Minireview

Sphingosine-1-phosphate: signaling inside and out

Sarah Spiegel[a,*], Sheldon Milstien[b]

[a] *Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, 353 Basic Science Building, 3900 Reservoir Road NW, Washington, DC 20007, USA*

[b] *Laboratory of Cellular and Molecular Regulation, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892, USA*

Received 5 May 2000

Edited by Gunnar von Heijne

Abstract Ample evidence indicates that sphingosine-1-phosphate (SPP) can serve as an intracellular second messenger regulating calcium mobilization, and cell growth and survival. Moreover, the dynamic balance between levels of the sphingolipid metabolites, ceramide and SPP, and consequent regulation of opposing signaling pathways, is an important factor that determines whether a cell survives or dies. SPP has recently also been shown to be the ligand for the EDG-1 family of G-protein-coupled receptors, which now includes EDG-1, -3, -5, -6 and -8. SPP is thus a lipid mediator that has novel dual actions signaling inside and outside of the cell.

Key words: Sphingosine-1-phosphate; EDG-1; Sphingosine kinase; Cell growth; Apoptosis

1. Introduction

Sphingosine-1-phosphate (SPP) is a bioactive sphingolipid metabolite whose importance in cell growth regulation was discovered in our lab in 1991 [1]. Over the ensuing decade, we have been constantly intrigued by the wide spectrum of biological processes, including calcium mobilization, cell growth, differentiation, survival, motility and cytoskeleton organization, that were found to be regulated by SPP [2]. Part of this puzzle now seems to be resolved as this sphingolipid metabolite is emerging as a member of a novel class of lipid mediators that can function as second messengers and as ligands of cell surface receptors. This review is thus focused on the dual actions of SPP.

2. Intracellular actions

Some confusion in the literature has arisen concerning the importance of signaling by sphingolipid metabolites because the net observed effect can be different dependent upon the type of cell, the nature of the stimulus being examined, and most importantly, the intracellular balance between sphingolipids that may mediate opposing pathways. Ceramide (*N*-acyl-sphingosine) is produced by stimulation of sphingomyelinases or of de novo biosynthesis and plays a prominent role in stress responses and programmed cell death, known as apoptosis [3]. In contrast, we have implicated SPP, a metabolite of ceramide, as a second messenger in cellular proliferation [4] and survival [5] and showed that SPP protects cells from ceramide-mediated apoptosis [5]. Sphingosine kinase, the enzyme that forms SPP from sphingosine, is activated by many stimuli as well as growth and survival factors, including platelet-derived growth factor and serum, nerve growth factor (NGF), muscarinic acetylcholine agonists, TNF-α, and cross-linking of the immunoglobulin receptors FcεR1 and FcγR1 (reviewed in [2]). While the intracellular targets of SPP have not yet been unequivocally identified, intracellular SPP has been shown to mobilize calcium from internal sources independently of inositol trisphosphate, as well as to affect many signaling pathways leading to proliferation, such as activation of ERK and inhibition of SAPK/JNK [5,6], and suppression of apoptosis [5–9]. As further evidence for the importance of intracellularly generated SPP, inhibitors of sphingosine kinase not only block formation of SPP in vivo, they selectively inhibit calcium mobilization and cellular proliferation and survival induced by various stimuli (reviewed in [2]). Thus, we have suggested that the dynamic balance between levels of ceramide and sphingosine, which mediate cell growth arrest, and SPP, which promotes proliferation and survival, and their regulation of opposing signaling cascades may be important in determining cell fate. For example, stress stimuli increase ceramide and sphingosine levels leading to apoptosis of T lymphocytes, whereas survival factors stimulate sphingosine kinase, leading to increased SPP levels, which suppress apoptosis [5]. In agreement, destruction of oocytes as a result of chemotherapy-induced ceramide elevation can be prevented by alteration of the apoptosis-associated sphingolipid-mediated signaling pathways [7]. Moreover, this ceramide/SPP rheostat is an evolutionarily conserved stress regulatory mechanism influencing growth and survival of yeast.

