F.Ü.Sağlık Bil.Dergisi 1998, 12 (1), 81-85

SPHINGOLIPIDS: A NOVEL CLASS OF SECOND MESSENGERS ACTING VIA INTRACELLULAR Ca^{2+}-RELEASE

[1]Ahmet AYAR [2]Vahit KONAR

[1]Fırat Üniversitesi, Tıp Fakültesi, Elazığ-TÜRKİYE
[2]Fırat Üniversitesi, Fen Edebiyat Fakültesi, Elazığ-TÜRKİYE

Geliş Tarihi: 15.07.1998

Sifingolipidler: Hücre içinden kalsiyum salınımına yol açarak etkili olan yeni bir sınıf ikincil haberciler

ÖZET

Sifingolipidler ökaryotik hücre membranlarında bulunan, önemli yapısal ve düzenleyici göreve sahip moleküllerdir. Membran yapısında bolca bulunmalarından dolayı, önceleri sadece yapısal görevleri olduğu sanılmaktaydı. Son on yılda, sifingolipidler hücre motilitesi ve proliferasyonunun kontrolü ile hücresel etkileşimlerde önemli rolleri olan yeni bir ikincil haberci grubu olarak ortaya çıktılar. Sifingolipid metabolitlerinin ayrıştırma ve tanımlanmasında kimyasal yöntemlerdeki yeniliklerin uygulanması ile sifingolipid sinyalleşmesinde önemli ilerlemeler sağlanmıştır. Sifingolipid yıkım ürünleri olan sifingosin, dihidrosifingosin, seramid, sifingosin-1-fosfat ve sifingomyelinin hücre proliferasyonu, hücre başkalaşımı ve hücre içi depolardan kalsiyum salınımına yol açtığı çeşitli çalışmalarla bildirilmiştir. Sifingolipidler ve metabolitlerinin rol oynadığı biyolojik olaylar çeşitli biyomedikal ve biyokimyasal laboratuarlarda halen devam eden sıcak bir ilgi görmektedir.

Anahtar Kelimeler: Sifingolipid,, sinyalleşme, İkincil haberciler, hücre içi kalsiyum salınımı, seramid.

SUMMARY

The sphingolipids are important structural and regulatory components of eukaryotik cell membranes. Initially, because of their abundant presence in membranes they thought to have primarily structural roles. In the last decade, sphingolipids have emerged as novel intracellular second messengers playing major roles in cellular interactions and control of cell proliferation and motility. Important progress has been achieved in sphingolipid signaling with utilization of recent great technological advances in separation and characterization of sphingolipid metabolites. Several investigators have suggested that sphingolipid metabolites sphingosine, dihydrosphingosine, ceramide, sphingosine-1-phosphate and sphingomyelin are playing important roles in cell proliferation, differentiation and release of calcium from intracellular stores. Furthermore, the biological processes that are mediated by sphingolipids and sphingolipid metabolites are currently receiving hot attention in the biomedical and biochemical laboratories.

Key Words: Sphingolipids, signaling, second messenger, intracellular calcium mobilization, ceramide.

INTRODUCTION

There are three main groups of lipid families expressed in eucaryotic cell membranes, the glycerophospholipids, cholesterol, and the sphingolipids. Second messenger role of glycerophospholipid substrates, diacylglycerol and inositol-phosphate is well known (1, 2, 3). Phosphatidylinositol hydrolysis products of inositol-phosphate and diacylglycerol function as second messenger by releasing Ca^{2+} (4) and activating protein kinase C (PKC) (5). Informations about several glycerophospholipid substrates are expanding rapidly (5, 6, 7).

There is as yet no report about second messenger or signaling role for cholesterol.

