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Review

Modulation of cell signalling by ceramides

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Contents

Abbreviations: Cer 1-P, ceramide 1-phosphate; DAG, diacylglycerol; MAP, mitogen-activated protein; PA, phosphatidate; PC, phosphatidylcholine; SM, sphingomyelin; Sph 1-P, sphingosine 1-phosphate; TNF-α, tumor necrosis factor-α

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1. Introduction

The aim of this review article is to emphasize the consequences of agonist-induced hydrolysis of phosphatidylcholine (PC) and sphingomyelin (SM), as well as the interactions between ceramide, and the different metabolites that can be generated from the two pathways. PC and SM are structurally related to each other and essentially differ, in that PC has a glycerolipid backbone whereas SM contains ceramide (N-acylated fatty acid linked to sphingosine). The turnover of PC and SM at the plasma membrane of cells plays a critical role in signal transduction and the regulation of cell functions. The interaction of extracellular agonists with specific membrane receptors results in the activation of specific enzymes that generate intracellular second messengers from these two lipid precursors. PC can be hydrolyzed via receptor-mediated stimulation of a specific PC-phospholipase C to produce diacylglycerol (DAG), or by the stimulation of phospholipase D to produce phosphatidate (PA). The latter can then be converted to lysoPA by the action of an A-type phospholipase, or to DAG by phosphatidate phosphohydrolase (Fig. 1). DAG can also be generated by phosphoinositide-specific phospholipases C acting on phosphatidylinositol 4,5-bisphosphate. The generation of PA, lysoPA and DAG is relevant for controlling many cellular functions. DAG is a well-known activator of protein kinase C, whereas PA and lysoPA stimulate tyrosine

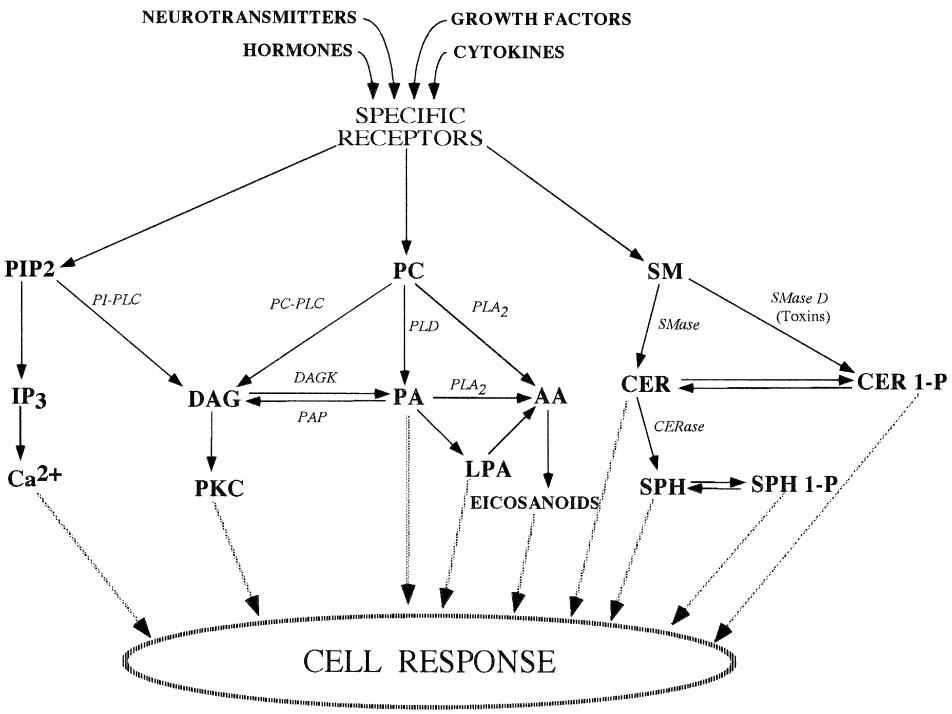

Fig. 1. Generation of bioactive glycerolipids and sphingolipids following agonist-stimulated activation of specific enzymes. The action of arthropode or bacterial sphingomyelinase D is also indicated. Abbreviations are as follows: AA, arachidonic acid; CER, ceramide; CER 1-P, ceramide 1-phosphate; DAG, diacylglycerol; DAGK, diacylglycerol kinase; IP_3, inositol 1,4,5-trisphosphate; LPA, lysophosphatidate; PA, phosphatidate; PAP, phosphatidate phosphohydrolase; PC, phosphatidylcholine; PIP_2, phosphatidylinositol 4,5 bisphosphate; PI-PLC, phosphatidylinositol-specific phospholipase C; PKC, protein kinase C; PLA_2, phospholipase A_2; PC-PLC, phosphatidylcholine-specific phospholipase C; PLD, phospholipase D; SM, sphingomyelin; SPH, sphingosine; SPH-1-P, sphingosine 1-phosphate.

kinases and activate the Ras–Raf–MAP kinase pathway. These features of DAG, PA and lysoPA are related to their ability to stimulate cell proliferation.

As a major phospholipid, SM is mainly localized to the outer leaflet of the plasma membrane of cells, although there may be functionally distinct pools in the inner leaflet and lysosomal–endosomal compartments [1–3]. SM can be hydrolyzed by receptor-mediated stimulation of SMases (C-type phospholipases) to generate phosphorylcholine and ceramide. The latter is now recognized to be a potent second messenger that is involved in the induction of cell differentiation, inhibition of cell proliferation, regulation of inflammatory responses, and induction of programmed cell death (apoptosis) [4–6]. Ceramides can be metabolized to sphingosine, sphingosine 1-phosphate (Sph 1-P) or ceramide 1-phosphate (Cer 1-P) (Fig. 1), and these metabolites have mitogenic properties [7–9]. Sphingosine inhibits phosphatidate phosphohydrolase [10–15] and protein kinase C [16–18] activities potently, and sphingosine [19] and Sph 1-P [20] stimulate the production of PA through the activation of phospholipase D. In contrast, ceramides are strong inhibitors of phospholipase D [21]. In rat fibroblasts, ceramides stimulate the degradation of PA, lysoPA [21], Sph 1-P [22] and Cer 1-P [23], and this could be part of the mechanism by which ceramides block the effects of these mitogenic phospholipids. Therefore, the interaction among the different signals that can be generated after agonist-stimulation of phospholipase D and SMase activities may be critical for the overall signal that is finally transmitted. In particular, the balance in the production of ceramides versus sphingosine, Sph 1-P, Cer 1-P, PA or lysoPA is important for regulation of phospholipase D activation, and for controlling the fate of cells towards cell division, cell cycle arrest, or cell death.