The sphingosine kinase/SPP signaling pathway is also critically involved in mediating TNF-α-induced endothelial cell activation [10]. Furthermore, the ability of high density lipoproteins (HDL) to inhibit cytokine-induced adhesion molecule expression correlates with its ability to reset the sphingolipid rheostat [10], which has important implications for the protective effects of HDL in the development of atherosclerosis and associated coronary heart disease. Recently, the decisive balance of sphingosine and SPP has also been shown to determine the allergic responsiveness of mast cells [11], where a high intracellular concentration of sphingosine acts to inhibit IgE plus antigen-mediated leukotriene synthesis and cytokine production by preventing activation of the mitogen-activated protein kinase ERK pathway and AP-1-mediated transcrip-

*Corresponding author. Fax: (1)-202-687 0260.
E-mail: spiegel@bc.georgetown.edu

0014-5793/00/$20.00
PII: S0014-5793(00)01670-7

tion [11]. In contrast, high intracellular levels of SPP, which is also secreted by allergically stimulated mast cells, activate ERK, leading to hexosaminidase and leukotriene release, and in combination with a calcium ionophore stimulate cytokine production [11]. Treatment of mast cells with SPP counteracted the inhibitory effects of sphingosine and induced AP-1, a response reminiscent of its effect on Swiss 3T3 fibroblasts [12]. Thus, it is likely that activation of sphingosine kinase is a critical event in the signaling cascades initiated at FcεRI.

In sum, these studies provide new insights into the biological functions of intracellularly generated SPP and emphasize the importance of sphingosine kinase, the enzyme that regulates its formation. Recently, we purified rat kidney sphingosine kinase to apparent homogeneity [13] and on the basis of peptide sequences derived from the purified enzyme, subsequently cloned and characterized the first mammalian sphingosine kinase (SPHK1) [14]. SPHK1 is predominantly a cytosolic enzyme, and a hydropathy plot of its predicted amino acid sequence did not reveal any signal peptide or hydrophobic transmembrane sequences. However, SPHK1 does contain several consensus binding sites for both calcium and calmodulin, although it is not yet clear whether they have a physiological regulatory role. Recently, two genes, *LCB4* and *LCB5*, were shown to encode sphingosine kinases in the yeast *Saccharomyces cerevisiae* [15]. Moreover, by database searches, we have identified homologs of SPHK1 in a number of other species, including worms, plants and mammals, demonstrating that the enzyme is encoded by a member of a highly conserved gene family. Comparison of the predicted amino acid sequences revealed five highly conserved domains, which might constitute critical portions of catalytic or substrate binding sites. Although these domains appear to be unique, several motifs in the C1 and C3 domains have some sequence homology with diacylglycerol kinase ζ [14], another lipid kinase that after phosphorylation translocates to and regulates the amount of diacylglycerol in the nucleus [16]. If SPHK1 behaves in a similar manner, then it might also function in the nucleus. Interestingly, transfection of HEK293 human embryonic kidney cells or NIH 3T3 mouse fibroblasts with SPHK1 cDNA resulted in a marked increase in sphingosine kinase activity and cellular SPP with a concomitant decrease of sphingosine and, to a lesser extent, of ceramide, although in no case was there detectable secretion of SPP into the medium [14,17]. Overexpression of sphingosine kinase also induced cell proliferation, by promoting the G_1 to S phase transition of the cell cycle, as well as inhibiting the apoptotic response to serum deprivation or ceramide [17]. Furthermore, we have recently succeeded in cloning a novel lipid phosphohydrolase that specifically degrades SPP and induces cell death [18]. Enforced expression of this SPP phosphatase in NIH 3T3 fibroblasts not only decreased SPP and enhanced ceramide levels, it also markedly diminished survival and induced the characteristic traits of apoptosis [18]. Collectively, our results suggest that sphingosine kinase and SPP phosphohydrolase may regulate the dynamic balance between sphingolipid metabolite levels in mammalian cells and consequently influence cell fate.

