The Role of Sphingolipids in Arachidonic Acid Metabolism

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Received November 22, 2013; Accepted January 17, 2014

Abstract. The arachidonic acid (AA) cascade is regulated mainly by the actions of two rate-limiting enzymes, phospholipase A2 (PLA2) and inducible cyclooxygenase-2 (COX-2). PLA2 acts to generate AA, which serves as the precursor substrate for COX-2 in the metabolic pathway leading to prostaglandin production. Amongst more than 30 members of the PLA2 family, cytosolic PLA2α (cPLA2α, group IVA) plays a major role in releasing AA from cellular membranes. Sphingolipids are a novel class of bioactive lipids that play key roles in the regulation of several cellular processes including differentiation, inflammatory responses, and apoptosis. Recent studies implicated a regulatory function of sphingolipids in prostaglandin production. Whereas ceramide-1-phosphate and lactosylceramide activate cPLA2α directly, sphingosine-1-phosphate induces COX-2 expression. Sphingomyelin has been shown to inhibit the activity of cPLA2α. In addition, several sphingolipid analogs including a therapeutic agent currently used clinically are also reported to be inhibitors of cPLA2α. This review explores the role of sphingolipids in the regulation of cPLA2α and COX-2.

Keywords: arachidonic acid cascade, cytosolic phospholipase A2α, cyclooxygenase, sphingolipid

1. Introduction

Arachidonic acid (AA) is a precursor of eicosanoids, including prostaglandins (PG) and thromboxanes, playing important roles in many physiological and pathological functions. The biosynthesis of these AA metabolites occurs mainly by activation of phospholipase A2 (PLA2) in response to a wide variety of stimuli. PLA2 catalyzes the hydrolysis of the sn-2 position of membrane glycerophospholipids to liberate free AA and lysophospholipids (1). Mammalian cells have structurally diverse forms of PLA2, including secretory PLA2, Ca2+-independent PLA2, and cytosolic PLA2 (cPLA2). Among these, group IVA PLA2 (cPLA2α) is highly selective for glycerophospholipids containing AA. The released AA is converted by the action of cyclooxygenase (COX)-1 and COX-2 to PG.

Sphingolipids are a class of lipids containing a backbone of sphingoid base. Not only do sphingolipids play a structural role in the cellular membrane, but they have also been implicated in various significant cell signaling pathways and physiological processes (2). Recent studies have revealed that sphingolipids regulate cPLA2α and COX-2 as shown below. This review provides an overview of our current understanding of the regulatory mechanisms of cPLA2α and COX-2 by sphingolipids.

2. Sphingolipid metabolism

The first necessary step in the de novo pathway of ceramide generation involves palmitoyl-CoA and amino acid serine condensation, via the action of the enzyme serine palmitoyl transferase, to form dihydrosphingosine (2). Following its synthesis, serine-derived dihydro sphingosine is acylated via action of the ceramide synthases to become dihydroceramide. Dihydroceramide is then desaturated to form ceramide. Ceramide may also be generated by the breakdown of membrane sphingomyelin (SM) or via degradation of complex glycosphingolipids by the action of sphingomyelinases and glucosyl ceramidases, respectively. Degradation of ceramide is
carried out by the ceramidases, whereby the acyl chain is removed from ceramide and the 18-carbon amino-alcohol compound sphingosine is formed. Sphingosine then serves as the substrate for the sphingosine kinases, which are responsible for phosphorylating sphingosine at the primary hydroxyl group, resulting in the production of sphingosine-1-phosphate (S1P). In lieu of being phosphorylated by sphingosine kinase to S1P, sphingosine can be recycled back to ceramide via ceramide synthase–mediated reacylation. Ceramide is also phosphorylated by the action of ceramide kinase (CerK) to form ceramide-1-phosphate (C1P) (Fig. 1).