2. Signalling through glycerolipid second messengers

2.1. Diacylglycerol

The role of DAG in cell signalling is well established. However, its action as a second messenger is mainly limited to the activation of protein kinase C [24,25]. When DAG is generated from the breakdown of phosphatidylinositol 4,5-bisphosphate, it is accompanied by the release of inositol trisphosphate (Fig. 1). The latter causes the release of Ca^{2+} from intracellular stores and increases the intracellular concentration of these cations in the cytosol. Ca^{2+} can then interact with specific Ca^{2+}/calmodulin kinases and thus regulate a variety of cellular functions. The simultaneous generation of DAG and Ca^{2+} leads to the activation of the protein kinase C isoforms α, β and γ, which have been involved in the regulation of cell division [17,18,24,25]. This increase in DAG generation lasts about 1 min and it is followed by a second and more sustained production of DAG that is derived from other phospholipids, mainly PC [26,27]. This production of DAG results directly from the activation of a PC-specific phospholipase C, or indirectly via the sequential activation of phospholipase D and phosphatidate phosphohydrolase to generate PA and DAG, respectively (Fig. 1). This second phase of DAG production takes place without an accompanying release of Ca^{2+}, and it might be related to the activation of Ca^{2+}-independent isoforms of protein kinase C. However, recent evidence suggests that the DAG generated by phospholipase C activity has distinct fatty acid composition and functions than the DAG derived from the coordinated action of phospholipase D and phosphatidate phosphohydrolase activities [28]. Phospholipase C activation generates primarily polyunsaturated DAG species, whereas the activation of phospholipase D results in the generation of saturated/monounsaturated DAG species. It has now been demonstrated that phospholipase D-derived DAG does not activate protein kinase C in porcine aortic endothelial cells [28].

2.2. Phosphatidic acid

The production of PA as a result of the agonist-stimulation of phospholipase D normally occurs in response to activation of tyrosine kinases by growth factors, or by protein kinase C [29]. PA is a bioactive lipid that has been implicated in the regulation of a variety of cellular functions. It is involved in the formation of hydrogen peroxide in neutrophils [30,31], stimulation of: (a) protein phosphorylation [32], (b) protein kinase C-ζ [33], (c) phosphatidylinositol 4-kinase [34], phospholipase C-γ [35], and monoacylglycerol acyltransferase [36]. More recently, Waite et

al. [37] have demonstrated that a PA-activated protein kinase is an important regulator of the neutrophil respiratory burst by phosphorylation of the NADPH oxidase component p47-*phox*, and Oguchi et al. [38] have shown that PA generated via activation of phospholipase D induces tyrosine phosphorylation of specific proteins. PA also regulates the biological action of cellular Ras [39–41], which plays a central role as a molecular switch in the signal transduction pathways that are associated with cell proliferation, differentiation and neoplasia [42]. Activation of Ras is a key step of the Ras–Raf–MAP kinase pathway for transmission of signals from the plasma membrane to the nucleus. The function of Ras in mammalian cells depends on its interaction with a cytoplasmic GTPase activating protein that stimulates its conversion to the inactive form [43]. It has been shown that PA binds and inhibits $p21^{ras}$ GTPase activating protein [39–41,44]. Therefore, the effect of PA results in an increased GTP-bound form of Ras and enhanced Ras activity. The latter effect would increase the activation of MAP kinase via stimulation of Raf. A further role for the phospholipase D pathway and PA in activating Raf is provided by the observation that PA binds to Raf and enables it to translocate to membranes where it becomes physiologically active [45]. Also, PA generated from phospholipase D activation has been related to the induction of cytosolic phospholipase A_2, the subsequent generation of arachidonic acid, and the formation of platelet activating factor [46]. This is particularly important since arachidonic acid is the precursor of eicosanoids. Both eicosanoids and platelet activating factor are important metabolites that are involved in inflammatory responses.

2.3. Lysophosphatidic acid

PA generated by the phospholipase D pathway can be converted to lysoPA via a phospholipase A type reaction (Fig. 1). LysoPA is released by activated platelets, and is thought to be a paracrine or autocrine signal. This lysophospholipid binds to a 38–40 kDa receptor that is coupled to multiple independent effector pathways via distinct G proteins. The α subunit of the heterotrimeric G_i directly inhibits adenylate cyclase, whereas the $\beta\gamma$ dimer is thought to activate Ras via an intermediary protein tyrosine kinase. The lysoPA receptor is also coupled to G proteins of the q family (G_q), causing the activation of phospholipase C-β, activation of Rho, and the stimulation of focal adhesion kinase and phosphorylation and reorganization of cytoskeletal proteins [47–49]. LysoPA has been shown to elicit diverse biological effects in different systems. These include platelet aggregation [47], neurite retraction [50] and local wound repair [47]. In addition, production of lysoPA through activation of secretory phospholipase A_2 on microvesicles may represent a pro-inflammatory pathway [51]. LysoPA can activate phospholipase C-γ, Ca^{2+} mobilization and arachidonate release [52]. Exogenous lysoPA and PA both activate phospholipase D, thus leading to the generation of intracellular PA [21,22], and they are potent mitogens in fibroblasts [21,22,47]. Finally, both lysoPA and PA decrease the activity of adenylate cyclase through pertussis toxin sensitive mechanisms and thereby lower the intracellular concentration of cAMP [21,52,53]. This latter effect has been suggested to be involved in the stimulation of the Ras–Raf–MAP kinase pathway, since low concentrations of cAMP facilitate the activation of Raf by Ras. These effects might contribute to the mitogenic actions of PA and lysoPA that have been described in cultured fibroblasts [21,44,47,52].

3. Signalling through sphingolipid second messengers

Sphingolipids have multiple biological activities. They are involved in the transformation, differentiation and proliferation of cells, and many have been described as potent second messengers and regulators of cell activation [4,5,7,8,17,18,44,54,55]. One of the most recently identified membrane lipids that can produce bioactive metabolites is SM [4–6]. The function of this phospholipid was considered for a long time to be structural in cells. However, it has been demonstrated that several agonists including tumor necrosis factor-α (TNF-α), 1-25 dihydroxyvitamin D_3, endotoxin, γ-interferon, interleukins, nerve growth factor, ionizing radiation, chemotherapeutic agents, and heat stimulate the hydrolysis of SM to produce ceramide and phosphorylcholine [4–6,55]. Other stimulators of SM hydrolysis in different cell

types include dexamethasone [56] and antibodies or ligands that engage Fas or CD28 receptors [57,58].

3.1. Sphingomyelinase activities

As mentioned before, SM can be hydrolyzed to ceramide and phosphorylcholine by receptor-mediated stimulation of SMase activity. SM is mainly localized to the outer leaflet of cell membranes and colocalizes with caveolin-reach domains [59–61]. However, there may also be functionally distinct pools in the inner leaflet of plasma membranes and lysosomal/endosomal compartments [1,2]. In this regard, Zhang et al. [62] have shown recently that transfection and induction of *B. cereus* SMase causes elevation of intracellular ceramides, cleavage of the death substrate poly (ADP-ribosyl)polymerase and cell death. However, exogenously applied *B. cereus* SMase, despite causing higher elevations in ceramide levels, was unable to induce poly (ADP-ribosyl)polymerase cleavage, or cell death. These observations support the existence of a signal-transducing pool of SM that is distinct from the pool accessible to exogenous SM. It is also important to emphasize that relatively high concentrations (above 100 mUnits/ml) of exogenous bacterial SMase may cause hydrolysis of PC and generation of DAG [63]. Therefore, the phospholipase C activity of SMase may limit its selectivity at high concentrations.