The sphingolipids are thought to be solely structural molecules since they are abundantly expressed in plasma membranes. Recently, increasing number of evidences is emerging for the role of sphingolipids and

their metabolic breakdown products as intracellular lipid second messengers (8, 9). Within the last decade, it has became clear that the sphingolipids are not only functioning as cyto-structural molecules but also as active partici pants in the regulation of variety of diverse cellular processes including regulation of transduction of extracellular stimuli across the plasma membrane, protein phosphorilation, receptor modulation, resulting in the physiological regulation of cell function, cell growth, differentiation, transformation, cell-cell contact, immune recognition and neoplastic transformation. (9, 10, 11). The original hypothesis on the roles of sphingolipids in transmembrane signal transduction came from the observation that sphingosine potently inhibited protein kinase (PKC) both *in vivo* and *in vitro* (9, 12).

Because PKC is a key regulatory enzyme of many physiological processes the above mentioned finding have stimulated a lot of interest in biological roles of sphingolipids and the discovery of many examples of sphingosine-mediated cellular phenomena which are apparently due to sphingolipid mediated inhibition of PKC (13). However, studies using several cell types have since been uncovered that sphingosine have more versatile roles and these roles do not solely depend on its effect on PKC (14, 15).

It is not clear how sphingolipids elicits their intracellular signaling cascades and physiological cellular responses. One possibility is that intracellular conversion of sphingolipids to sphingolipid metabolic breakdown products. Indeed, the use of recent great technological advances in separation and characterization of sphingolipids has revealed that the second messenger effects of sphingolipids resides not only in the parent molecules but also in their metabolic derivatives, such as sphingosine, sphingosine-1-phosphate and ceramide (16,17,18). Some of the effects of sphingolipid derivatives in different types of cells are given in table 1.

Ceramide (N-acyl erythrosphingosine) and sphingosine-1-phosphate are among the well-studied sphingolipid metabolic breakdown products with second messenger roles, such as transduction, cell proliferation, differentiation and apoptosis (19,20). Ceramide has been shown to promote apoptotic cell death in the human myeloid leukemia cell lines (21).

The pathway by which sphingolipid breakdown product sphingosine is generated is not clear yet but it is likely that the enzyme sphingomyelinase hydrolysis sphingomyelin to ceramide, which is the precursor of sphingosine (22).

In addition to already mentioned inhibitory effect of sphingosine on PKC it has also reported to cause inhibition of calmodulin requiring enzymes (23), activation of phospholipase D (24), and activation of Ca^{2+} release from intracellular stores (25). Although the mechanism by which sphingosine releases Ca^{2+} from intracellular stores is still not elucidated, it has been suggested that sphingosine-1-phosphate is the active product causing Ca^{2+} release in smooth muscle cells and fibroblasts (26, 27). On the other hand, sphingosine have also reported to inhibit Ca^{2+}-induced Ca^{2+}-release (CICR) in skeletal muscle (28) and resulted in decrease of ryanodine binding to ryanodine receptor (RyR) in cardiac sarcoplasmic reticulum (29).

Furthermore, sphingosine-1-phosphate has been reported to activate inward Ca^{2+}-activated chloride currents in *Xenopus oocyte* (30). Sphingosine-1-phosphate dependent activation of DNA synthesis in *Swiss 3T3 cells* was recently reported to be inhibitable by pertussis toxin (31). Pertussis toxin is known to be a functional uncoupler of guanine nucleotide binding protein (G-protein) from its receptor (32). These findings suggest that G-protein is involved in regulation of the mitogenic effect of sphingosine-1-phosphate. In a recent study, it has been shown that sphingosine-1-phosphate plays a major role in regulation of morphogenetic differentiation via activation of G-protein. (33).