3. Regulatory mechanisms of cPLA2α activity

cPLA2α is widely expressed in mammalian cells and mediates the production of functionally diverse lipids in response to extracellular stimuli. cPLA2α is highly selective for glycerophospholipids containing AA (1). cPLA2α is regulated mainly by binding Ca2+, phosphorylation on serine residues, and interaction with lipids. The binding of Ca2+ to the C2 domain of cPLA2α triggers translocation of cPLA2α from the cytosol to the perinuclear membranes including the Golgi apparatus, endoplasmic reticulum, and nuclear envelope. The translocation of cPLA2α to the perinuclear membranes is important for its functional coupling with COXs, which reside there. The activation of cPLA2α in cells requires sustained phosphorylation of Ser505 by extracellular signal-regulated kinase 1/2. The main role of the Ser505 phosphorylation of cPLA2α is to promote membrane penetration of hydrophobic residues in the active-site rim by inducing a conformational change in the enzyme; these enhanced hydrophobic interactions allow sustained membrane interaction of cPLA2α. Full activation of cPLA2α is achieved by additional phosphorylation at Ser515 and Ser727 by Ca2+-calmodulin kinase II and mitogen-activated protein kinase-activated protein kinases, respectively. Phosphatidylinositol 4,5-bisphosphate binds with high affinity and specificity to cPLA2α, facilitating membrane binding and activity. The binding site of phosphatidylinositol 4,5-bisphosphate includes four lysine residues (Lys488, Lys541, Lys543, and Lys544), which are located in the highly basic region of the catalytic domain on the side close to the membrane. In addition, sphingolipids regulate the activity of cPLA2α as shown below.
4. Regulatory mechanisms of cPLA₂α activity by sphingolipids

4.1. Role of C1P in the activity of cPLA₂α

The first report on the regulation of AA release and the production of PG by C1P was by Chalfant and co-workers (3). These authors demonstrated that C1P potently and specifically stimulates AA release and PG synthesis in A549 lung adenocarcinoma cells. In the same report, the authors showed that CerK via the production of C1P is a mediator of AA release in response to interleukin-1β (IL-1β) and calcium ionophore A23187. In a later report, the same group demonstrated that the mechanism of C1P-stimulated AA release occurs through direct activation of cPLA₂α (4). In addition, C1P has been shown to induce the translocation of cPLA₂α and its C2 domain to the Golgi apparatus. Our laboratory has also reported that C1P activates cPLA₂α directly and by a protein kinase C–dependent pathway (5). Subsequently, Chalfant’s group showed that C1P increases the affinity of cPLA₂α for membranes through interaction with the C2 domain of cPLA₂α (6). This enhanced association of cPLA₂α with membrane leads to an increase in the enzymatic activity of the enzyme. In addition, the C1P interaction site has been identified in a cationic β-groove of the C2 domain using the solved structure of cPLA₂α in conjunction with site-directed mutagenesis (7). Specifically, the amino acids Arg⁵⁷, Arg⁵⁸, and Arg⁶⁹ are required for the interaction of cPLA₂α with C1P. The mutation of these residues abolishes the ability of the enzyme to be activated and translocated in response to probably C1P formed by A23187 or ATP (8). Recently, Arg⁵⁹, Arg⁶¹, and His⁶² (an RxRH sequence) have also been shown to be essential for the in vitro affinity of C1P and translocation in response to A23187 (9). These studies demonstrate that C1P is required for the translocation of cPLA₂α to intracellular membranes in response to stimuli and the subsequent production of AA.

4.2. Role of ceramide in the activity of cPLA₂α

Ceramide has been shown to interact directly with cPLA₂α via the C2 domain, which causes improved membrane binding of cPLA₂α, resulting in enhanced AA release and PG synthesis (10). To determine the functions of ceramide in cells, ceramide analogs consisting of sphingosine linked by an amide bond to a short-chain fatty acid, such as acetic acid (C2-ceramide), hexanoic acid (C6-ceramide), and octanoic acid (C8-ceramide), are often used because they are cell-permeable. Both C2- and C8-ceramides were shown to enhance the A23187-induced release of AA in Chinese hamster ovary cells, which was inhibited by treatment with an inhibitor of cPLA₂α (11). Exogenous ceramide is metabolized by various enzymes including CerK, ceramidases, SM synthases, and glucosylceramide synthases. The release of AA induced by C6-ceramide is inhibited by treatment with CerK inhibitor in RBL-2H3 rat mast cells (12). Thus, it seems that the release of AA induced by the exogenous addition of ceramide is in part due to C1P generation.

