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Minireview

Molecular diversity of sphingolipid signalling

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Abstract Sphingolipid breakdown products are now being recognized to play a dual role in cellular signalling, acting as intracellular as well as extracellular signalling molecules. Both types of action may even be found with one sphingolipid species. The recent demonstration of G protein-coupled receptors with high affinity for sphingosine 1-phosphate and sphingosylphosphorylcholine has been followed by the discovery of several novel sphingolipid actions, such as regulation of heart rate, oxidative burst, neurite retraction or platelet activation. Ligand profiles and concentration-response relationships suggest the existence of putative sphingolipid receptor subtypes. Against this background, several observations on supposed sphingolipid second messenger actions deserve a new evaluation.

Key words: Sphingolipid; Sphingosine 1-phosphate; Sphingosylphosphorylcholine; G protein-coupled receptor; Signal transduction

1. Introduction

Many extracellular signalling molecules such as hormones, neurotransmitters and growth factors act via specific cell surface receptors which often regulate cellular functions by inducing breakdown of certain complex plasma membrane lipids. Since the 1980s, several second messenger molecules derived from membrane glycerophospholipids have been identified [1]. However, lipid signalling is not restricted to second messenger functions. Several lipid messengers derived from glycerophospholipids act as extracellular signalling molecules, such as the eicosanoids, platelet-activating factor and lysophosphatidic acid (LPA). Despite their lipophilic character, which should possibly allow easy access to the cell interior, these lipids act via specific cell surface receptors, which are mainly of the G protein-coupled receptor superfamily [2].

Much less is known about the signalling properties of another major group of ubiquitously occurring membrane lipids, the sphingolipids. Sphingolipids are composed of three basic constituents, a sphingoid base, i.e. a long chain aliphatic 2-amino-1,3-diol, which predominantly is sphingosine, an amide-linked fatty acid, and a polar head group. The headgroup distinguishes between neutral sphingolipids like ceramide, phosphosphingolipids like sphingomyelin, and glycosphingolipids like cerebrosides and gangliosides. The complex structure of the members of this lipid class ideally suits them for a diversity of cellular functions [3]. Sphingolipid degradation products which are presently regarded as signalling molecules are ceramide, ceramide 1-phosphate, sphingosine, sphingosine 1-phosphate (SPP) and sphingosylphosphorylcholine (SPPC). Galactosylsphingosine (psychosine) as well as glucosylsphingosine (glucopsychosine, GPS) should perhaps be added to the list. Fig. 1 gives a short overview of the structures and some of the metabolic pathways producing and degrading the respective molecules, which are probably subject to complex cellular regulations. A more comprehensive presentation of sphingolipid metabolism can be found in [4].

Sphingolipid breakdown products have been implicated as second messengers in the regulation of cell growth, differentiation and programmed cell death. Most details are known about the sphingomyelin cycle leading to receptor-mediated production of ceramide. Several intracellular targets of ceramide possibly involved in ceramide-induced apoptosis have recently been identified. This topic is reviewed in [5–8] and will not be covered here. Publications dealing with second messenger actions of sphingosine, SPP and SPPC have been summarized in [4,9–13]. Very recently, however, high-affinity plasma membrane receptors for SPP and SPPC have been discovered [14–17]. In parallel, progress is made in characterizing the regulation of SPP production [18] and defining intracellular SPP and/or SPPC targets [19,20]. Sphingolipids have thus to be regarded as *intra*cellular as well as *inter*cellular messengers. This will be the topic of the present minireview.

2. G protein-coupled sphingolipid receptors

2.1. Evidence for specific G protein-coupled sphingolipid receptors

Hints pointing to the existence of sphingolipid receptors had been obtained from sporadic reports that the actions of sphingosine, SPP or SPPC could be attenuated by treatment with pertussis toxin (PTX). This toxin prevents receptor coupling to G proteins of the G_i family, thus blocking cellular signalling by these receptors [21]. For example, PTX partially inhibited sphingosine-induced inositol phosphate accumulation in dermal fibroblasts [22]. In thyroid FRTL-5 cells, PTX attenuated the increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in response to SPPC but not to sphingosine [23]. In Swiss 3T3 fibroblasts, PTX inhibited 70–80% of SPP-stimulated mitogen-activated protein kinase activity and reduced SPP-induced inositol phosphate production, increase in $[Ca^{2+}]_i$, DNA synthesis as well as DNA binding activity of activator protein-1 [24,25]. Here, SPP was also found to inhibit forskolin-stimulated cAMP production in a PTX-sensitive manner, which is a typical response of G_i-coupled receptors. In addition to the PTX sensitivity, the high potency of

