the potent biological actions of the eicosanoids, cells keep this fatty acid at very low levels, by promoting its esterification into cellular lipids. As a matter of fact the availability of free AA is well described to constitute a rate-limiting step in the generation of eicosanoids by mammalian cells [3,4]. In addition, free AA may also exert signaling functions by itself, e.g. as an inducer of apoptosis [5].

Under physiological conditions, AA is generally found esterified into the *sn*-2 position of glycerophospholipids, particularly choline glycerophospholipids (PCs) ethanolamine glycerophospholipids (PEs), and phosphatidylinositol (PI). The production of free AA is a highly regulated process that represents a balance between two competing reactions, namely, phospholipid deacylation by phospholipase A_2 (PLA_2) enzymes and reacylation and transfer into various phospholipid pools by acyltransferases and transacylases [6]. Depending on the state of the cell (i.e. resting or activated) one kind of reaction will dominate over the other. Thus, in resting cells, reacylation dominates, and hence, the bulk of cellular AA is found in the esterified form. In stimulated cells, the dominant reaction is the PLA_2-mediated deacylation, which results in dramatic releases of free AA that is now available for eicosanoid synthesis. However, under activation conditions AA reacylation is still very significant, as manifested by the fact that only a minor fraction of the AA released by PLA_2 is available for eicosanoid synthesis, and the remainder is effectively incorporated back into phospholipids by acyltransferases [5,6]. In this regard, various studies have shown that the rate of AA incorporation into cellular phospholipids is slightly increased following cellular stimulation [7–11]. Such an increase is generally thought to be important for the replenishment of the intracellular pools of AA being exhausted as a result of cellular stimulation [6]. However, increased influx of exogenous AA into phospholipids can also occur under conditions where no endogenous AA release occurs [12,13], implying that this may actually be an independent process.

2. Regulation of AA incorporation into phospholipids

The pathways for AA incorporation into various classes of glycerophospholipids have been described in detail in various cells, particularly those involved in inflammatory reactions such as neutrophils and macrophages [6,14]. Two distinct pathways appear

Abbreviations: AA, arachidonic acid; ACS, acyl-CoA synthetase; PA, phosphatidic acid; PC, choline glycerophospholipids; PE, ethanolamine glycerophospholipids; PI, phosphatidylinositol; PIP_2, phosphatidylinositol 4,5-bisphosphate; PS, phosphatidylserine; PG, phosphatidylglycerol; PLA_2, phospholipase A_2; $cPLA_2\alpha$, group IVA cytosolic phospholipase $A_2\alpha$; $iPLA_2$, calcium-independent phospholipase A_2; $iPLA_2$-VIA, group VIA calcium-independent phospholipase A_2; $sPLA_2$, secreted phospholipase A_2; LPAAT, lysoPA:acyl-CoA acyltransferase; LPCAT, lysoPC:acyl-CoA acyltransferase; LPEAT, lysoPE:acyl-CoA acyltransferase; LPIAT, lysoPI:acyl-CoA acyltransferase; MBOAT, membrane bound *O*-acyltransferase; AGPAT, acyl-glycerol phosphate acyltransferase

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to exist for the initial incorporation of AA (Fig. 1). The first one is a high-affinity pathway that incorporates low concentrations of AA into phospholipids via direct acylation reactions catalyzed by coenzyme A-dependent acyltransferases. This is thought to be the major pathway for AA incorporation into phospholipids under physiological conditions [6]; thus, the PLA_2-dependent availability of lysophospholipid acceptors may constitute a critical regulatory factor [15,16]. The second pathway operates under high levels of free AA, which may be pathophysiological, and leads to the incorporation of the fatty acid primarily via the *de novo* route for phospholipid biosynthesis, resulting ultimately in the accumulation of AA into triacylglycerols and diarachidonoyl phospholipids [6]. This "high-capacity, low affinity" pathway is thought to primarily operate after the high-affinity deacylation/reacylation pathway has been saturated due to the high AA concentrations [6].

Once the AA has been incorporated into phospholipids, a remodeling reaction carried out by CoA-independent transacylase transfers AA from choline glycerophospholipids (PCs) to ethanolamine glycerophospholipids (PEs), in a process that generally takes several hours in primary cells but is strikingly fast in tumor cell lines, where it takes only minutes [17–20]. In inflammatory cells, a major consequence of the CoA-independent transacylase-driven remodeling reactions is that, despite PC being the preferred acceptor for exogenous AA, under equilibrium conditions, AA is more abundant in PE than in PC [6,14].

2.1. Lysophospholipid regulation of AA incorporation

For the efficient incorporation of AA into phospholipids, two kinds of lysophospholipid acceptors should be readily available in the cell. Lysophospholipids, particularly lysoPC, are needed for the initial incorporation of AA into phospholipids via the Lands pathway, and lysophospholipids are again required, particularly lysoPE, for AA remodeling between phospholipids via CoA-independent transacylation reactions (Fig. 2) [15]. Given that AA preferentially incorporates into the *sn*-2 position of phospholipids, the lysophospholipid acceptors used for AA incorporation and remodeling are of the 2-lyso type, i.e. those produced by PLA_2s.

It is likely that several PLA_2 forms may contribute to the 2-lysophospholipid pool utilized for AA incorporation and remodeling and that their identity varies between cell types and tissues. In phagocytic cells, a significant part of the steady-state level of lysoPC appears to be maintained by the continuing action of Ca^{2+}-independent group VIA phospholipase A_2 (iPLA_2-VIA) on cellular phospholipids [21,22]. Thus, a decrease in the activity of the iPLA_2-VIA frequently results in the diminished production of lysoPC and hence in the inhibition of AA incorporation into phospholipids [21,22]