Evidences are ever increasing for the role of sphingolipids and sphingolipid metabolic breakdown products in release of Ca^{2+} from intracellular stores. In a recent study, intracellular photorelease of 'caged' sphingolipid dihydrosphingosine has been shown to release Ca^{2+} from intracellular stores in cultured rat sensory neurons (34). The use of "caged" dihydrosphingosine, a photolabile compound that releases dihydrosphingosine upon irradiation with a near ultraviolet light, in this patch clamp study had the advantages of increased solubility and prevention of dilution or prevention of membrane impermeability since it was directly applied to the intracellular environment by including in the patch pipette solution (35). In that study, release of Ca^{2+} from intracellular stores were determined by activation of Ca^{2+}-activated chloride, ($I_{Cl(Ca)}$), and Ca^{2+}-activated non-selective cation, (I_{CAT}), currents. Intracellular Ca^{2+}-releasing effect of sphingosine, sphingosine-1-phosphate, ceramide, sphingomyelin and dihydrosphingosine has been shown by several studies in many cell types (26, 27, see also Table 1).

The prominent delay of activation of release of Ca^{2+} from intracellular stores even after intracellular photorelease of "caged dihydrosphingosine" is likely due to conversion of sphingolipids to more active products (34). Since direct application of sphingolipid breakdown products are shown to have a potent and direct effect in mediating Ca^{2+}-release from intracellular stores, sphingolipids must be enzymatically converted most probably to sphingosine-1-phosphate and mediates Ca^{2+}-release from intracellular stores that also include

inositol 1,4,5-triphosphate, (IP_3), sensitive stores, at least in smooth muscle cells (36,25). Sphingosine-1-phosphate has also reported to release Ca^{2+} from internal stores via an IP_3-independent pathway in Swiss 3T3 fibroblasts (27). "Caged dihydrosphingosine" induced Ca^{2+}-release is also independent of IP_3-sensitive stores possible from caffeine/ryanodine sensitive intracellular stores since ryanodine potently inhibited its Ca^{2+}-releasing ability (34,36). The finding that sphingolipids and their metabolites retain their Ca^{2+}-releasing ability when recordings performed in Ca^{2+}-free extracellular recording medium supports the idea that these agents releases Ca^{2+} from intracellular stores rather than causing entry of Ca^{2+} from extracellular environment via voltage and ligand-gated Ca^{2+}-channels. Additionally, sphingolipid induced Ca^{2+}-release potently blocked by intracellular Ca^{2+}-release channel blockers dantrolene and heparin (34, 25, 37), and also by increasing intracellular Ca^{2+}- buffering capacity of cells or by intracellular photorelease of "caged Ca^{2+} chelators" diazo-2 (34, 36, 37, 38).

Table 1. Some of the effects of well known sphingolipid products on cellular functions

Signal	Cell or Tissue Type	Response	References
Sphingosine	Dermal Fibroblasts	Stimulates Ca^{2+}-release from internal stores	39
	Swiss 3T3 cells	Promotes cell growth	40
	Rat ventricular myocytes	Inhibits L-type-Ca^{2+} channel currents	41
Sphingosine-1Phosphate	Bovine Aortic Endothelial Cells	Stimulates Ca^{2+}-release from internal stores	42
	Rat Glioma Cells	Stimulates Ca^{2+}-release from internal stores	43
	Thyroid FTRL 5 cells	Stimulates Ca^{2+}-release from internal stores	44
Ceramide	Cultured hippocampal neurons	Promotes cell growth	45
	Neutrophil	Inhibits superoxide generation	46
	Rat basophilic leukemia (RBL-2H3) cells	Inhibits Phospholipase D	47
		Induces apoptosis	48
	Myocardial cells	Causes ischemia/reperfusion induced apoptosis (*in vitro*)	49
Dihydrosphingosine	Rat thymocytes	Inhibits capacitative Ca^{2+} influx	50
	Human leukemia cells	Inhibits cell growth	51

CONCLUDINGREMARKS

The present review was undertaken to highlight some aspects of sphingolipids, which are emerging as important second messengers in signal transduction either by their direct effect or via the generation of their metabolites sphingosine, sphingosine-1-phosphate, sphingomyelin and ceramide, which have been shown to modulate a variety of biological processes. The discovery of the sphingolipids as Ca^{2+}-releasing agents is an important new development in current knowledge of cellular signaling.