4.3. Role of lactosylceramide (LacCer) in the activity of cPLA₂α

LacCer is a member of the glycosphingolipid family and is known to be a bioactive lipid in various cell physiological processes. LacCer stimulates PECAM-1 (platelet endothelial cell adhesion molecule-1) expression in the adhesion and diapedesis of monocytes/lymphocytes, which is inhibited by treatment with inhibitors of Ca²⁺-independent PLA₂ or cPLA₂α (13). Recently, our laboratory has reported that down-regulation of cPLA₂α using RNA interference abolishes the ability of LacCer to induce AA release in cells, suggesting that cPLA₂α is the key PLA₂ downstream of LacCer (14). Treatment of cells with LacCer induces the translocation of full-length cPLA₂α from the cytosol to the Golgi apparatus. LacCer also induces the translocation of the C2 domain of cPLA₂α in the same manner. Interestingly, LacCer interacts directly with full-length cPLA₂α and with the C2 domain in a Ca²⁺-independent manner. Furthermore, LacCer directly activates cPLA₂α in vitro. Thus, LacCer is a novel direct activator of cPLA₂α.

4.4. Role of SM in the activity of cPLA₂α

Our laboratory has reported that SM inhibits the activity of cPLA₂α and the release of AA (15). In that report, we showed that A23187-induced AA release was increased in LY-A cells that are defective in the ceramide transport protein CERT for SM synthesis, compared with that in control cells. On the other hand, increasing the cellular SM level by treatment with an acid sphingomyelinase inhibitor decreased the release of AA in response to stimuli including A23187 or platelet-activating factor (PAF). In addition, in vitro study indicated that SM disturbed the binding of cPLA₂α to glycerophospholipids. These results suggest that SM at the bio-membrane plays important roles in regulating the cPLA₂α-dependent release of AA by inhibiting the binding of cPLA₂α to glycerophospholipids.

5. Regulation of COX-2 expression by S1P

S1P exerts a variety of biological responses through extracellular specific receptors or intracellular mechanisms as a secondary messenger. The S1P family of
G protein–coupled receptors, of which there are five (S1P₁–S1P₅), couple to different alpha subunits of heterotrimeric G proteins such as Ga₅, Ga₁₂, and Ga₁₅₁₃. S1P receptor expression patterns, along with the Ga α subunits to which each receptor couples, dictate the activation of different downstream targets that occur upon receptor activation, including the activation of Rac, extracellular signal-regulated kinase, phosphatidylinositol-3 kinase, adenylyl cyclase, phospholipase C, Rho, and c-jun N-terminal kinase, resulting in various cellular responses (16).

Various investigators have demonstrated that S1P regulates COX-2 expression. Davaille et al. (17) have reported that S1P treatment causes strong induction of COX-2, which peaks after 3 h and remains elevated for at least 8 h. In contrast, COX-1 expression is not affected by S1P. Subsequently, Ki et al. (18) have reported that Ga₁₂ specifically regulates nuclear factor-κB–mediated COX-2 induction by S1P downstream of S1P₁, S1P₃, and S1P₅, in a process mediated by the e-c-Jun N-terminal kinase–dependent ubiquitination and degradation of IκBα. Although they showed that S1P-induced COX-2 induction was not attenuated by RNA interference of S1P₂, another group has shown that S1P₂ activation induces COX-2 expression (19). In transforming growth factor β1–stimulated fibroblasts, S1P formed by the receptor stimulation induces COX-2 expression via S1P₁ (20). Thus, subtypes of S1P receptor for COX-2 induction may depend on cell types or conditions. In addition, S1P has been shown to activate nucleocytoplasmic shuttling of the mRNA-stabilizing protein HuR, which in turn stabilizes COX-2 mRNA and elicits COX-2 expression (21). Thus, S1P may increase COX-2 expression by inducing transcription of COX-2 mRNA and by stabilization of its mRNA.

6. The role of sphingolipid metabolism in the inflammatory response

Pro-inflammatory cytokines, including IL-1β and tumor necrosis factor-α (TNF-α), regulate both cPLA₂α and COX-2 via sphingolipid metabolism. The abilities of IL-1β or TNF-α to induce COX-2 expression are abrogated by RNA interference of sphingosine kinase 1, which can be rescued by replenishment of S1P, indicating that the sphingosine kinase 1/S1P pathway is required upstream of the induction of COX-2 (22, 23). IL-1β leads to the formation of C1P via CerK, resulting in the activation of cPLA₂α. Postoperative ileus, a major cause of morbidity after abdominal surgery, is characterized by intestinal dysmotility and inflammation. The levels of S1P and of C1P are increased in intestinal smooth muscle cells from a rat model of intestinal surgical manipulation (24).