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Abbreviations: SPP, sphingosine 1-phosphate; SPPC, sphingosylphosphorylcholine; GPS, glucopsychosine; DHS, DL-*threo*-dihydrosphingosine; LPA, lysophosphatidic acid; PTX, pertussis toxin; PDGF, platelet-derived growth factor

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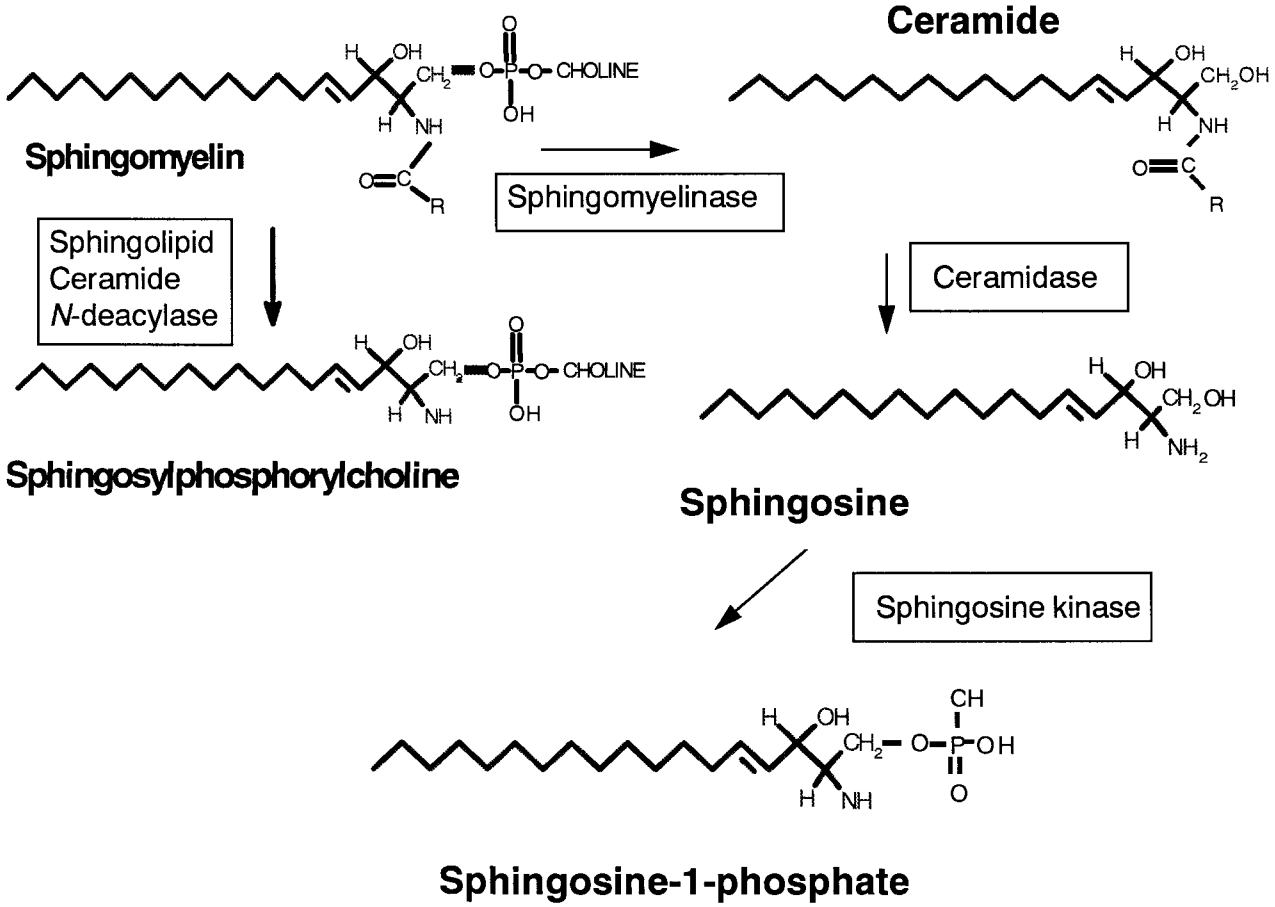

Fig. 1. A summary of sphingomyelin metabolism generating biologically active sphingolipid metabolites.

exogenously added SPP, in the low nanomolar range, found in some cell types suggested the participation of specific membrane receptors in SPPs action. For example, SPP was reported to inhibit platelet-derived growth factor (PDGF)-induced migration of human aortic smooth muscle cells with an EC_{50} of 16 nM [26]. Similarly, inhibition of B16 melanoma cell motility was maximal at 10–100 nM SPP [27,28]. Finally, the observation made in several cell types that the action of SPP was subject to desensitization pointed towards a receptor-mediated process.