Earlier studies on the initial incorporation of AA into glycerophospholipids in mouse macrophages indicated that the process was essentially Ca^{2+}-independent [12], suggesting that the PLA_2 putatively responsible for generating lysophospholipid acceptors for AA incorporation would correspond to that of an iPLA_2-like enzyme [12]. Such an activity was later identified to belong to iPLA_2-VIA in studies carried out with murine P388D_1 macrophage-like cells [23,24]. However, evidence has also been provided to indicate that iPLA_2-VIA does not serve this function in other cell types [25], suggesting that, like other iPLA_2-regulated processes, the involvement of iPLA_2-VIA in phospholipid AA incorporation may depend on cell type and, in particular, on the expression level of iPLA_2-VIA (i.e. how much the enzyme contributes to the steady-state lysophospholipid pool of a given cell). Based on studies of iPLA_2 inhibition by the inhibitor bromoenol lactone (BEL), the iPLA_2-VIA contribution ranges from ~90% in rat submandibular ductal cells [26], to 50%–60% in phagocytic cells [16,23,24,27–30], and to only 20%–25% in rat uterine stromal cells [31]. Studies in rat pancreatic islets, where iPLA_2 inhibition by BEL does not result in diminished AA incorporation into phospholipids [25], have estimated that iPLA_2-VIA contributes to at least 20% of the steady-state lysophospholipid levels of these cells. Given that rat pancreatic islets maintain cellular lysophospholipid levels at high levels, it seems possible that the amount of lysophospholipid present in these cells even after iPLA_2 inhibition by BEL is still high enough to account for a normal rate of AA incorporation into phospholipids. In agreement with this view, studies on AA incorporation utilizing cells overexpressing iPLA_2-VIA have indicated that the excess amount of lysophospholipid produced under those conditions does not increase the rate of fatty acid incorporation [16,32,33].

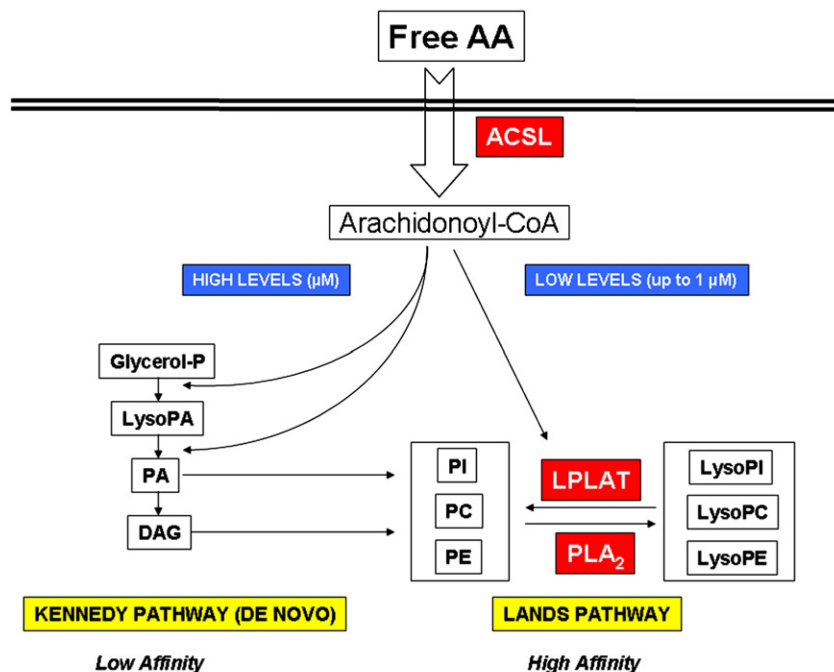

Fig. 1. Pathways for the incorporation of arachidonic acid into glycerolipids. LPLAT, lysophospholipid acyl-CoA acyltransferase.

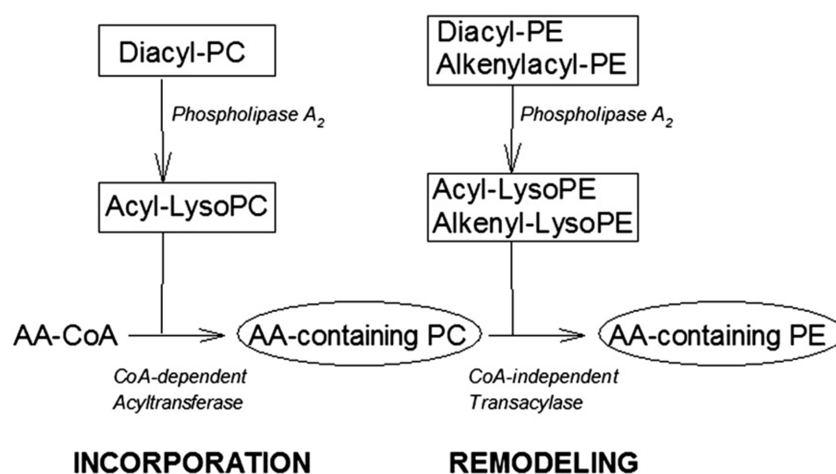

Fig. 2. Arachidonic acid incorporation into and remodeling among phospholipids. Adapted with permission from Ref. [15] (© The Biochemical Society).

On the other hand, we believe that it is important to note that a slowed rate of AA incorporation into phospholipids due to diminished availability of lyso acceptors subsequent to $iPLA_2$ inhibition does not necessarily imply that the profile or amount of AA-containing phospholipids may have to change under equilibrium conditions, as has been assumed in a number of papers. The distribution of AA among phospholipid classes ultimately depends on transacylation reactions that are essentially $iPLA_2$-independent and do not change whether $iPLA_2$ is inhibited or not (see below).

Very few studies have focused on the PLA_2 enzyme providing lysophospholipid acceptors for AA remodeling reactions via CoA-independent transacylases. The nature of such a PLA_2 has been investigated in peripheral T lymphocytes [13] and U937 macrophages [15] by measuring the transfer of AA from PC to PE in the presence of different PLA_2 inhibitors. Inhibitors of group IVA cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$) and $iPLA_2$-VIA failed to exert any detectable effect on the transfer of AA from PE to PC in either cell type, raising the possibility that the PLA_2 implicated in this pathway might be an as yet undefined PLA_2. The Ca^{2+}-independent nature of the response suggests the involvement of an $iPLA_2$-like activity different from the group VIA enzyme. An $iPLA_2$ activity that is not inhibited by BEL, and therefore is not a group VI enzyme, was recently identified in U937 macrophage-like cells [15,34]. This activity appears to prefer PE as substrate, consistent with a presumed role in providing lysoPE acceptors for transacylation reactions [15].

2.2. Acyl-CoA synthetases utilizing AA

The first enzymatic step for the incorporation of AA into phospholipids is catalyzed by the enzyme acyl-CoA synthetase (ACS), which activates the carboxyl group of AA by coupling a CoA moiety to it via a thioester linkage.