The effects of these agents may occur via mechanisms that are dependent on PKC, G-protein and production of more active metabolites. Sphingosine has also been shown to act in a mechanism of PKC-independent targets, which are insulin, tyrosine kinase, diacylglycerol kinase and phospholipase D.

In conclusion, although abundant evidences strongly suggest that sphingolipid and sphingolipid breakdown products may be a novel class of second messengers; many questions remain to be answered about this new family of intracellular signaling molecules. Is there any other active sphingolipid breakdown products to be discovered? Are the effects of these agents similar or fundamentally different in all cell and tissue types?

Future studies will need to address the regulation of enzymes of sphingolipid synthesis and conversion of sphingolipids to their metabolites during the signal generation.

This subject is promise to be a fertile ground for research in the field of decreasing apoptosis, programmed cell death, and development of mutations or development of cancer.

REFERENCES

1. Majerus PW, Connolly TM., Deckmyn H., et al. The metabolism of phosphoinositide derived messenger molecules. Science 1986; 234: 1519-1526.
2. Rhee SG, Suh PG, Ryu SH, et al. Studies of inositol phospholipid-specific phospholipase C. Science 1989; 244: 546-560.
3. Liscovitch M. Crosstalk among multiple signal-activated phospholipases. Trends Biochem Sci 1992; 17 (10):393-399.
4. Berridge M, and Irvine RF. Inositol phosphates and cell signaling Nature 1989; 341: 197-205.
5. Bell RM, Ganong B, Loomis C. et al. In Enzymes of Lipid metabolism (Freysz L, Dreyfus H Massarelli R & Gatt S. eds.) 1986; 693-700.
6. Billah MM, Pai P-K, Mullmann TJ, et al. Sphingosine inhibits phosphatidate phosphohydrolase in human neutrophils by a protein kinase C-independent mechanism. J Biol Chem 1991; 266(4):2013-2016.
7. Dennis EA, Rhee SG, Billah MM, et al. Role of phospholipase in generating lipid second messengers in signal transduction. FASEB J. 1991; 5: 2068-2077.
8. Kan CC, and Kolesnick RN. A synthetic ceramide analog, D-threo-1-phenyl-2morpholino-1-propanol, selectively inhibits adherence during macrophage differantiation of human leukemia cells. J. Biol. Chem 1992; 267 (14): 9663-9667.
9. Hannun YA, and Bell RM. Functions of sphingolipids and sphingolipid breakdown products in cellular regulation. Science 1989; 243: 500-507.
10. Olivera A, Zhang H, Carlson RO, et al. Stereospecificity of sphingosine-induced intracellular calcium mobilization and cellular proliferation. J Biol Chem 1994:269(27):17924-17930.
11. Olivera, A, and Spiegel S. Sphingosine-1-phosphate as a second messenger in cell proliferation induced by PDGF and FCS mitogens. Nature1993, 365: 557-560.
12. Merrill AH, Sereni AM, Stevens VL, et al. Isolation and idendification of alternative riboflavin-binding proteins fromhuman plasma. J. Biol. Chem. 1986, 261: 12610-12615.
13. Hannun YA, and Bell RM. Lysoophingolipids inhibit protein kinase C: imolications for the sphingolipidoses. Science 1987; 235: 670-674.
14. Spiegel S, Olivera A, and Carlson RO. The role of sphingosine in cell growth regulation and transmembrane signaling. Advances in Lipid Res. 1993; 25: 105-129.
15. Olivera A, and Spiegel S. Ganglioside GM1 and sphingolipid breakdown products in cellular proliferation and signal transduction pathway. Glyconjugate Journal 1993; 9: 109-117.
16. Zhang H, Desai NN, Olivera A. et al. Sphingosine-1-phosphate, a novel lipid, involved in cellular proliferation. J. Cell Biol. 1991; 114: 155-167.
17. Kolesnick R, and Golde DW. The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. Cell 1994, 77: 325-328.
18. Pushkareva MYu, Khan WA, Alessenko AV, et al. Sphingosine activation of protein kinases in Jurkat T cells. In vitro phosphorylation of endogenous protein substrates and specificity of action. J. Biol. Chem. 1992 ; 267 (21):15246-15251
19. Hannun, YA, Obeid LM, and Dbaibo GS. Ceramide-a novel second messenger and lipid mediator. Lipid Second Messengers 1996; 8: 177-204.
20. Hannun YA, and Obeid LM. Ceramide: an intracellular signal for apoptosis. Trends in Biochim. Soc. 1995; 20: 73-77.
21. Pushkerava M, Obeid LM, and Hannun YA. Ceramide: an endogenous regulator of apoptosis and growth suppression. Immunology Today 1995; 16: 294-297.
22. Slife CW, Wang E, Hunter R, et al. Free sphingosine formation from endogenous substrates by a liver plasma membrane system with a divalent cation dependence and neutral pH optimum. J. Biol. Chem 1989; 2664: 10371-10377.
23. Jeffersson AB, and Schulman H. Sphingosine inhibits calmodulin-dependent enzymes. J. Biol. Chem. 1998; 263(30): 15241-15245.
24. Desai NN, Zhang H, Olivera A, et al. Sphingosine-1-phosphate, a metabolite of sphingosine, increases phosphatidic acid levels by phosphalipase D activation. J. Biol. Chem.1992; 267:23122-23128.
25. Ghosh TK, Bian J, and Gill DL. Intracellular calcium release mediated by Sphingosine derivatives generated in cells. Science 1990; 248: 1653-1656.
26. Ghosh TK, Bian J, and Gill DL. Sphingosine-1-phosphategenerated in the edoplasmic reticulum activates reelase of stored calcium..J. Biol. Chem 1994; 269: 22628-22635.
27. Mattie M, Brooker G, and Spiegel S. Sphingosine-1-phosphate, a putative second messenger, mobilizes calcium from internal stores via an Inositol Triphosphate-independent pathway. J. Biol. Chem. 1994; 269: 3181-3188.