Although these data were suggestive of the existence of specific membrane receptors for these sphingolipids, they could not resolve the question whether sphingolipids act by direct G protein activation or by receptor stimulation. This dilemma was solved by the observation that SPP activated muscarinic K^+ current ($I_{K(ACh)}$) in atrial myocytes [29]. The channel is directly inhibited by βγ-subunits released from G_i proteins which in turn are activated by plasma membrane receptors such as muscarinic acetylcholine receptors, thus representing a signalling system composed of only three membrane components [30]. SPP activated this current with a high potency ($EC_{50} \sim 1$ nM) in a completely PTX-sensitive manner [29]. This observation thus made it possible to identify the site of SPPs acton. Using inside-out patches, it was demonstrated that SPP activated the current only in the presence of GTP, required for G_i activation, and, most importantly, only when SPP was applied to the extracellular face of the plasma membrane [14]. The high potency of SPP combined with the absence of LPA receptors strongly suggests that SPP is an endogenous ligand of this G_i-coupled membrane receptor.

Further proofs for sphingolipid plasma membrane receptors followed soon. In N1E-115 neuronal cells, SPP caused neurite retraction with an EC_{50} of 1.5 nM [17]. This action of SPP was not PTX-sensitive, was subject to desensitization and was only observed when SPP was applied extracellularly, whereas microinjection of SPP had no effect. In atrial myocytes, $I_{K(ACh)}$ was not only activated by SPP but also by SPPC. Importantly, this action was highly stereoselective, the naturally occurring *erythro*-isomer being ~400 times more potent than the *threo*-isomer of SPPC [31]. This observation also strongly argues against a non-selective detergent action of the amphipathic sphingolipids; detergent-like actions, however, may well occur at much higher concentrations. Sphingolipid receptors are apparently not restricted to those for SPP and SPPC only. In insulinoma cells, GPS inhibited L-type Ca^{2+} currents, however, only when applied extracellularly, whereas inclusion of GPS into the patch pipette solution had no effect. This Ca^{2+} channel inhibition was PTX-insensitive, but could be blocked by intracellular GDPβS, indicating involvement of PTX-insensitive G proteins [32].

2.2. *Occurrence and functions of sphingolipid receptors*

Until recently, sphingolipids were considered to be involved only in regulation of mitogenesis, cell differentiation and cell growth or apoptosis [4,12,13]. Testing the concept of specific

Table 1
Putative sphingolipid receptor subtypes

Receptor subtype	Sphingolipid potency (EC_{50})			PTX	Cell type	Response	Reference
	SPP	SPPC	GPS, PS				
I	1.2 nM	1.5 nM	>10 μM	+	atrial myocytes	activation of $I_{K(ACh)}$	[14,31]
II	2.0 nM	0.5 μM	>10 μM	+	HEK-293 cells	$[Ca^{2+}]_i$ increase	[14]
	0.8 nM	0.3 μM	>10 μM	+	endothelial cells		[16]
				+	VSMC		[26]*
				+	dermal fibroblasts		*
	1.5 nM	n.d.	n.d.	−	N1E-115	neurite retraction	[17]
IIIa	>10 μM	1.5 μM	~8 μM	+	neutrophils	O_2^- production	[15]*
				+	HL-60 cells[a]	$[Ca^{2+}]_i$ increase	
IIIb	>10 μM	5 μM	4 μM	−	insulinoma cells	inhibition of VOCC	[32]

PS, psychosine; PTX, + and −, PTX-sensitive and -insensitive; HEK-293, human embryonic kidney; VSMC, vascular smooth muscle cells; N1E-115, mouse neuronal cells; VOCC, voltage-operated calcium channels; *own unpublished observations; n.d., not determined; [a]but see [56,57], in which PTX-sensitive $[Ca^{2+}]_i$ increase by SPP, SPPC and PS in HL-60 cells has been reported. The difference is probably due to different subclones of HL-60 cells.