There are at least four different SMases that can be identified by their specific in vitro pH optima and activation requirements: (1) a membrane Mg^{2+}-dependent neutral SMase (pH optimum 7.0–7.5); (2) a cytosolic Mg^{2+}-independent neutral SMase that can be activated by arachidonic acid [64]; (3) an acidic SMase (pH optimum 4.5–5.0) that requires DAG for activation [65] and (4) an alkaline SMase (pH optimum 9.0) which is dependent on bile salts for activation. Neutral and acidic SMases are involved in cell signalling, whereas alkaline SMase is thought to play an essential role in the digestion of dietary SM and gallbladder disease [66].

3.2. Ceramides

It is now well established that ceramides are second messengers for cell regulation. They participate in signal transduction by activating specific serine/threonine kinases [5,67], or by stimulating protein phosphatases [4]. Since natural ceramides are not permeant to cell membranes, many of the studies to determine the role of ceramides in signal transduction have been carried out by using short-chain cell-permeable analogs, or by exogenous addition of bacterial SMase to cells in culture. Cell-permeable ceramides have been shown to stimulate protein kinase C-ζ [68], an atypical protein kinase C that is insensitive to DAG, leading to the rapid induction of a nuclear transcription factor system NF-kB [69,70]. Recently, ceramides have been suggested to be required for activation of stress-activated protein kinases (SAPKs), which are also known as Jun nuclear kinases (JNKs) [71,72] leading to stimulation of NF-kB-dependent gene transcription, and to apoptosis. However, the molecular mechanisms by which ceramides induce the activation of SAPK/JNK are unknown. Recently, Shirakabe et al. [73] have shown that cells treated with agents and stresses that induce the generation of intracellular ceramides, or cell-permeable ceramides, activate a novel member of mammalian MAP kinase named TAK1. Interestingly, arachidonate or DAG, which can stimulate neutral cytosolic or acidic SMases respectively, failed to activate TAK1 [73]. It has been shown that cell-permeable ceramides specifically bind to and activate protein kinase c-Raf, leading to the activation of the MAP kinase cascade, stimulation of phospholipase A_2 and the release of arachidonic acid which are required for acute inflammatory processes [74]. Cell-permeable ceramides induce the transcription of cyclooxygenases 1 and 2, and αB-crystallin, a heat shock protein [59,74,75]. Also, exogenously applied SMase, or cell-permeable ceramides have been shown to interfere with insulin-induced tyrosine phosphorylation of the insulin receptor substrate-1 and to cause insulin resistance by the inactivation of the MAP kinase cascade via stimulation of protein phosphatase 2A [76]. In addition, cell-permeable ceramides suppress the expression of c-*myc* protooncogene by interfering with transcription elongation [77]. Ceramides have also been implicated in the regulation of the tyrosine kinase cascade including $p125^{FAK}$ and paxillin by a mechanism distinct from that of platelet derived growth factor [78]. More recently, natural ceramides have been shown to mimic many of the effects of TNF-α when presented to cells in a mix-

ture of ethanol and dodecane [79]. The tyrosine kinase inhibitor herbimycin A was found to inhibit TNF-α- or natural ceramide-induced internucleosomal DNA fragmentation, but not early apoptotic morphological changes, suggesting that ceramide-induced tyrosine phosphorylation is a later event involved in the process of apoptosis [80,81]. Also, increasing intracellular ceramide levels by hydrolysis of SM with exogenous SMase or by addition of cell-permeable or natural ceramides resulted in increased P23 tyrosine phosphorylation. This indicates that P23 may be a downstream effector of the ceramide signalling pathway [81]. Other potential molecular targets for ceramide-mediated signalling include the retinoblastoma gene product (pRb), a nuclear phosphoprotein that modulates cell cycle progression [82–84], interleukin converting enzyme [85,86] and phospholipase D [21,22].

The cellular concentrations of C_2- or C_6-ceramide in cells exposed to 1 to 10 μM C_2- or C_6-ceramide is approximately 10 to 100 pmol/nmol of phospholipid, which is comparable to the concentrations achieved after prolonged response to TNF-α or serum deprivation [55]. The effects of cell-permeable ceramides are specific to D-*erythro*-ceramide analogs. While the latter are potent, biologically active molecules their dihydro analogs are inactive [87]. Dihydroceramide is a naturally occurring ceramide that lacks the 4–5 *trans* double bond in the sphingoid base backbone. Studies with the fluorescence analog of dihydroceramide, 6-[*N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]hexanoyl-dihydroceramide (C_6-NBD-DH-ceramide) led Kok et al. [87] to demonstrate that the incorporation of dihydroceramide into more complex sphingolipids occur prior to addition of the 4-*trans* double bond. This would minimize the accumulation of bioactive ceramide as an intermediate of the de novo biosynthetic pathway that could be fatal for cells [87]. More recently, the same group conclusively established the pathway of ceramide synthesis via desaturation of dihydroceramide in an in vitro system using rat liver microsomes [88].

Ceramides are responsible for many biological effects. They: (a) induce the differentiation of human leukemia promyelocytic HL-60 cells [63,89,90]; (b) inhibit DNA synthesis and cell proliferation in different cell types [1–3,21,22,59]; and (c) induce apoptosis [91]. Interestingly, cell-permeable ceramides have been demonstrated to act in distal axons, but not in cell bodies, as potent inhibitors of neurite growth [92]. In this respect, cell-permeable ceramides have been shown to decrease the concentration of tau proteins by stimulating the activity and levels of calpain I, a Ca^{2+}-activated protease, in PC12 cells. Tau proteins are localized predominantly in axons and they are involved in axonal growth and maintenance [93].