28. Sabbadini RA, Betto R, Teresi A, et al. The effect of sphingosine on sarcoplasmic reticulum membrane calcium release. J. Biol. Chem. 1992; 267: 15475-15484.

29. Dettbarn CA, Betto R, Salviati G, et al. Modulation of cardiac sarcoplasmic reticulum ryanodine receptor by sphingosine. J. Mol. Cardiol. 1994; 26: 229-242.

30. Duriex ME, Carlisle SJ, Salafranca MN., et al. Responses to sphingosine-1-phosphate in *Xenopus laeves oocytes* : similarities with lysophatidic acid signalling. American J. Physiol. 1993: C1360-C1364.

31. Goodemote KA, Mattie ME, Berger A, et al. Involvement of a pertussis toxin sensitive G-protein in mitogenic signaling pathways of sphingosine-1-phosphate. J. Biol. Chem. 1995, 270: 10272-10277.

32. Masters SB, Sullivan KA, Miller RT, et al. Carboxyl terminal domain Gs α specifies coupling of receptors to stimulation of adenylyl cyclase. Science 1988, 241: 448-451.

33. Lee M-J, Van Brocklyn JR, Thangada S, et al. Sphingosine-1-phosphate as ligand for the G-Protein-Coupled Receptor EDG-1. Science 1998; 279: 1552-1555.

34. Ayar A, Thatcher NM, Zehavi U, et al. Mobilization of intracellular calcium by intracellular flash photolysis of caged dihydrosphingosine in cultured neonatal rat sensory neurones. Acta Biochimica Polonica 1998; 45(2): 311-326.