All enzymatically active ACSs contain at least two conserved amino acid sequence domains: a covalent AMP-binding domain (motif I) consisting of 10 residues highly conserved from bacteria to humans [35] and a 36- to 37-residue domain (motif II) containing a sequence that is thought to be essential for binding of the substrate [36]. The latter has been used to assign ACSs to subfamilies [35,36]. Up to now, 26 different ACS isoforms, each encoded by a separate gene, have been identified in the genome of human cells [36], although 4 of them are still considered as candidates to exhibit ACS activity based on the presence of the two distinctive motifs (medium-chain ACS-2A, short-chain ACS-3, medium-chain ACS-5, and ACS family-4). Twenty-two of these ACSs are classified into 5 subfamilies considering the chain length of the fatty acid of their preferred acyl groups (short-chain ACSs, medium-chain ACSs, long-chain ACSs, very long-chain ACSs, and "bubblegum" ACSs); the other four proteins do not belong to any subfamily and are denominated ACSF (ACS family) (Table 1). The ACS enzymes displaying some preference for AA are typically those of the ACSL family. Thus, a summary of the properties of the other ACS families is given below and the long-chain acyl-coenzyme A synthetase (ACSL) family is reviewed in more detail.

The family of short-chain acyl-coenzyme A synthetases (ACSSs) is composed by three enzymes (ACSS1, 2, and 3) capable of activating acetate, propionate, or butyrate. It has been described in bovine, murine, and human tissues that this family uses mainly acetate as a substrate, presenting a weak selectivity for propionate or butyrate [37,38].

The medium-chain acyl-coenzyme A synthetase family (ACSMs) consists of six enzymes, all localized almost exclusively in the mitochondrial matrix. These ACSs activate C4-10 fatty acids, although the selectivity can drastically differ between each isoform [39]. The metabolism of medium-chain fatty acids is poorly understood, but it is thought to play an important role in energy generation, given that the medium-chain fatty acids are probably generated from long- and very long-chain fatty acids by peroxisomal β-oxidation, and further degradation via mitochondrial β-oxidation after transportation into the mitochondrial matrix. [40].

The very long-chain acyl-coenzyme A synthetase family (ACSVLs) is composed of six membrane integral proteins (ACSVL-1 to -6) that are capable of activating long-chain, branched-chain and very long-chain fatty acids containing more than 22 carbons. Members of this family are also designated as fatty acid transporter proteins (FATPs) and are thought to be involved in translocation of long- and very long-chain fatty acids across the plasma membrane [41]. Thus, these proteins could play a dual role in the transport and esterification of their substrates, with the exception of ACSVL3/FATP3, which has been demonstrated not to exhibit fatty acid transport activity [42].

The first member of the "bubblegum" acyl-coenzyme A synthetases (ACSBGs) was originally discovered in the *Drosophila* mutant "*bubblegum*", characterized by neurodegeneration and high tissue levels of saturated very long-chain fatty acids [43]. Overexpression of human ACSBG1 led to the finding that this enzyme activates both long- and very long-chain fatty acids [35]. More recently, a second member (ACSBG2) has been located in murine and human testis and brainstem, showing a high degree of homology to ACSBG1 [44].

The long-chain acyl-coenzyme A synthetases (ACSLs) are the best characterized of the ACS enzymes and play a key role in remodeling of membranes and *de novo* lipid synthesis. To date, five ACSL isoforms have been described in mammalian cells, ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6 [45], with at least two spliced transcript variants per

Table 1
Biological features of human ACS families.

ACS nomenclature	Other names	Tissue specificity	Subcellular location	Substrate	Acyl-CoA selectivity	TC sensitivity
Short-chain ACS family						
ACSS1 (2 variants[a])	ACAS2L, AceCS2L	Heart	Mitochondrial matrix		2:0	Insensitive
ACSS2 (2 variants)	ACAS2, ACS, ACSA, AceCS	Liver	Cytoplasm	C(2–4)	2:0	Insensitive
ACSS3 (2 variants)		Lung	Mitochondria[b]		2:0[b]	Insensitive
Medium-chain ACS family						
ACSM1 (2 variants)	BUCS1, MACS1	Liver, kidney	Mitochondrial matrix		8:0	ND
ACSM2A	ACSM2, MACS2	Liver, kidney	Mitochondrial matrix		Broad range	ND
ACSM2B (2 variants)	ACSM2, HXMA	Liver	Mitochondrial matrix	C(4–10)	Broad range	Insensitive
ACSM3 (2 variants)	SA, SAH	Liver, kidney	Mitochondrial matrix, peroxisome		4:0	ND
ACSM4		–	Mitochondrial matrix		Broad range	ND
ACSM5 (2 variants[a])	MACS3	Liver, kidney, heart, pancreas	Mitochondrial matrix		Broad range	ND
Long-chain ACS family						
ACSL1 (3 variants)	ACS1, FACL1, FACL2, LACS, LACS1, LACS2	Heart, liver, adipose	Plasma membrane, ER, vesicle, mitochondrial membrane		16:0>18:1>18:2	Sensitive
ACSL3 (2 variants)	ACS3, FACL3, LACS3	Brain, liver, small intestine	ER, lipid droplets		14:0>12:0>20:4>20:5	Sensitive
ACSL4 (2 variants)	ACS4, FACL4, LACS4	Steroidogenic tissue, liver	Peroxisome, ER, mitochondrial membrane	C(12–20)	20:4>20:5	Sensitive
ACSL5 (3 variants)	ACS2, ACS5, FACL5	Uterus, spleen liver, small intestine	ER, mitochondrial outer membrane, plasma membrane		Broad range of saturated fatty acids C16–C18 unsaturated fatty acids	Insensitive
ACSL6 (5 variants)	ACS2, FACL6, LACS2, LACS5	Brain, reticulocytes	Plasma membrane, mitochondria		22:6>20:4	Insensitive
Very long-chain ACS family						
ACSVL1	FATP-2, SLC27A2, FACVL1, VLACS, VLCS	Liver, kidney, small intestine	RE and peroxisomes		16:0, 24:0, THCA, phytanic acid, pristanic acid	Sensitive[b]
ACSVL2 (2 variants)	FATP-6, SLC27A6, FACVL2, VLCS-H1	Heart, placenta	Cell membrane, sarcolemma		18:1, 20:4, 24:0	ND
ACSVL3 (3 variants)	FATP-3, SLC27A3, VLCS-3	Testis, adrenal gland, ovary, brain, lung, kidney	Mitochondrial membrane	C(18–26)	16:0, 18:1, 24:0[b]	ND
ACSVL4	FATP-4, SLC27A4,	Small intestine, skin, brain, adipose, muscle, heart, liver, kidney	ER membrane		24:0, (16:0)[b]	Insensitive (24:0)[b]
ACSVL5	FATP-1, SLC27A1, FATP	Heart, adipose, muscle, brain	Plasma and intracellular membrane, cytoplasm		16:0, 18:1, 24:0	Sensitive (16:0)[b]
ACSVL6	FATP-5, SLC27A5, BAL, ACSB, BACS, VLCS-H2	Liver	ER membrane		Primary (cholic and chenodeoxycholic) and secondary (deoxycholic and lithocholic) bile acids, THCA, (24:0)	Insensitive[b]
Bubblegum ACS family						
ACSBG1	BG1, BGM, lipidosin	Brain, adrenal gland and testis	Cytoplasm, cytoplasmic vesicle, microsome, ER	C(14–24)	18:1>20:5=20:4=18:0	ND
ACSBG2 (4 variants[a])	BGR, BGR-like, BRGL	Adult testis	Cytoplasm, mitochondria		18:1=18:2	Insensitive
Other ACSs (ACSF)						
ACSF1 (3 variants[a])	AACS, SUR-5	Kidney, heart, brain	Cytoplasm, cytosol		Acetoacetate	ND
ACSF2	ACSMW	Adipose	Mitochondria		8:0 (medium-chain)	ND
ACSF3 (2 variants)		–	Mitochondria		24:0 (very long-chain)	ND
ACSF4 (5 variants[a])	AASDH, LYS2, U26	Liver, kidney, pancreas, spleen, testis	–		–	ND