Regarding apoptosis, De Maria et al. reported recently that the apoptotic signals that follow ceramide accumulation after cross-linking of the apoptosis-inducing CD95 (also called Fas or APO-1) are mediated by ganglioside GD3. The accumulation of ganglioside GD3, in turn, depended on the activation of a family of cysteine proteases called caspases [94]. Ceramide-induced apoptosis may not be restricted to the ceramides that are derived from SM hydrolysis. The anticancer drug daunorubicin stimulates the elevation of ceramides and causes apoptosis in p388 and U937 cells [95]. This daunorubicin-stimulated elevation of ceramides did not result from the breakdown of SM but rather from de novo synthesis via the activation of ceramide synthase. This was concluded from studies with fumonisins, which are natural and specific inhibitors of ceramide synthase [96,97]. Fumonisin B_1 blocked sphinganine *N*-acyltransferase activity, and the daunorubicin-stimulated elevation of ceramides and apoptosis. These naturally occurring inhibitors of sphingolipid metabolism are proving to be powerful tools for studying the role of sphingolipids in cell regulation and disease [96,97]. In addition, palmitate and stearate have been shown to also induce apoptosis by stimulating the de novo synthesis of ceramides. The palmitate-mediated build-up of ceramide and the subsequent induction of apoptosis can be blocked in the presence of fumonisin B_1 or ISP1 (a pharmacological inhibitor that interferes with ceramide synthesis [98]. Also, the inhibition of carnitine palmitoyltransferase I by etomoxir, which led to a further increase in ceramide synthesis, was shown to enhance palmitate-induced cell death [98]. Other important inhibitors that can be used to manipulate the endogenous concentration of ceramides are D-threo-1-phenyl-decanoylamino-3-morpholino-1-propanol (PDMP), which inhibits further incorporation of ceramide into glycolipids, and D-erythro-2-(*N*-myristoylamino)-1-phenyl-1-propanol

(D-MAPP) which inhibits ceramide metabolism through ceramidase [99–102].

Kinetic studies have shown that ceramides can be generated within minutes or within hours, depending upon the extracellular stimuli [55]. In most cases, the magnitude of the response has been found to be moderate with increases in the range 20–50%. However, Smith and Merrill [103] have shown that larger increases in the production of ceramides and other sphingolipids occur after changing the medium to cells in culture. This observation is extremely important when determining the role of sphingolipid metabolites in cell regulation. Further studies from the same group showed that changing J774A.1 cells to new medium resulted in up to 10-fold increases in sphingoid bases. This caused modifications in protein kinase C activity and in the expression of JE and Mn^{2+}-dependent superoxide dismutase, two genes that are induced by TNF-α [104]. Regarding ceramide, the most dramatic change (about 15-fold) was observed after deprivation of serum in leukemia cells [55].

The changes in intracellular levels of ceramides are normally calculated from measurements of SMase activity, or by following their conversion to Cer 1-P by treatment with bacterial DAG kinase. However, in a recent report, Watts et al. [105] showed that DAG kinase increased the phosphorylation of substrates that comigrated with ceramide standards. This was caused by an enhancement of DAG kinase activity rather than increases in the levels of cellular ceramides as substrates per se. Therefore, the DAG kinase assay may not be an appropriate method for determinations of ceramide mass. Alternatively, ceramide mass can be accurately quantitated by using high-performance liquid chromatography [87,103] or by mass spectrometric techniques [105].

Another important enzyme that can control the intracellular concentration of ceramide is ceramidase (Fig. 1). In a recent report, Nikolova-Karakashian et al. [106] demonstrated a bimodal regulation of this enzyme activity by interleukin-1β. Low concentrations of interleukin-1β ($\sim$ 2.5 ng/ml) activated both SMase and ceramidase activities, which resulted in the generation of sphingosine. By contrast, higher concentrations ($\geq$ 5 ng/ml) of interleukin-1β only caused SMase activation, allowing ceramides to accumulate. Sphingosine and ceramide often induce opposing or different effects (see below) and therefore, control of the balance between the formation of these two interconvertible metabolites may be an important factor in controlling cellular functions.

3.3. Sphingosine and sphingosine 1-phosphate

Long-chain sphingoid bases became the focus of attention with the discovery that sphingosine inhibited protein kinase C [16]. Cells normally contain low levels of free long-chain bases (1–10 nmol/g of wet tissue, or 10–100 pmol/10^6 cells) and their levels have been found to change in response to various treatments ([104], and references therein). However, there has been no direct link between changes in endogenous sphingoid bases and inhibition of protein kinase C [104]. The greatest changes in free sphingosine have been seen after cells in culture are changed to new medium [103]. Further studies have revealed numerous targets for sphingosine, indicating that sphingosine itself or its derivatives may play alternative roles in cell signalling. Several protein kinase C isoforms are regulated by DAG, and it was shown subsequently that sphingosine can decrease the formation of DAG by inhibiting both the Mg^{2+}-dependent and independent phosphatidate phosphohydrolase activities [10,11,13,15]. In addition, sphingosine is able to decrease DAG concentrations by stimulating the activity of DAG-kinase [107,108] with the subsequent generation of PA. This increase in PA can be further enhanced by sphingosine, since it can rapidly activate phospholipase D in some cell types [109]. However, in NG 108-15 neural-derived cells [19] and in rat fibroblasts [22], activation of phospholipase D by sphingosine requires several hours to occur. These combined actions increase the accumulation of PA relative to DAG, which could also decrease protein kinase C activation.

Sphingoid bases are known to be potent inhibitors of cell growth [8]. However, it has been demonstrated that in Swiss 3T3 cells [110,111], mesanglial cells [112], CHO cells [113], and rat fibroblasts [21,22] sphingosine, or Sph 1-P stimulate cell proliferation. This effect was shown to be independent of protein kinase C [110] and stereospecific for the D-(+)*erythro*-sphingosine stereoisomer [114,115]. Sphingosine or Sph 1-P has also been shown to increase the intracellular concentration of Ca^{2+} [116,117], and

Sph 1-P is a potent activator of phospholipase D [20,118]. Some of the effects of sphingosine have been suggested to be caused by its conversion to Sph 1-P [20]. However, this may not always be the case since, for instance in rat fibroblasts, Sph 1-P stimulates phospholipase D activity rapidly and potently, whereas sphingosine is a slow and weak activator [22]. Also, sphingosine was more potent than Sph 1-P in activating DNA synthesis in rat fibroblasts [22]. In addition, treatment of fibroblasts with Fumonisin B_1 which causes the accumulation of sphingoid bases, stimulated DNA synthesis in the absence of sphingoid base-phosphate formation [119]. Therefore, it appears that sphingoid bases per se can stimulate DNA synthesis. Also, sphingosine-induced Ca^{2+} mobilization may be independent of conversion to Sph 1-P [116]. These findings suggest that sphingosine and Sph 1-P may act through different mechanisms to control cell activation.

It has been suggested that cAMP may play a role in the regulation of cell proliferation by various mitogens [52]. Inhibition of adenylate cyclase has been linked to the mitogenic effect of lysoPA in rat fibroblasts [52]. Sphingosine [22,110] and Sph 1-P [22,120] have also been shown to decrease cAMP concentrations at mitogenic concentrations. However, in Swiss 3T3 cells, cAMP is a positive effector of cell proliferation [8,121], and therefore, the precise role of cAMP in regulating cell growth remains controversial.