3. Extracellular actions: SPP is a ligand for the EDG-1 family of G-protein-coupled receptors (GPCRs)

Interest in SPP has accelerated recently with our discovery that it is a ligand of the G-protein-coupled cell surface receptor EDG-1 [6,19]. This rapidly led to the identification of several other related receptors, named EDG-3, -5, -6 and -8, demonstrating that EDG-1 belongs to a family of GPCRs that bind SPP with high affinity and specificity (reviewed in [20]). We also found that sphinganine-1-phosphate, which is structurally similar to SPP and only lacks the *trans* double bond at the 4 position, binds to EDG-3, EDG-5 [21] and EDG-6 [22]. In contrast, lysophosphatidic acid (LPA) and sphingosylphosphorylcholine, two other serum borne lysophospholipids, do not bind to this subfamily of receptors and it is well established that LPA binds to another family of EDG receptors, which includes EDG-2, -4 and -7 (reviewed in [23]). The EDG-1 family of receptors are differentially expressed, mainly in the cardiovascular and nervous systems, and are coupled to a variety of G-proteins and thus can regulate diverse signal transduction pathways culminating in pleiotropic responses depending on the cell type and relative expression of EDG receptors (reviewed in [20]).

Although the biological functions of the EDG-1 family of GPCRs are not completely understood, our recent studies have implicated EDG-1 in the regulation of cell migration [24]. Cell migration is crucial for embryonic development, the inflammatory immune response, wound healing, and tumor formation and metastasis. We found that binding of SPP to cells overexpressing the serpentine receptor EDG-1 or to endothelial cells which constitutively express EDG-1 activates a pertussis toxin-sensitive G_i protein crucial for chemotaxis. In agreement, it has been demonstrated that activation of $G\alpha_i$-coupled receptors and the subsequent release of $G_{\beta\gamma}$ dimers is required to initiate signal transduction leading to directed cell migration [25]. EDG-6 is expressed mainly in lymphocytes which are normally exposed to high concentrations of SPP in serum. Because the receptors for known leukocyte chemoattractants, including the chemokines, are GPCRs, it is tempting to speculate that EDG-6 may also function as a lymphocyte chemokine receptor.

Regulation of cell motility by SPP/EDG-1 signaling may have important implications for the function of SPP in angiogenesis. Endothelial cells play a critical role in angiogenesis, i.e. new vessel formation from pre-existing ones, or neovascularization [26]. They migrate, proliferate and assemble into tubes with tight cell–cell junctions to form the vessel. Mural support cells (pericytes and smooth muscle cells (SMC)) are then required to seal and provide modulatory functions for the vessel. Recently, we have demonstrated that SPP has appropriate properties to be considered as a bona fide angiogenic factor. For example, it stimulates chemokinetic and chemotactic motility of vascular endothelial cells and other cell types including SMC, and stimulates angiogenesis in vitro, acting similarly to the known angiogenic factor basic fibroblast growth factor (bFGF) [24]. Because bFGF and SPP have an additive effect on formation of capillary-like tubes by endothelial cells invading collagen gels, SPP may be a specific type of angiogenic factor. Interestingly, SPP was even more potent than the known angiogenic factors, bFGF and vascular endothelial growth factor (VEGF) [27]. To determine whether SPP regulates angiogenesis in vivo, two groups have recently utilized the Matrigel implant model in athymic mice [28,29]. SPP dramatically enhanced bFGF-induced angiogenesis; vascular density and the appearance of mature vascular structures were greatly increased [29]. In agreement with our in vitro studies

[24], SPP also potentiated VEGF-induced in vivo angiogenesis [29]. Collectively, these studies demonstrate that SPP may play an important role in platelet-induced angiogenesis and define SPP as a novel regulator of this process. It is possible that SPP also plays a role in normal blood vessel formation and in injury, when local production of SPP could be increased by release from activated platelets, and extravasation of intravascular fluid could also present SPP into tissues at concentrations sufficient to promote angiogenesis and wound healing. Elucidation of the molecular mechanisms by which SPP regulates angiogenesis might provide clues for development of a new class of therapeutic agents to either promote or block mature neovessel formation through effects on SPP/EDG-1 signaling.