THCA: trihydroxycholestanoic acid; ND: not determined; TC: triacsin C.
[a] No experimental confirmation available.
[b] Checked in murine.

isoform. Based on sequence homologies, the ACSL enzymes have been subdivided into two major groups, ACSL1/ACSL5/ACSL6 and ACSL3/ACSL4 [46]. *ACSL1* was the first cloned human ACSL family gene [47]. Originally it was considered to be different from *ACSL2* [48], but later it was found to be the same gene, which went on to be denominated *ACSL1*. As a consequence, the rat *ACS2* gene was renamed *Acsl6*, because of its high homology with human *ACSL6*. A remarkable characteristic of *ACSL1* is that the rodent protein is one residue longer than the human protein (699 and 698 amino acids, respectively). This enzyme is predominantly located in heart, liver, and adipose tissue and uses a wide range of fatty acids, although with a slight preference for palmitic, oleic, and linoleic acids [49].

ACSL3 is one of two ACSL isoforms highly expressed in the brain [50,51]. It is located in the endoplasmic reticulum and lipid droplets [52]. ACSL3 presents a marked selectivity for AA and eicosapentaenoic acid over other unsaturated fatty acids, although its preference also for myristic acid and lauric acid makes this isoform less specific than ACSL4 with regard to AA and eicosapentaenoic acid.

ACSL4 shows close homology to ACSL3, sharing 68% of their amino acids. It is expressed predominantly in steroidogenic tissue and located in peroxisomes and mitochondrial membrane. With regard to substrate preference, murine and human cell ACSL4 utilizes AA and eicosapentaenoic acid with marked preference over all other fatty acids, indicating a critical function in AA metabolism [53,54].

ACSL5 is the only ACSL located in the outer mitochondrial membrane, suggesting a preferential role in activating acyl groups for mitochondrial β-oxidation. ACSL5 is highly expressed in small intestine, and to a lesser extent in liver, and uses a wide range of saturated and unsaturated fatty acids [55].

Together with ACSL3, ACSL6 is the major ACSL expressed in brain. Murine and human ACSL6 show a clear preference for docosahexaenoic acid (22:6) and AA [56,57]. The fact that this isoform presents a preference for the most abundant polyunsaturated fatty acids in brain suggests an important role in the synthesis of lipids in neuronal membranes, which experience a rapid phospholipid turnover. In addition, ACSL6 is also present in the plasma membrane of mature erythrocytes, where it activates long-chain fatty acids for remodeling of lipids and acylation of proteins [58].

In addition to the differences in fatty acid preference, subcellular location and tissue distribution, ACSLs also show different responsiveness to pharmacological inhibitors. It has been shown that ACSL1, ACSL3, and ACSL4 are sensitive to triacsin C, while ACSL5 and ACSL6 are not [51,56,59]. Moreover, thiazolidinediones, a type of oral insulin-sensitizing agents formerly used to treat type 2 diabetes, can specifically inhibit the activity of ACSL4 [56,60]. The initial experiments with these inhibitors suggest that the various ACSL isoforms can drive acyl-CoAs to different lipid metabolism pathways with some selectivity. It was described in human fibroblasts that triacsin C inhibits the *de novo* synthesis of diacylglycerol, triacylglycerol, cholesterol esters, and phospholipids from glycerol but not the reacylation of fatty acids into phospholipids [61]. These findings have led to the suggestion that triacsin C-sensitive ACSLs supply acyl-CoA for the *de novo* synthesis of glycerolipids, whereas isoforms resistant to triacsin C would be involved in reacylation of phospholipids and β-oxidation.

Studies in human monocyte-derived macrophages have indicated that rosiglitazone, a type of thiazolidinedione, inhibits the incorporation of oleic acid into diacylglycerol and triacylglycerol, but not into phospholipids, whereas triacsin C inhibits the partitioning of these fatty acids into all lipid classes [60]. These data confirm a role for ACSL4 in the partitioning of fatty acids into diacylglycerol and triacylglycerol and suggest additional roles for ACSL1 and ACSL3. On the other hand, studies in rat demonstrate that ACSL5, a triacsin C-resistant form, is also implicated in activating acyl-CoA for the *de novo* synthesis of triacylglycerol [62].

2.3. Lysophospholipid acyltransferases

Mammalian cells contain a number lysophospholipid acyltransferases that exhibit distinct acyl-CoA and lysophospholipid acceptor specificities. The recent availability of genomic information and sequence data has led to the identification of many lysophospholipid acyltransferases that may potentially be involved in AA recycling, either specifically or as part of a general function in homeostatic phospholipid metabolism.