Sphingosine and Sph 1-P are generated intracellularly by the action of certain growth factors, such as platelet-derived growth factor, and serum [122–124]. Platelet-derived growth factor or serum, but not epidermal growth factor, stimulate sphingosine kinase activity and elevation of Sph 1-P in Swiss 3T3 cells [125]. This latter effect defines divergence in the signalling pathways of platelet-derived growth factor and epidermal growth factor receptors leading to MAP kinase activation [125]. Sph 1-P is stored in high concentrations in human platelets, and it is released into the blood stream upon activation by physiological stimuli [126]. It has been postulated that Sph 1-P may play a critical role in platelet aggregation and thrombosis [126]. The effects of Sph 1-P may involve a previous interaction with G_i/G_0-couple receptors in the plasma membrane [120], subsequent activation of the MAP kinase cascade [127], and activation of the transcription factor activator protein-1 (AP-1) in Swiss 3T3 fibroblasts [128]. The cellular levels of sphingosine and Sph 1-P are ultimately determined by their rate of formation from hydrolysis of ceramide and phosphorylation/dephosphorylation processes by specific kinases and phosphatases (Fig. 1).

3.4. Ceramide 1-phosphate

Another possible fate for ceramide is its conversion to Cer 1-P. Dressler and Kolesnick [129] identified a novel pathway in which ceramide originating from the action of neutral SM, but not glucosylceramidase, is converted to Cer 1-P by a Ca^{2+}-dependent kinase. Cer 1-P has been found in HL-60 cells [129] and brain tissue [130]. Shinghal et al. [130] identified a Cer 1-P phosphatase in rat brain, suggesting that Cer 1-P might regulate some aspects of synaptic vesicle functioning, and Boudker and Futerman [131] characterized a plasma membrane phosphatase that preferentially hydrolyzes Cer 1-P. Furthermore, Cer 1-P can be converted to ceramide by the action of a phosphatidate phosphohydrolase that is specifically located in the plasma membrane of cells [132]. Interestingly, the majority of Cer 1-P phosphatase activity in synaptosomal fractions also partitions with plasma membranes [130]. This activity has characteristics similar to phosphatidate phosphohydrolase and therefore, it is possible that dephosphorylation of Cer 1-P in brain tissue is mediated by the same enzyme that acts on PA. These observations indicate that Cer 1-P can be turned over, and that it may play an important role in cell activation. However, little is known about its physiological effects. The pathological and toxic effects of Cer 1-P have been recognized since a SMase D was identified as the active principle of the venom of the brown recluse spider *Loxosceles reclusa*. SMase D can also be produced by some bacteria including *Corynebacterium pseudotuberculosis* and *Vibrio damsela* [133]. However, SMase D can also hydrolyze lysoPC to LPA and choline [133]; therefore, Cer 1-P may not be the only active component of these venoms. Although Cer 1-P can be produced directly from SM by the toxins of these organisms, there is as yet no evidence for this action occurring as a result of a mammalian enzyme (Fig. 1). It was recently reported that syn-

thetic short-chain Cer 1-P might have applications as novel pharmacological agents for altering cell signalling processes, particularly those leading to cell proliferation and apoptosis [23]. In the latter study, short-chain Cer 1-Ps were presented in a sonicated form in water to subconfluent cultures of rat fibroblasts, and found to stimulate the synthesis of DNA. However, natural (long-chain) Cer 1-P was ineffective under the same conditions; therefore the effects of short-chain Cer 1-Ps were regarded as pharmacological in nature. More recently [9], it has been shown that natural Cer 1-P can be dispersed into aqueous solutions when dissolved in an appropriate mixture of methanol/dodecane (49:1, v/v). This solvent mixture facilitates the interaction of this phospholipid with cells in culture. Under those conditions, natural Cer 1-P caused a potent stimulation of DNA synthesis that was accompanied by an increase in the levels of proliferating-cell nuclear antigen. Interestingly, Berger et al. have shown recently [134] that long-chain Cer 1-P (*N*-palmitoyl-sphingosine 1-phosphate), which was complexed to bovine serum albumin, stimulated the incorporation of thymidine into DNA by about 7.9 fold in Swiss 3T3 fibroblasts. In contrast, *N*-palmitoyl-dihydrosphingosine 1-phosphate, which lacks the 4–5 *trans* double bond but retains the stereochemical configuration of Cer 1-P had very little effect on stimulating the synthesis of DNA. These results demonstrate the specificity of Cer 1-P to stimulate the synthesis of DNA [134]. Also, preliminary observations indicate that Cer 1-P can partially reverse the morphological changes that are induced in rat fibroblasts after serum deprivation, and that Cer 1-P can decrease the detachment of serum-starved cells from culture dishes (A. Gómez-Muñoz and U.P. Steinbrecher, unpublished work). A possible involvement of Cer 1-P in blocking or retarding the apoptotic changes induced by serum deprivation of cells in culture still remains to be investigated.

4. Interaction between ceramide and other glycerolipid or sphingolipid metabolites in signal transduction

There are several sites at which the signalling pathways of glycerolipids and sphingolipids can interact with each other to control signal transduction (Fig. 2). As mentioned before, sphingosine can inhibit phosphatidate phosphohydrolase activities causing PA to accumulate, therefore decreasing the production of DAG. This would potentiate the inhibiting effect of sphingosine on protein kinase C, which is the intracellular target of DAG. Sphingosine can also favour an increased PA:DAG ratio in some cell types by stimulating DAG kinase [107,108] and phospholipase D activities [19,22,109]. Also, sphingosine and Sph 1-P stimulate the DNA binding activity of AP-1 [120,128], and this may be a convergence point where sphingolipid signals are integrated with the intracellular second messengers derived from glycerolipids [8].

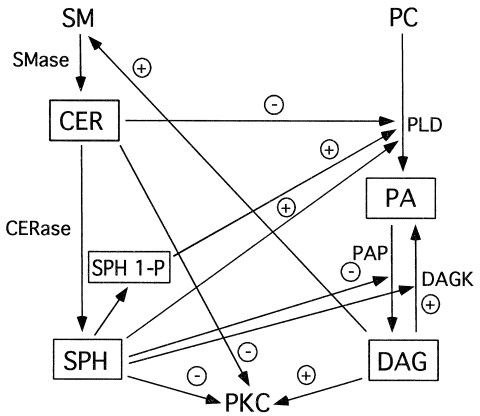

Fig. 2. Interaction between glycerolipid and sphingolipid signalling pathways. Signal transducing metabolites are shown in boxes. The ⊖ or ⊕ symbols indicate inhibitory or stimulatory actions by the different signalling molecules. Abbreviations are as follows: CER, ceramide; DAG, diacylglycerol; DAGK, diacylglycerol kinase; PA, phosphatidate; PAP, phosphatidate phosphohydrolase; PC, phosphatidylcholine; PKC, protein kinase C; PLD, phospholipase D; SM, sphingomyelin; SPH, sphingosine; SPH-1-P, sphingosine 1-phosphate. PA can be generated by the action of PLD on phospholipids, such as PC, or by the action of DAGK which phosphorylates DAG. Conversely, PA can be acted upon by PAP with the subsequent generation of DAG which in turn, activates PKC. DAG can also stimulate SMase activity to generate CER from SM. CER can give rise to SPH by the action of CERase activity. SPH can then be phosphorylated to SPH 1-P by SPH kinase activity. Both SPH and SPH 1-P can stimulate PLD activity, whereas CER is a potent inhibitor of PLD activation and can also inhibit some protein kinase C isoforms. SPH inhibits PAP and PKC, and stimulates DAGK. These actions by SPH lead to increased concentrations of PA.