Less is known of the functions of the other members of the EDG-1 family. EDG-5 is expressed in the cardiovascular system [30] and in the brain during embryogenesis, where its expression is temporally regulated such that high levels of expression are found in neuronal cell bodies during early stages of differentiation and in axons during their outgrowth [31]. This led to the suggestion that EDG-5 may play an important role in neuronal development and may steer axons by regulating their growth and inhibiting their extension [31]. In agreement with this hypothesis, we found that enforced expression of EDG-5 [21] or EDG-8 [32] in PC12 cells caused a decrease in NGF-induced neurite outgrowth and increased the fraction of cells with rounded morphology. Because PC12 cells express EDG-5 [31] and EDG-8 [33] constitutively, it is likely that SPP-induced neurite retraction in PC12 cells is mediated through these receptors. Moreover, EDG-5 or EDG-8 may be the unidentified cell surface receptors responsible for SPP-induced cell morphology alterations and remodeling of the actin cytoskeleton, particularly in neurons. Thus, SPP synthesized by target tissues could help to guide axons by regulating axon extension or stabilization through binding to EDG-5 and/or EDG-8. It is also possible that SPP might play a role during normal brain development or after traumatic injury by acting through EDG-5 and possibly EDG-8 to affect neuritogenesis.

In conclusion, as EDG-1, -3, -5, -6 and -8 are widely expressed in most cells and tissues, important questions that should be addressed in the future are their roles in mediating various biological responses to SPP. It is still an enigma why a simple sphingolipid metabolite such as SPP would have so many receptors. Gene knockouts might provide clues to substantiate the importance of their physiological functions. Moreover, identification of additional SPP receptors which mediate different responses to SPP will be an exciting area of future research. SPP analogs with different specificities for the different SPP receptors should be useful to determine which receptors mediate specific biological responses to SPP. Identification of SPP agonists and antagonists may provide the basis for development of novel therapeutics. Finally, a challenging but important task is the identification of the intracellular targets of SPP.

Acknowledgements: We thank the many members of the Spiegel laboratory for their contributions to the studies that were quoted in this review, especially Dr. Ana Olivera. This work was supported by research Grants from the National Institutes of Health (GM43880 and CA61774).