Two families of lysophospholipid acyltransferase enzymes have been recognized, namely the membrane bound *O*-acyltransferase (MBOAT) family and the 1-acyl-glycerol-3-phosphate *O*-acyltransferase (AGPAT) family [63–65]. While the MBOAT family comprises members specifically involved in the Lands cycle of phospholipid fatty acid remodeling, members of the AGPAT family are typically involved in the *de novo* pathway for phospholipid biosynthesis, but some members may also be involved in remodeling reactions.

The MBOAT family includes acyltransferases that can use not only lysophospholipids as acceptors, but also diacylglycerol, cholesterol, or even a protein [63–65]. Only the MBOAT enzymes using lysophospholipids as acceptors will be considered in this review. Characteristic features of the MBOAT lysophospholipid acyltransferases include the existence of several membrane-spanning domains and a conserved His residue in a hydrophobic region that could constitute the catalytic site [66].

Members of the AGPAT family were first hypothesized to utilize lysoPA specifically as acceptor and thus were classified as acyltransferases of the *de novo* phospholipid biosynthetic pathway. It was realized later that these enzymes possess broader substrate specificity being able to utilize other lysophospholipids such as lysoPC and lysoPE. Common structural features of AGPAT acyltransferases include the presence of four conserved domains (motifs I–IV) that are important for catalytic activity and substrate binding [67,68].

2.3.1. Acyltransferases using lysoPA as acceptor

Three lysoPA acyltransferases have been cloned and characterized, namely LPAAT1 [69–71], LPAAT2 [69,72], and LPAAT3 [73]. LPAAT1 and LPAAT2 may utilize several acyl-CoA as donors [74] and are expressed in a wide number of tissues. LPAAT3 shows selectivity for AA and, interestingly, also possesses LPIAT activity [73].

2.3.2. Acyltransferases using lysoPC as acceptor

To date, three enzymes, called LPCAT1, LPCAT2, and LPCAT3, have been found to utilize preferentially lysoPC as acceptor. LPCAT1 (also known as AGPAT9) [63,64] was identified and characterized independently by two different groups in murine alveolar type II cells [75,76]. LPCAT1 is highly expressed in lung, where it is suggested to play an important role in the synthesis of surfactant phospholipids, particularly dipalmitoyl glycerophosphocholine, the major component of pulmonary surfactant. Activity assays show high selectivity for medium-chain saturated acyl-CoAs (6:0-16:0) and lysoPC substrates, although the enzyme also displays significant activity towards lysoPA and lysoPG [76]. LPCAT1 appears to play an important role in the remodeling of PC in erythrocytes [77]. Recently it has been described that LPCAT1 is implicated in platelet-activating factor synthesis under Ca^{2+} independent, non-inflammatory conditions [78]. Human LPCAT1 is also abundant in lung, and it seems to have the same properties as mouse LPCAT1. Other authors have described upregulation of human LPCAT1 in colorectal cancer adenocarcinomas. [79].

LPCAT2 has recently been cloned and characterized in mouse, and is believed to constitute the main enzyme involved in the formation of platelet-activating factor under inflammatory conditions [80]. The enzyme belongs to the AGPAT family, and is highly expressed in inflammatory cells, mainly in resident macrophages and casein-induced neutrophils. It shows marked preference for lysoPC. Using RAW264.7 cells overexpressing LPCAT2, it was found that under resting cell conditions the enzyme shows activity for acetyl-CoA and, strikingly, for arachidonoyl-CoA. Under these conditions, the enzyme appears to have more affinity for arachidonoyl-CoA than for acetyl-CoA. However, upon receptor stimulation the acetyltransferase activity of LPCAT2 was found to be significantly increased, while the arachidonoyl-CoA acyltransferase was not [80].

LPCAT3, also known as MBOAT5, is expressed at high levels in all murine tissues, especially testis [81]. The enzyme shows selectivity for lysoPC, although it can also utilize lysoPE and lysoPS. As for fatty acyl donors, the enzyme utilizes AA and linoleic acid with preference over other fatty acids [81]. In humans, the enzyme is expressed at high levels in liver, pancreas and adipose tissue. In terms of specificity a preference for linoleic acid over AA was noted [82,83].

2.3.3. Acyltransferases using lysoPE as acceptor

To date, three different LPEAT acyltransferases have been found, designated as LPEAT1 (also known as MBOAT1), LPEAT2 (also known

as AGPAT7), and MBOAT2. LPEAT1 was extensively characterized in mouse and exhibits preference for oleoyl-CoA. The enzyme can also utilize lysoPS as an acceptor, although lysoPE is the preferred substrate [81]. The human enzyme displays similar properties, although in this case a higher preference for lysoPS over lysoPE was found [83].

LPEAT2 was identified in human tissues by Cao et al. [84]. The enzyme is highly expressed in brain and inflammatory cells. It has selectivity for long-saturated acyl-CoAs as donors (16:0, 18:0, 18:1) and shows selectivity towards lysoPE, although the enzyme can also utilize lysoPC, lysoPG, and lysoPS. Because the brain is a tissue highly enriched in PE, LPEAT2 has been suggested as a crucial enzyme in PE remodeling, and it could be implicated in neurological disorders, such as Alzheimer or multiple sclerosis.

MBOAT2 has been extensively characterized in mice and is highly expressed in epididymis, brain, testis, and ovary, and it shows preference for oleyl-CoA as donor and can use both lysoPE and lysoPC as acceptors. Human MBOAT2 has clearly been shown to exhibit preference for lysoPE over lysoPC, and also for oleoyl-CoA [82]. Mouse MBOAT2 has also been called LPCAT4 because it utilizes lysoPC and lysoPE equally well [81].

2.3.4. Acyltransferases using lysoPI as acceptor

MBOAT7 was identified in *Caenorhabditis elegans* as an acyltransferase specific for lysoPI [85]. In addition, MBOAT7 exhibits high selectivity for AA and eicosapentaenoic acid, making it an obvious candidate for mediating AA recycling into PI via the Lands pathway. The homolog in humans is also called BB1/LENG4 and displays the same substrate specificity as the *C. elegans* enzyme [82].

The other LPIAT described to date is LPAAT3 which, as indicated above, may utilize either lysoPA or lysoPI as acceptors [73].