The apoptotic and anti-apoptotic properties of ceramide and DAG respectively, are well documented. Ceramide elevation induces cell death in many cell types, and this action can be overcome by the presence of DAG [7]. It is likely that DAG abrogates the ceramide-initiated signals leading to apoptosis while allowing signals for other events to proceed. In this regard, when both DAG and ceramide are combined, cell proliferation prevails over cell death [7]. Interestingly, DAG generated via a PC-specific phospholipase C has been shown to stimulate acidic SMase activity with the subsequent generation of intracellular ceramides and activation of the transcription factor NF-kB [70]. However, there is evidence that dissociates NF-kB activity from an increase in cell ceramide concentrations [135]. Also, kinetics studies have demonstrated that the activation of NF-kB by TNF-α or interleukin-1 occurs prior to the accumulation of ceramides caused by these agents [55]. The association between acidic SMase stimulation and NF-kB activation has also been questioned by Zumbansen and Stoffel [136] who have demonstrated that TNF-α can activate NF-kB in acid SMase deficient mouse fibroblasts.

4.1. Ceramides inhibit the agonist-stimulation of phospholipase D

Control of phospholipase D activity may be critical for the regulation of cell activation. The stimulation of phospholipase D activity by serum, thrombin, lysoPA, PA, Sph 1-P, or phorbol esters is inhibited by the cell-permeable C_2- and C_6-ceramides (*N*-acetyl- and *N*-hexanoyl-sphingosine, respectively) [21–23]. Similarly, ceramide-induced inhibition of phospholipase D can be seen in permeabilized fibroblasts that were treated with GTPγS [21]. Ceramides have also been shown to inhibit the activation of phospholipase D in other cell systems. For instance, C_2-ceramide and sphingoid bases inhibited diradylglycerol formation by the phospholipase D pathway in neutrophils [137] and C_6-ceramide, inhibited the accumulation of DAG in senescent cells [138]. The generation of DAG in response to mitogens occurs predominantly in response to phospholipase D catalyzed hydrolysis of membrane PC [138]. Senescent cells lack phospholipase D activity, which could be due to the high concentration of ceramides that are found in 'old' cells compared with 'young' cells [139,140].

Another important observation is that cell-permeable ceramides are able to stimulate the generation of intracellular long-chain ceramides [22], and this could potentiate the action of the exogenous short-chain analogues. The generation of intracellular ceramides might be, at least, part of the mechanism whereby synthetic short-chain ceramides exert their effects [22]. Some of the actions of ceramides on cell signalling are caused by changes in the serine/threonine phosphorylation state of target proteins caused by activation of kinase activity [5,67], or by increasing phosphoprotein phosphatases [141,142]. The latter effect can be blocked by okadaic acid, but the addition of this compound to the fibroblasts did not reverse the ceramide-induced inhibition of phospholipase D [22]. Okadaic acid at 0.5 and 1.0 μM, in fact, decreased the activation of phospholipase D by Sph 1-P. In cell-free systems, phospholipase D activity is dependent on the presence of both membrane and cytosolic components. These latter consist of small molecular weight G-proteins of the Ras super-family, such as ADP-ribosylation factor (ARF) and Rho [143–146]. ARF was first identified as a cofactor necessary for the ADP-ribosylation of the α subunit of heterotrimeric G-proteins (i.e., G_s), by cholera toxin [147] and is implicated in vesicular transport in the Golgi [148] and in endocytosis. Rho proteins regulate the assembly of focal adhesion complexes and actin stress fibers in fibroblasts. They inhibit phorbol ester-induced and integrin-dependent aggregation in lymphocytes and play a critical role in coupling of G-protein-linked chemoattractant receptors to integrin-mediated adhesion in leukocytes (reviewed by Gomez-Muñoz et al. [15]). Although Rho can activate phospholipase D in cell-free systems, its physiological involvement has been questioned and the major stimulation by G-proteins was concluded to be mediated by ARF [149]. The mechanism whereby ceramides inhibit phospholipase D activation has been examined in a reconstituted assay system. The addition of cell-permeable ceramides (C_2- or C_8-ceramide) or natural long-chain ceramides to the lipid substrates used in that assay resulted in a decrease in phospholipase D activity [150]. By contrast, dihydro-C_2-ceramide had no significant effect on phospholipase D activation. These results demonstrate the

specificity of the ceramide action and indicated that the inhibition was not caused by a nonspecific action of the lipids on phospholipase D. In the same report, the authors demonstrated that the activation of phospholipase D that was stimulated by recombinant ARF and Rho was also decreased by C_2-ceramide, and that cell-permeable ceramides displaced both ARF and Rho from the membrane fraction into the cytosol. These effects were not dependent on the stimulation of kinases or phosphatases by ceramides, since the effect was not modified significantly by the additions of either apyrase to destroy any endogenous ATP, or by okadaic acid to inhibit phosphoprotein phosphatases [151]. In previous work, the inhibition of phospholipase D has been ascribed to the effects of ceramides in inhibiting the translocation of protein kinase C-α to the membrane fraction [152], since this protein can activate phospholipase D by ATP-dependent and independent mechanisms [143,147,151]. An inhibitory effect of C_2-ceramide on phospholipase D activation was also observed in rat basophilic leukemia (RBL-2H3) cells [153]. In the same work, the authors demonstrated that the translocation of protein kinase C-α, β_1, β_2, but not δ and ε isozymes, from cytosol to membrane fraction was specifically prevented during treatment with C_2-ceramide. In other work, the inhibition of phorbol ester-induced activation of phospholipase D by ceramide was also observed, but this effect was not accompanied by a decrease in protein kinase C in the membrane fraction [154]. It can, therefore, be concluded that the inhibition of phospholipase D is a target for the action of ceramides. There may be a variety of mechanisms that mediate this action via the regulation of low molecular weight G-proteins and protein kinase C. Furthermore, the effect of ceramides on the association of G-proteins with membranes could have important implications for the regulation of cell activation, cell motility and vesicle trafficking. As mentioned before, ARF is involved in vesicle movement and Rho is implicated in the organization of the cytoskeleton. Therefore, the interaction of ceramides with small molecular weight G-proteins could contribute to the inhibitory effect of ceramides on vesicle transport [155], and on the modulation of assembly of focal adhesion complexes and actin stress fibers in some cell types.

Regarding cell proliferation, it should be stressed that although PA is mitogenic, the activation of phospholipase D need not be related directly to cell growth [15,21,23]. For example, Cer 1-P stimulates DNA synthesis in the absence of any activation of phospholipase D [9,23], and endothelin, which is more efficient than lysophosphatidate in generating phosphatidate from phosphatidylcholine via phospholipase D, is a poor mitogen [156]. It is therefore unlikely that the stimulation of the phospholipase D pathway alone may provide a complete mitogenic signal, and that other events such as the stimulation of tyrosine kinases or a decrease in cAMP concentrations might be necessary for cell proliferation to occur.