References

[1] Zhang, H., Desai, N.N., Olivera, A., Seki, T., Brooker, G. and Spiegel, S. (1991) J. Cell Biol. 114, 155–167.
[2] Spiegel, S. (1999) J. Leukoc. Biol. 65, 341–344.
[3] Kolesnick, R. and Hannun, Y.A. (1999) Trends Biochem. Sci. 24, 224–225.
[4] Olivera, A. and Spiegel, S. (1993) Nature 365, 557–560.
[5] Cuvillier, O., Pirianov, G., Kleuser, B., Vanek, P.G., Coso, O.A., Gutkind, S. and Spiegel, S. (1996) Nature 381, 800–803.
[6] Van Brocklyn, J.R. et al. (1998) J. Cell Biol. 142, 229–240.
[7] Perez, G.I., Knudson, C.M., Leykin, L., Korsmeyer, S.J. and Tilly, J.L. (1997) Nat. Med. 3, 1228–1232.
[8] Edsall, L.C., Pirianov, G.G. and Spiegel, S. (1997) J. Neurosci. 17, 6952–6960.
[9] Cuvillier, O., Rosenthal, D.S., Smulson, M.E. and Spiegel, S. (1998) J. Biol. Chem. 273, 2910–2916.
[10] Xia, P., Gamble, J.R., Rye, K.A., Wang, L., Hii, C.S.T., Cockerill, P., Khew-Goodall, Y., Bert, A.G., Barter, P.J. and Vadas, M.A. (1998) Proc. Natl. Acad. Sci. USA 95, 14196–14201.
[11] Prieschl, E.E., Csonga, R., Novotny, V., Kikuchi, G.E. and Baumruker, T. (1999) J. Exp. Med. 190, 1–8.
[12] Su, Y., Rosenthal, D., Smulson, M. and Spiegel, S. (1994) J. Biol. Chem. 269, 16512–16517.
[13] Olivera, A., Kohama, T., Tu, Z., Milstien, S. and Spiegel, S. (1998) J. Biol. Chem. 273, 12576–12583.
[14] Kohama, T., Olivera, A., Edsall, L., Nagiec, M.M., Dickson, R. and Spiegel, S. (1998) J. Biol. Chem. 273, 23722–23728.
[15] Nagiec, M.M., Skrzypek, M., Nagiec, E.E., Lester, R.L. and Dickson, R.C. (1998) J. Biol. Chem. 273, 19437–19442.
[16] Topham, M.K., Bunting, M., Zimmerman, G.A., McIntyre, T.M., Blackshear, P.J. and Prescott, S.M. (1998) Nature 394, 697–700.
[17] Olivera, A., Kohama, T., Edsall, L.C., Nava, V., Cuvillier, O., Poulton, S. and Spiegel, S. (1999) J. Cell Biol. 147, 545–558.
[18] Mandala, S.M., Thornton, R., Poulton, S., Galve-Roperh, I., Peterson, C., Olivera, A., Bergstrom, J., Kurtz, M.B. and Spiegel, S. (2000) Proc. Natl. Acad. Sci. USA (in press).
[19] Lee, M.J., Van Brocklyn, J.R., Thangada, S., Liu, C.H., Hand, A.R., Menzeleev, R., Spiegel, S. and Hla, T. (1998) Science 279, 1552–1555.
[20] Spiegel, S. and Milstien, S. (2000) Biochim. Biophys. Acta (in press).
[21] Van Brocklyn, J.R., Tu, Z., Edsall, L.C., Schmidt, R.R. and Spiegel, S. (1999) J. Biol. Chem. 274, 4626–4632.
[22] Van Brocklyn, J.R., Gräler, M.H., Bernhardt, B., Hobson, J.P., Lipp, M. and Spiegel, S. (2000) Blood (in press).
[23] Goetzl, E.J. and An, S. (1998) FASEB J. 12, 1589–1598.
[24] Wang, F., Van Brocklyn, J.R., Hobson, J.P., Movafagh, S., Zukowska-Grojec, Z., Milstien, S. and Spiegel, S. (1999) J. Biol. Chem. 274, 35343–35350.
[25] Arai, H., Tsou, C.L. and Charo, I.F. (1997) Proc. Natl. Acad. Sci. USA 94, 14495–14499.
[26] Folkman, J. (1995) Nat. Med. 1, 27–31.
[27] English, D., Kovala, A.T., Welch, Z., Harvey, K.A., Siddiqui, R.A., Brindley, D.N. and Garcia, J.G. (1999) J. Hematother. Stem Cell Res. 8, 627–634.
[28] Lee, O.H., Kim, Y.M., Lee, Y.M., Moon, E.J., Lee, D.J., Kim, J.H., Kim, K.W. and Kwon, Y.G. (1999) Biochem. Biophys. Res. Commun. 264, 743–750.
[29] Lee, M.J., Thangada, S., Claffey, K.P., Ancellin, N., Liu, C.H., Kluk, M., Volpi, M., Sha'afi, R.I. and Hla, T. (1999) Cell 99, 301–312.
[30] Okazaki, H., Ishizaka, N., Sakurai, T., Kurokawa, K., Goto, K., Kumada, M. and Takuwa, Y. (1993) Biochem. Biophys. Res. Commun. 190, 1104–1109.
[31] MacLennan, A.J., Marks, L., Gaskin, A.A. and Lee, N. (1997) Neuroscience 79, 217–224.
[32] Malek, R.L., Toman, R.E., Edsall, L.C., Wong, S., Chiu, J., Letterle, C.A., Van Brocklyn, J.R., Spiegel, S. and Lee, N.H (2000) J. Biol. Chem. (in press).
[33] Glickman, M., Malek, R.L., Kwitek-Black, A.E., Jacob, H.J. and Lee, N.H. (1999) Mol. Cell. Neurosci. 14, 141–152.