2.3.5. Other lysophospholipid acyltransferases

LCLAT1, cloned in mice, is involved in the remodeling of cardiolipin, a glycerophospholipid abundant in mitochondria [86]. LCLAT1 possesses both acyl-CoA:monolysocardiolipin acyltransferase and acyl-CoA:dilysocardiolipin acyltransferase activities and uses oleic and linoleic acids with preference as donors.

LPGAT1 was identified in human as an acyltransferase using PG as acceptor [87]. PG is a major component of lung surfactant; thus, LPGAT1 is presumed to play an important key role in lung physiology. The enzyme appears to show some selectivity for palmitic, stearic, and oleic acids.

3. Role of PLA_2 in AA mobilization

Stimulation of cells via receptor agonists frequently results in the activation of phospholipid hydrolysis by phospholipase A_2 enzymes. An immediate consequence of this is the net accumulation of free AA that can be used for various cellular functions, e.g. the biosynthesis of eicosanoids. Various routes for AA release have been described, including a phospholipase C/diacylglycerol lipase pathway or the inhibition of phospholipid AA reacylation; however, there is general agreement that the PLA_2-mediated hydrolysis of phospholipids is the major pathway controlling AA mobilization in stimulated cells and that all major AA-containing phospholipid classes, namely PC, PE, and PI, contribute to this release [88–91].

Mammalian cells contain multiple structurally diverse PLA_2 enzymes capable of hydrolyzing sn-2 fatty acids from phospholipids. PLA_2s have been systematically classified according to their nucleotide sequence. In the latest update to this classification, the PLA_2 enzymes were classified into 15 group types, according to their primary sequence [92]. Additionally, a 16th PLA_2 group has been reported very recently [93]. However, a second classification of the PLA_2 enzymes, sometimes more useful, also exists which categorizes the enzymes into five major families attending to biochemical commonalities [89,94]. These families are the Ca^{2+}-dependent secreted enzymes, the Ca^{2+}-dependent cytosolic enzymes, the Ca^{2+}-independent cytosolic enzymes, the platelet-activating factor acetyl hydrolases, and the lysosomal PLA_2s. Of these families, the first two have been repeatedly implicated in AA mobilization in response to a variety of immunoinflammatory stimuli [88,89,95]. Today, it is firmly established that the calcium-dependent cytosolic group IVA $PLA_2\alpha$ ($cPLA_2\alpha$) is the critical enzyme in AA release [96] and that, depending on cell type and stimulation conditions, a secreted PLA_2 – in particular, that belonging to groups IIA, V, and X – may also participate by amplifying the $cPLA_2\alpha$-regulated response [17,97–100]. In addition, recent data have also indicated that the Ca^{2+}-independent cytosolic PLA_2 (group VI enzymes) can also mediate AA release under certain conditions [101,102].

A number of excellent reviews have recently been published covering various aspects of $cPLA_2\alpha$ biochemistry, including structure, catalysis, regulation by Ca^{2+} availability, and physiological/pathophysiological functions [96,102–106]. Thus, in the following sections, we will focus on recent data on the cellular regulation of $cPLA_2\alpha$ by phosphorylation and anionic lipids.

3.1. Role of phosphorylation reactions in regulating $cPLA_2\alpha$ activity in cells

$cPLA_2\alpha$ can be phosphorylated on multiple residues under activation conditions, but only three of them, namely, Ser^{505}, Ser^{727}, and Ser^{707}, appear to be relevant to the regulation of AA mobilization in agonist-stimulated cells [107–109]. Phosphorylation of $cPLA_2\alpha$ at these sites only modestly increases the activity of the enzyme *in vitro*, thus suggesting that such phosphorylation serves other regulatory functions in cells.

Phosphorylation of $cPLA_2\alpha$ at Ser^{505} was the first to be described [107] and is still the most extensively studied and perhaps the most controversial from a functional point of view. Phosphorylation of $cPLA_2\alpha$ at Ser^{505} stably increases enzyme activity by 1.5- to 2-fold and promotes a significant mobility shift of the protein in acrylamide gels [107]. Depending on cell type and stimulus, the phosphorylation reaction is catalyzed by extracellular-regulated kinases p42 and p48 [107], p38 [110], and SAPK/JNK [111,112]. By studying *in vitro* membrane affinity of different phosphorylation-site mutants of $cPLA_2\alpha$, Das et al. [113] have recently described that mutation at Ser^{505} results in a lower affinity for PC membranes than the wild type enzyme, which is due to a faster desorption from the membrane. This difference is very much enhanced at low Ca^{2+} concentrations compared with high Ca^{2+} concentrations during the assays (2.5 and 50 μM, respectively) [113]. In experiments with cells, this different behavior can also be observed at low intracellular Ca^{2+} concentrations during cellular activation, a situation that is observed when the cells are stimulated with 2 μM Ca^{2+} ionophore (0.4 mM intracellular Ca^{2+}). The interpretation of these results is that phosphorylation at Ser^{505} enhances hydrophobic interaction of the enzyme with the membrane, by promoting membrane penetration of the hydrophobic residues Ile^{399}, Leu^{400}, and Leu^{552}. This effect probably occurs through a conformational change of the protein [113]. Other studies, however, have not found differences between the membrane translocation behavior of the wild type enzyme and the mutated enzyme in Ser^{505} [114,115]. Recently, it has been described that in lung fibroblasts activated with phorbol esters or serum, Ser^{505} phosphorylation does not work to lower the Ca^{2+} threshold levels necessary for $cPLA_2\alpha$ translocation, but only acts to increase the catalytic activity of the enzyme [116].

$cPLA_2\alpha$ phosphorylation at Ser^{727} was first described in Sf9 cells overexpressing $cPLA_2\alpha$ [108] and later in agonist-stimulated human platelets [111]. In platelets, this phosphorylation is required for a full AA mobilization response, and the kinase involved appears to be MNK-1 or a closely related kinase [117]. Phosphorylation of $cPLA_2\alpha$ at Ser^{727} increases the cellular activity of the enzyme by a mechanism

that is not mediated by increased membrane affinity [117] but by a mechanism related with the interaction of cPLA$_2$α with a tetramer of p11 and annexin 2A in the cytosol [118]. *In vitro* activity assays and membrane binding measurements by surface plasmon resonance analyses showed that, in resting conditions, cPLA$_2$α interacts with a tetramer of p11 and annexin 2A via the hydroxyl group of Ser^{727}, inhibiting the targeting of the enzyme to cellular membranes and the AA release. When cells are stimulated and phosphorylation in Ser^{727} occurs, the tetramer is displaced from cPLA$_2$α and the enzyme can then interact with cellular membranes and effect phospholipid hydrolysis [118]. This mechanism has yet to be confirmed in agonist-stimulated cells.