4.2. Selective modulation of protein kinase C isoforms by ceramides

Protein kinase C consists of a family of structurally related serine/threonine kinases that are implicated in diverse cellular functions. Some protein kinase C isoforms (α, β_1, β_2 and γ) are dependent upon Ca^{2+} for activity, whereas the δ, ε, σ and μ isoforms are Ca^{2+}-independent. A common feature, however, that is shared by all of these isoforms, is that they require DAG to be activated. By contrast, the atypical protein kinase C isoforms, ζ and λ are independent of Ca^{2+} and they are not activated by DAG [7]. Among all of these protein kinase C isoforms, only the α, δ and ε isoforms have been shown to be regulated by ceramides, and they have been linked to the inhibition of phospholipase D by ceramides [152]. More recently, cell-permeable ceramides have been shown to induce the translocation of protein kinase C-δ and ε from the membranes to the cytosol. Interestingly, exogenous SMase, TNF-α or anti-Fas antibody, all of which can induce apoptosis, similarly induced cytosolic translocation of PKC-δ and ε [157]. These observations suggest that cytosolic translocation of PKC-δ and ε plays an important role in ceramide-mediated apoptosis.

4.3. Inhibition of the mitogenic effects of phosphatidate, lysophosphatidate, sphingosine 1-phosphate and ceramide 1-phosphate by ceramides

As discussed before, ceramides are potent inhibitors of cell division and can induce programmed

cell death. By contrast PA, lysoPA, Sph 1-P and Cer 1-P stimulate cell proliferation. It was, therefore, investigated whether ceramides would inhibit the effects of the mitogenic lipids. In these experiments C_{2-} and C_6-ceramides were used to mimic the effects of the natural long-chain ceramides. The cell-permeable ceramides inhibited the stimulation of DNA synthesis that was induced by PA, lysoPA [21], Sph 1-P [22], C_2- or C_8-Cer 1-P [23], and natural (long-chain) Cer 1-P [9]. More recently, natural ceramides have also been shown to inhibit the natural Cer 1-P-stimulated synthesis of DNA [9]. These effects of ceramides were also accompanied by a decrease in cell division [23]. The effects of the ceramides on the stimulation of DNA synthesis by the bioactive lipid phosphates were relatively specific since they were unable to block the induction of DNA synthesis by insulin, epidermal growth factor or platelet-derived growth factor [22]. Interestingly, relatively high concentrations of insulin potentiate the mitogenic effect of Sph 1-P and protect the stimulation of DNA synthesis by Sph 1-P against the inhibition by C_2-ceramide [22]. This observation indicates that insulin may be an important factor in regulating ceramide action. However, the concentrations of insulin used in these experiments were relatively high, and it is not clear at present if this effect of insulin has physiological relevance.

Since ceramides can be rapidly metabolized in cells, it was important to define whether the effects observed were due to ceramide itself or to some potential by-product of ceramide metabolism, especially sphingosine. Therefore, the effect of sphingosine on PA- or lysoPA-induced DNA synthesis was evaluated. Rather than blocking the stimulation of DNA synthesis, sphingosine increased the incorporation of thymidine into DNA further in the presence of PA or lysoPA [21]. Although these effects were not strictly additive, these results indicate that sphingosine stimulated DNA synthesis by a mechanism that is distinct from that used by PA and lysoPA. This conclusion is supported by the observation that the increased DNA synthesis that is induced by sphingosine is only inhibited by about 16% by cell-permeable ceramides, whereas that stimulated by PA, lysoPA, Cer 1-P and Sph 1-P is inhibited almost completely [9,21–23]. It can then be concluded that the inhibition of DNA synthesis by ceramide does not depend upon its metabolism. One mechanism whereby ceramides block the mitogenic effect of the bioactive lipid phosphates in rat fibroblasts might be by inhibiting the decrease of cAMP accumulation. However, the cell-permeable ceramides were unable to reverse the effects of PA, lysoPA, sphingosine and Sph 1-P in lowering cAMP levels in the presence or absence of forskolin [21,22]. This implies that the mechanism by which ceramides inhibit the mitogenic effect of PA, lysoPA, or Sph 1-P is independent of interaction with adenylate cyclase. It is also significant that Cer 1-P did not change the activity of adenylate cyclase in the fibroblasts after 1 min to 2 h of incubation [9,23].

LysoPA and PA also increased the activity of MAP kinase up to 5- to 6-fold in rat fibroblasts [23]. Therefore, the interaction of C_2-ceramide on this activation was examined to see whether this would explain the inhibition of DNA synthesis. There was no significant interaction between lysoPA and C_2-ceramide in modifying the stimulation of MAP kinase activity; therefore, the inhibition of lysoPA-induced DNA synthesis by ceramide could not be explained by changes in MAP kinase activity. In contrast to PA, lysoPA and Sph 1-P, there was no change in MAP kinase activity after 1 min to 2 h after treatment with Cer 1-P [9,23]. These results demonstrate that exogenous PA and Cer 1-P, which are structurally related, exhibit different effects on signal transduction via cAMP and MAP kinase despite their similar effects on DNA synthesis and cell division [15].

4.4. Ceramides stimulate the metabolism of phosphatidate, lysophosphatidate, sphingosine 1-phosphate and ceramide 1-phosphate

Ceramides can also control cell signalling by modifying the metabolism of PA, lysoPA, Sph 1-P and Cer 1-P. This could contribute to the antagonism of ceramides towards the mitogenic effects of the bioactive lipid phosphates. Pretreatment of rat fibroblasts with cell-permeable ceramides stimulated the degradation of exogenous PA and lysoPA and decreased the interaction of these lipids with the cells [21]. Therefore, the inhibition of the interaction of the exogenous phospholipids with cell membranes by ceramides and the enhanced degradation of PA and lysoPA could be important steps in decreasing the

mitogenic signal of these phospholipids. These changes were related to an increased activity of the plasma membrane phosphatidate phosphohydrolase [21]. However, the mechanism for this latter effect of ceramide on the phosphohydrolase is unknown. Subsequent work showed that ceramides also stimulate the dephosphorylation of Sph 1-P and Cer 1-P when these agonists are added externally to cells [22,23]. Thus, the action of ceramides in these latter cases is to promote the degradation of these two mitogenic lipid phosphates and to increase their conversion to ceramide. This action decreases the relative concentrations of the mitogenic lipids relative to ceramides. Gómez-Muñoz et al. [22] provided preliminary evidence that the phosphohydrolase that degrades Sph 1-P is identical to phosphatidate phosphohydrolase. This prediction was confirmed in subsequent work with the Mg^{2+}-independent- and *N*-ethylmaleimide-insensitive phosphatidate phosphohydrolase that was purified from rat liver plasma membranes [132].