Our laboratory has reported recently that TNF-α–induced AA release is abolished by RNA interference of cPLA₂α or glucosylceramide synthase (14). TNF-α induces the translocation of cPLA₂α to the perinuclear regions, which is inhibited by treatment with inhibitor of glucosylceramide synthase. TNF-α induces the activation of β₁,4-galactosyl transferase and the formation of LacCer, which is generated via the galactosylation of glucosylceramide produced from ceramide. In addition, TNF-α and IL-1β activate sphingomyelinase that hydrolyzes SM to ceramide. Thus, it is supposed that, in response to pro-inflammatory cytokines, the level of SM as an inhibitor of cPLA₂α is decreased, and C1P and LacCer as activators are increased in the membrane, thereby inducing the catalytic ability of cPLA₂α to release AA (Fig. 2). In addition, the induction of COX-2 in response to cytokine-stimulated S1P formation exhibits a significant synergistic response in the production of PG.

7. Sphingolipid analogs as inhibitors of cPLA₂α

7.1. FTY720

FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol) is structurally similar to sphingosine and the lead compound from which it was derived, ISP-1 (myriocin), was isolated from the culture broth of Isaria sinclairii (25). Also known as Fingolimod, or by the trade name Gilenya™, FTY720 is currently used clinically to quell the symptoms and slow the progression of multiple sclerosis. FTY720 is phosphorylated by sphingosine kinase 2, and a large body of evidence suggests that FTY720-phosphate, an analog of S1P, is the biologically active form. FTY720-phosphate can bind to all known S1P receptors, except S1P₅, and has been shown to regulate S1P₁ actions that are crucial for lymphocyte migration and trafficking.

FTY720 has also been shown to inhibit antigen-induced release of AA and secretion of PGD₂ (26).
Interestingly, these effects are independent of its phosphorylation and S1P receptor functions. In addition, FTY720 but not FTY720-phosphate inhibits recombinant cPLA2α activity. Thus, the development of nonphosphorylatable analogs of FTY720 that still target cPLA2α but would not affect lymphocyte trafficking and homing through S1P receptors might lead to novel potent and specific therapeutics.

### 7.2. C1P analogs

A C1P analog, 1-methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl-disodium-phosphate, named phospho-ceramide analogue-1 (PCERA-1), was synthesized by Zor and co-workers (27). PCERA-1 has been shown to be an immune modulator with the ability to downregulate the production of pro-inflammatory cytokines such as TNF-α and IL-12p40 and to up-regulate the production of the anti-inflammatory cytokine IL-10, in lipopolysaccharide-stimulated macrophages and in vivo. In addition, PCERA-1 suppresses lipopolysaccharide-induced AA release and PGE2 production in RAW264.7 macrophages by inhibiting the enzymatic activity of cPLA2α (28). The inhibitory activity of PCERA-1 on the release of AA is attributed to its dephosphorylated derivative, ceramide analogue-1 (CERA-1), which directly inhibits cPLA2α.

Our laboratory has also synthesized novel di-methyl (DM) or di-ethyl (DE) phosphate ester analogs of C1P with N-acyl chains of different lengths, and found that short-N-acyl C1P analogs such as C2-DM-C1P, C5-DM-C1P, C6-DM-C1P, and C2-DE-C1P inhibited the release of AA mediated by cPLA2α in cells (29, 30). These analogs inhibit the enzymatic activity of cPLA2α directly. Interestingly, C2-DE-C1P inhibits the translocation of cPLA2α in response to A23187 or PAF. In addition, C2-DE-C1P disturbs the binding of the enzyme to glycerophospholipids in the lipid–protein overlay assay. Thus, C1P analogs may be useful for the development of therapeutics for various AA metabolism–related diseases.

### 8. Concluding remarks

This review describes mechanisms by which various bioactive sphingolipids can differentially modulate the AA metabolism. Table 1 lists regulators of cPLA2α activity and COX-2 expression. C1P and LacCer mediate cPLA2α activation by causing the translocation of cPLA2α to the Golgi apparatus, whereas SM inhibits cPLA2α activity. S1P increases COX-2 expression by inducing transcription of COX-2 mRNA and by stabilization of its mRNA. Various sphingolipid analogs can act as cPLA2α as inhibitors. Pro-inflammatory cytokines increase cPLA2α activity and COX-2 expression via sphingolipid metabolism as shown in Fig. 2. However, much remains to be understood and discovered with regard to the regulatory mechanisms of sphingolipid metabolism enzymes. Hopefully, such approved understanding will enable the development of agents aimed at decreasing the potential contribution of AA metabolism to human pathology.

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