Phosphorylation of cPLA$_2$α on Ser^{515} was first described as the only residue phosphorylated by calcium/calmodulin-dependent protein kinase II *in vitro* and lead to an increase in enzymatic activity of about 2-fold [109]. The biological role of this phosphorylation has been defined very recently in norepinephrine-stimulated vascular smooth muscle cells by using a specific antibody against the phosphorylated residue [119]. Phosphorylation of cPLA$_2$α at Ser^{515} by calcium/calmodulin-dependent protein kinase II appears to be a pre-requisite for further phosphorylation of the enzyme at Ser^{505} by ERK1/2, and both phosphorylated sites are required for a full AA mobilization response to norepinephrine. In this system, mutation of Ser^{505}, Ser^{515}, or both (Ser^{505}/^{515}) to Ala does not change the ability of the mutated enzyme to translocate to the nuclear envelope [119].

3.2. Cellular regulation of cPLA$_2$α by anionic phospholipids

The activating effect of anionic phospholipids on cPLA$_2$α was first described by Leslie and Channon [120] in studies utilizing a partially purified enzyme from RAW264.7 cells. It was shown that PS, PA, PI, and phosphatidylinositol-4,5-bisphosphate (PIP$_2$) have the capability to increase cPLA$_2$α activity *in vitro* when incorporated into the vesicle substrate. PIP$_2$ was the best activator, reaching a 7-fold increase in activity at 1 mol% [120]. This effect could be further enhanced to 20-fold by coincubating with diacylglycerol or PE, decreasing at the same time the requirement of Ca^{2+} for enzyme activity from millimolar to nanomolar [120].

The activating effects of PIP$_2$ were later confirmed by Mosior et al. [121] utilizing human recombinant cPLA$_2$α. Binding of cPLA$_2$α to large unilamellar vesicles of PC was enhanced 20-fold in the presence of 1% PIP$_2$, with a concomitant increase in activity of the same magnitude. The stoichiometry of binding was 1:1, and just 1 molecule per 2000 lipid molecules in the membranes was enough to double the binding of the cPLA$_2$α [121]. The binding effect produced by PIP$_2$ was so important that it supported measurable association with vesicles and activity even in the absence of Ca^{2+} in the reaction mixture (presence of EGTA). Other related phospholipids like PI(3,4,5)P$_3$ and PI(3,4)P$_2$ also enhanced PLA$_2$α activity in their assay but at 60% and 63% of the PIP$_2$. However, other anionic lipids such as PI, PS or PA had little or opposite effects on the binding of cPLA$_2$α to the lipid vesicles [121]. Following on these observations, Das and Cho [122] identified a polybasic cluster in the catalytic domain of cPLA$_2$α that, at least partially, accounted for PIP$_2$ binding (Lys^{541}, Lys^{543}, Lys^{544}, and Arg/Lys^{488}). Mutations of this cluster eliminate the specific activation of the cPLA$_2$α promoted by PIP$_2$. However, no effect of PIP$_2$ on the affinity of the enzyme for vesicles was appreciated in experiments of surface plasmon resonance [122].

In a cellular context, the first evidence for the regulation of cPLA$_2$α by PIP$_2$ was obtained in a macrophage-like cell line activated by UV radiation [123]. Inhibition of phosphoinositide increases during cellular activation also inhibited the release of AA. The biological relevance of these observations was that UV activated cPLA$_2$α in the cells in the absence of any apparent change in the intracellular Ca^{2+} concentration [123]. In this regard, Das and Cho [122] also observed that sequestration of cellular PIP$_2$ by overexpressing a pleckstrin homology domain of the phospholipase C-δ$_1$ considerably decreased the amount of AA released by cellular activation with ionophore. Later it was also demonstrated that exogenous PIP$_2$ shuttled into the cells by coupling it to cationic carriers promoted the translocation of cPLA$_2$α to those membranes where PIP$_2$ was localized, mainly perinuclear membranes [124]. The effect was observed at basal intracellular Ca^{2+} concentrations (50 nM), but it did not occur in the presence of EGTA, suggesting that the process of cPLA$_2$α translocation by PIP$_2$ is not Ca^{2+}-independent. In support of this, a mutant in the Ca^{2+}-binding site of the enzyme (D43N) did not translocate in response to PIP$_2$ [124]. It was also observed that intracellular increases in PIP$_2$ lowered the Ca^{2+} requirements for enzyme translocation to intracellular basal levels. Furthermore, mutations in the cationic cluster Lys^{541}, Lys^{543}, Lys^{544}, and Lys^{488} did not change the translocation pattern of the enzyme to intracellular membranes but inhibited the release of AA, indicating a non-productive membrane binding [124].

In contrast with these observations, by using yeast-based assay that tests the ability of proteins to bind to membrane lipids, Le Berre et al. [125] have found that only the Ca^{2+}-binding domain of cPLA$_2$α interacts with lipids, including PIP$_2$. Because inhibitory effects on cPLA$_2$α activity were observed by overexpressing the pleckstrin homology domain of PLC-δ$_1$, and PIP$_2$-specific 5′-phosphatase in stimulated cells, the conclusion that cPLA$_2$α activity can be modulated by sequestration or depletion of cellular PIP$_2$, but not by direct binding, was made [125].

Another anionic phospholipid that appears to have profound effects on the activity and physical state of cPLA$_2$α is ceramide 1-phosphate. Pettus et al. [126] demonstrated that ceramide kinase and its product ceramide 1-phosphate mediated the activation of cPLA$_2$α during cellular stimulation with IL-1 or calcium ionophore. It was also observed that ceramide 1-phosphate induces the translocation of cPLA$_2$α to intracellular membranes, mainly Golgi and perinuclear membranes [127]. Moreover, the C2 domain of the enzyme itself is also translocated in response to ceramide 1-phosphate. In vitro studies demonstrated that ceramide 1-phosphate binds cPLA$_2$α through the C2 domain (at the cationic β-groove Arg^{57}, Lys^{58}, Arg^{59}) and that such an interaction increases the enzymatic activity in a calcium-dependent manner [127,128]. By using surface-dilution kinetics and surface plasmon resonance, it has been described as well that ceramide 1-phosphate activates cPLA$_2$α not by affecting the Michaelis–Menten constant but by increasing the residence time of the enzyme on membranes, decreasing the dissociation constant of the enzyme to membrane PC [129,130].