4.5. Potential role of phosphatidate phosphohydrolase activity in signal transduction

An important enzyme that can regulate the levels of PA and DAG in cells is phosphatidate phosphohydrolase (Figs. 1 and 3). This enzyme exists in two distinct forms. Phosphatidate phosphohydrolase-1 is a Mg^{2+}-dependent activity that is found in the cytosol and has the ability to translocate to the endoplasmic reticulum or mitochondria (reviewed by Martin et al. [158]). Translocation of this phosphohydrolase isoform occurs as a result of an accumulation of fatty acids, acyl CoA-esters or PA in the cell. This enzyme

Fig. 3. Phosphatidate phosphohydrolase can degrade phosphatidate, lysophosphatidate, ceramide 1-phosphate and sphingosine 1-phosphate. Abbreviations are as follows: CER, ceramide; CER 1-P, ceramide 1-phosphate; DAG, diacylglycerol; LPA, lysophosphatidate; MAG, monoacylglycerol; PA, phosphatidate; PAP, phosphatidate phosphohydrolase; SPH, sphingosine; SPH-1-P, sphingosine 1-phosphate.

is thought to play an essential role in glycerolipid synthesis since it degrades PA to DAG which is the precursor for triacylglycerol, PC and phosphatidylethanolamine. Phosphatidate phosphohydrolase-1 could potentially translocate to the inner surface of the plasma membrane and degrade the PA generated by phospholipase D activation. This would implicate Phosphatidate phosphohydrolase-1 in signal transduction processes. However, evidence for a role of this enzyme in cell signalling has not been provided yet. Phosphatidate phosphohydrolase-2 is an integral plasma membrane protein which does not require Mg^{2+} for activity [11]. This enzyme is thought to be involved in signal transduction. Phosphatidate phosphohydrolase-2 has been purified from porcine thymus membranes [159], and from rat liver plasma membranes [160,161]. The porcine thymus phosphatidate phosphohydrolase-2 was first reported to be an 83-kDa protein [159]. A similar enzyme activity of the same molecular weight was also obtained from rat liver by a similar purification protocol [162]. However, re-evaluation of the enzyme purification procedure in porcine thymus membranes revealed a minor 35-kDa protein that was further identified as the phosphatidate phosphohydrolase isoform bound to plasma membranes. The phosphohydrolase activity purified from rat liver plasma membranes was identified as a 31-kDa protein on SDS gels [132]. More recently, Kanoh's group succeeded in cloning the cDNA of the 35-kDa phosphatidate phosphohydrolase-2 and subsequent determination of its primary structure. The protein contains several putative membrane-spanning domains and two *N*-glycosylation sites [163].

The degradation of PA by phosphatidate phosphohydrolase-2 generates DAG which is also a second messenger. However, as mentioned before, phospholipase D-derived DAG does not activate protein kinase C [28]; therefore, the main function of phosphatidate phosphohydrolase-2 in signal transduction may be terminating signalling by PA. Interestingly, lysoPA can also be degraded by phosphatidate phosphohydrolase activity to produce monoacylglycerol, which lacks biological activity when added to cells. Phosphatidate phosphohydrolase also degrades Cer 1-P and Sph 1-P (Fig. 3) [22,132]. Therefore, this action of the Mg^{2+}-independent phosphatidate phosphohydrolase may terminate the signals from PA,

lysoPA, Cer 1-P and Sph 1-P, but will also generate DAG, ceramide and sphingosine, which are also bioactive. It can then be concluded that phosphatidate phosphohydrolase could play an important role in cell signalling by modifying the balance of the bioactive lipids that are implicated in cell activation and in controlling cell growth. Furthermore, the mutual competition between all of these lipids could be an additional site of interaction between the glycerolipid and sphingolipid signalling pathways (Fig. 2).

Interestingly, both Mg^{2+}-dependent and Mg^{2+}-independent phosphatidate phosphohydrolase activities are decreased in *ras*-transformed fibroblasts compared to non transformed cells. There is also an increased production of PA relative to DAG following activation of phospholipase D in the transformed cells [164]. The latter also have an increased DAG kinase activity, which can further enhance the formation of PA [165]. This high levels of PA may be important in producing the low cAMP concentrations that are observed in the transformed fibroblasts and which may facilitate the activation of Raf by Ras [166,167]. Such effects might be part of the mechanism for enabling the transformed cells to continue to divide, whereas the non-transformed fibroblast attain growth arrest when they reach confluence. Elevation of intracellular cAMP concentration results in decreased levels of PA and DAG, enhancement of ceramide production, and inhibition of cell division [165]. This latter observation is particularly important since ceramides can block the stimulation of DNA synthesis by PA and lysoPA specifically, in rat fibroblasts [21].

5. Concluding remarks

PC and SM are phospholipid precursors of important bioactive lipids. The interaction between the sphingolipid and glycerolipid metabolites that can be derived from the agonist-stimulated breakdown of PC and SM may be relevant for controlling signal transduction. In particular, the control of ceramide levels seems to be critical for regulating the production of PA, DAG, and possibly lysoPA through the phospholipase D pathway. Ceramides block agonist-stimulated phospholipase D activation whereas sphingosine, an immediate metabolite of ceramide, stimulates phospholipase D activity. This latter effect of sphingosine may result in the accumulation of high levels of PA relative to DAG, since sphingosine is a potent inhibitor of phosphatidate phosphohydrolase and can also stimulate DAG kinase. Although activation of phospholipase D may not be essential for mitogenesis, PA, DAG and lysoPA are positive signals for cell growth. The sphingolipid metabolites, sphingosine, Sph 1-P and Cer 1-P are also mitogenic. However, ceramides are potent inhibitors of cell proliferation. In particular, ceramides block the mitogenic effects of PA, lysoPA, Sph 1-P and Cer 1-P. Part of this negative regulation of cell growth by ceramides may involve increased degradation of the bioactive lipids, possibly by enhancing the activity of a common phosphohydrolase. These results suggest that the overall signal that a cell receives depends upon the balance in the production of bioactive lipids. Activation of the SMase pathway to form ceramides may yield an apoptotic signal. However, conversion of ceramide to sphingosine, Sph 1-P or Cer 1-P would stimulate cell division. Therefore, the enzymes that are involved in the metabolism of ceramides are relevant for controlling the overall signal that will finally be transmitted. The existence of different SMases, which results in the generation of ceramides in different cell compartments, adds difficulty to understand the role of ceramides as intracellular second messengers. Ceramides may also interact with tyrosine kinase cascades by modulating the levels of phosphorylation of target proteins that are involved in the epidermal growth factor and insulin signalling cascades.

It can be concluded that a better understanding of the interactions between the different metabolites that can be generated from the phospholipase D and SMase pathways will be essential for elucidation of the mechanisms that regulate cell functions, in particular cell proliferation and death.

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