35. Scott RH, Ayar A, and Crawford JH. Flash photolysis of caged compounds: their use in investigating intracellular messengers. The Physiological Society Magazine 1997: 26: 16-18.

36. Kondo T, Konishi F, Inui H, et al. Differing signal transduction elicited by three isoforms of platelet-derived growth factor in vascular smooth muscle cells. J. Biol Chem. 1993; 268: 4458-4464.

37. Scott RH, Ayar A, Thatcher NM, et al. Mobilization intracellular calcium by intracellular flash photolysis of caged dihydrosphingosine in cultured neonatal rat sensory neurones. International Symposium on Structure, Metabolism and Functions of Glygosphingolipids and Sphingolipids 1997; P20.

38. Zucker RS. Effects of photolabile calcium chelatorson fluerescent calcium indicators. Cell Calcium 1992, 13: 29-40.

39. Chao CP, Laulederkind SJF, and Ballou LR. Sphingosine mediated phosphatidylinositol metabolism and calcium mobilization. J. Biol. Chem. 1994; 269: 5849-5856.

40. Zhang H, Buckley NE, Gibson IC, et al. Sphingosine stimulates cellular proliferation via a protein kinase C-independent pathway. J. Biol. Chem 1990; 265: 76-81.

41. McDonough PM, Yasui K, Betto R, et al. Control of cardiac Ca^{2+} levels. Inhibitory actions of sphingosine on Ca^{2+} transients and L-type Ca^{2+} channel conductance. Cicr. Res. 1994; 75(6): 981-989.

42. Meyer Z-H D, van Koppen CJ, Windorfer B. et al. Calcium signalling by G-protein-coupled sphingolipid receptors in bovine aortic endothelial cells. Naunyn Schmi. Arch. Pharmacol 1996; 354(4): 397-403.

43. Tas PW, and Koschel K. Sphingosine-1phosphate induces Ca^{2+} signal in primary rat astrocytes and a Ca^{2+} signal and shape changes in C6 rat glioma cells. J. Neurosci. Res. 1998; 52(4):427-434.

44. Tornquist K, Saarinen P, Vainio M., et al. Sphingosine-1-phosphate mobilizes releases sequestered calcium, activates calcium entry, and stimulates deoxyribonucleic acid synthesis in thyroid FTRL-5 cells. Endocrinology 1997; 138 (10): 4049-4057.

45. Futerman AH. The roles of ceramide in the regulation of neuronal growth and development. Biochemistry 1998; 63: 74-83.

46. Wong K, Li XB, and Hunchuk N. N-acetylsphingosine (C2-ceramide) inhibited neutrophil superoxide formation and calcium influx. J. Biol. Chem. 1995; 270 (7): 3056-3062.

47. Nakamura Y, Nakashima S, Ojio K, et al. Ceramide inhibits IgE-mediated activation of phospholipase D, but not of phospholipase C, in rat basophilic leukemia (RBL-2H3) cells. J. Immunol 1996; 156(1): 256-262.

48. Frago LM, Leon Y, de la Rossa EJ, et al. Nerve growth factor and ceramide modulate cell death in early developing inner ear. J. Cell Sci. 1998; 111 (5): 549-556.

49. Bielawska AE, Shapiro JP, Jiang L, et al. Ceramide is involved in triggering of cardiomyocyte apoptosis induced by ischemia and reperfusion. Am. J. Pathol. 1997; 151 (5): 1257-1263.

50. Gamberucci A, Fulceri R, and Benedetti A. Inhibition of store-dependent capacitative Ca^{2+} influx by unsaturated fatty acids. Cell Calcium 1997; 21 (5): 375-385.

51. Tamiya-Koizumi K, Murate T, Suzuki M, et al. Inhibition of DNA primase by sphingosine and its analogues paralels with their growth suppression of cultured human leukemia cells. Biochem. Mol. Biol Int. 1997; 41 (6): 1179-1189.