3.3. sPLA$_2$'s role in AA release

There are much data suggesting that certain sPLA$_2$ forms are involved in mediating AA mobilization in a variety of cells, most notably those involved in immunoinflammatory reactions, like macrophages and mast cells [89,94]. However, the mode how sPLA$_2$ participates in this process is still a very controversial issue. This is due in part to the fact that most of the evidence implicating sPLA$_2$ in AA mobilization derives from studies utilizing exogenous enzymes or cells overexpressing certain sPLA$_2$ forms, and limited information is available on the role of the "endogenous" relevant sPLA$_2$.

Exogenous sPLA$_2$, particularly that belonging to groups IIA, V, and X, or sPLA$_2$ overexpressed in various cells can amplify the essential role of cPLA$_2$α in eicosanoid biosynthesis by augmenting the release of AA and other fatty acids under various experimental conditions [98,131–135]. The sPLA$_2$ enzymes could potentially be involved in the cPLA$_2$α-dependent AA mobilization through three pathways, one involving re-internalization via caveolin-rich domains [136,137], the second involving direct interaction with PC-rich outer membrane domains [136,138,139], and the third one involving an undefined intracellular action prior secretion of the enzyme [140]. Nonetheless,

$sPLA_2$s may also act to release AA in a $cPLA_2\alpha$-independent manner, as demonstrated by studies in cells from mice lacking $cPLA_2\alpha$ by genetic disruption [141].

Regarding the endogenous enzyme, studies using mice in which the gene encoding group V $sPLA_2$ was deleted have provided conclusive evidence for the role of this enzyme in eicosanoid production by macrophages and mast cells *in vivo* [142]. Interestingly, the effect of $sPLA_2$ is observed in cells on a C57BL/6 genetic background, while in cells on a BALB/c background, no $sPLA_2$ effect could be ascertained [143]. These data provide evidence that two different phenotypes may exist in cells regarding the involvement of $sPLA_2$ in eicosanoid generation. Whether these two phenotypes may also manifest in cells depending on culture conditions is unknown at present.

On the other hand, studies on the role of $sPLA_2$ in AA release are complicated by the existence of cross-talk between the $sPLA_2$ and the main effector of the response, the $cPLA_2\alpha$. This cross-talk may work in both directions, i.e. $cPLA_2\alpha$ may regulate the action of $sPLA_2$ or *vice versa*.

In some cell types such as human and murine macrophage-like cell lines, $cPLA_2\alpha$ has been shown to regulate $sPLA_2$ activity by a mechanism involving the rapid generation of hydroperoxyeicosatetraenoic acid [144,145]. At long incubation times, activation of $cPLA_2\alpha$ is also required for the increased expression of group V $sPLA_2$ that is characteristically induced by immunoinflammatory stimuli such as lipopolysaccharide and, as with the short-term response, may involve the production of hydroperoxyeicosatetraenoic acid [146–150].

The regulation of $cPLA_2\alpha$ by $sPLA_2$ has been characterized in detail in some instances. In murine mesangial cells, an adenoviral infection technique was used to stably express group IIA and/or group V $sPLA_2$ into the cells [100]. $cPLA_2\alpha$ was found to effect the AA release and, when present, both $sPLA_2$ forms amplified the $cPLA_2\alpha$-mediated response, thus resulting in increased AA mobilization [100]. Moreover, a correlation was found to exist between the expression level of $cPLA_2\alpha$ and the magnitude of AA release. Such a correlation did not occur between the expression level of $sPLA_2$ and the extent of AA release. Recent work in mouse mast cells from mice lacking group V $sPLA_2$ by genetic deletion has provided conclusive evidence that $sPLA_2$ modulates the activity of $cPLA_2\alpha$ by regulating its phosphorylation via extracellular-regulated kinases [151]. Utilizing inhibitors, similar observations have also been made in recent work with murine macrophage-like cells [152,153].

3.4. $iPLA_2$'s role in AA release

The group VIA PLA_2 ($iPLA_2$-VIA) is ubiquitously expressed and has the potential to participate in AA release under some conditions [101,102]. However, the role of this enzyme in AA release has traditionally been inferred from studies using the inhibitor BEL, a compound that manifests high selectivity for $iPLA_2$ *in vitro* but not *in vivo* [154–156]. In some studies, BEL was found to inhibit the AA release, but in others, notably in phagocytes, no significant effect was detected [89]. It appears likely that the involvement of group $iPLA_2$-VIA in AA release is markedly cell- and stimulus-dependent, as most of the roles attributed to this enzyme in cell physiology appear to be [22,101,157]. Since various $iPLA_2$-VIA splice variants co-exist in cells [22,101,157], it is possible that the enzyme is subject to multiple regulatory mechanisms that differ among cell types and stimulation conditions. This in turn could also explain the multiplicity of functions that this enzyme appears to serve depending on cell type.

Recently, mice with targeted disruption of the gene encoding for $iPLA_2$-VIA have been generated [158]. Use of cells from these animals has reinforced the idea that the involvement of $iPLA_2$ in AA mobilization notably differs depending on cell type and stimulation conditions. Thus, peritoneal macrophages from $iPLA_2$-VIA null mice appear to release AA in response to zymosan in a manner that is indistinguishable from that of cells from wild type animals [159]. In contrast, however, $iPLA_2$-VIA appears to be crucial for AA mobilization in macrophages upon free cholesterol loading [159] and for the eicosanoid response of macrophages stimulated via class A scavenger receptors [160].

4. Conclusions

In the last two decades, much effort has been made to elucidate the mechanisms by which AA is liberated and incorporated into phospholipids. This review has dealt with the relatively high number of enzymes with acyl-CoA synthetase, CoA-dependent acyltransferase, or phospholipase A_2 activities that have been described to participate in the regulation of cellular AA availability. Some of these enzymes show a marked selectivity for AA. Clearly, this is a very complex issue involving multiple enzymes and pathways, and there is still much to be learned about the interplay between some of the AA-utilizing enzymes and the regulatory mechanisms involved.

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