membrane receptors for sphingolipids has already been fruitful in that it has led to the discovery of several novel sphingolipid actions. Thus, SPP and SPPC, both potently activating atrial $I_{K(ACh)}$, may be involved in regulation of heart rate [29,31]. The physiological role of the observed sphingolipid effects on vascular cells deserves specific attention. SPP (and SPPC) potently increased $[Ca^{2+}]_i$ in endothelial cells of different origin such as bovine aorta [16] and human umbilical vein (own unpublished observations) as well as in vascular smooth muscle cells [26], suggesting that SPP plays a delicate role in hemodynamics and regulation of vascular tone and blood pressure in vivo, which needs to be demonstrated. A source for SPP in the vascular system may be thrombin-activated platelets, which have recently been shown to release internally stored SPP extracellularly upon stimulation [33,34]. Platelets themselves are also activated by SPP, however, apparently by binding to the platelet LPA receptor [34]. Induction of neurite retraction by SPP observed in N1E-115 cells may be a hint towards a role of this sphingolipid in neuropathology, with blood-borne SPP possibly contributing to damage of neuronal cells under conditions of blood-brain barrier breakdown where axon regeneration is often impaired [17].

Another cellular function potently affected by SPP is cell motility, which is essential for many physiological and pathological processes such as wound healing, angiogenesis and tumor metastasis [27,28,35]. Even mitogenesis induced by SPP, previously thought to be an intracellular SPP action, may be mediated by a membrane receptor, as the mitogenic response and DNA synthesis induced by SPP in Swiss 3T3 cells was inhibited by PTX [24,25]. Similarly, as demonstrated in airway smooth muscle cells, SPP-induced activation of extracellular signal-regulated kinase, involved in mitogenesis [36,37] and suppression of ceramide-mediated apoptosis [38], was dependent on PTX-sensitive G proteins [39]. Finally, SPPC-induced activation of mitogen-activated protein kinase in Swiss 3T3 cells was reported to involve a PTX-sensitive G protein [40].

Based on the observation that SPPC efficiently stimulates superoxide production in HL-60 granulocytes and human neutrophils, it is likely that sphingolipids, specifically SPPC and its receptors, also play a role in host defense against invading microorganisms [15]. In line with this hypothesis is the recent observation that a certain *Pseudomonas* strain secretes an enzyme capable of cleaving glycosphingolipids as well as sphingomyelin to produce SPPC [41]. SPPC may even be a candidate for clinical application, as it has been reported to be a potent wound healing agent in animal models in vivo [42]. Finally, in the studies on the regulation of L-type Ca^{2+} channels in insulinoma cells by GPS and psychosine, it was observed that these sphingolipids also affect insulin secretion ([32] and own unpublished observations), suggesting that they may modulate insulin secretion in pancreatic beta cells as well. Thus, sphingolipids by activating specific membrane receptors can apparently regulate a wide variety of cellular functions and it can be anticipated that in the near future many additional sphingolipid receptor-regulated processes will be discovered.

2.3. *Putative sphingolipid receptor subtypes*

Although a sphingolipid receptor has not been cloned so far or positively identified by specific binding, the present functional data strongly support the notion of sphingolipid receptor subtypes. A preliminary classification is given in Table 1. Included are only data which are comprehensive enough to allow a rather firm statement. Atrial myocytes apparently contain a G_i protein-coupled receptor, which is activated with high affinity by both SPP and SPPC, as shown by full cross-desensitization of these two sphingolipids [31]. A second high-affinity SPP receptor, binding SPPC with ~500-fold lower affinity (or alternatively two separate receptors for SPP and SPPC), seems to be expressed rather widely. Activation of this receptor leads to a PTX-sensitive increase in $[Ca^{2+}]_i$ in a variety of cell types [14,16]. Whether or not the SPP receptor described in N1E-115 cells belongs to this subtype is not certain, as the SPP action was PTX-insensitive and data on SPPC were not reported [17]. In human neutrophils and HL-60 granulocytes, SPPC, but not SPP, stimulated superoxide production and increased $[Ca^{2+}]_i$, strongly suggesting the existence of a distinct SPPC receptor, which was shown to couple to PTX-sensitive G_i proteins [15]. Finally, G protein-coupled sphingolipid receptor-mediated inhibition of L-type Ca^{2+} channels in insulinoma cells was induced by GPS, psychosine and SPPC in a PTX-insensitive manner, thus arguing for a further distinct receptor subtype [32]. Thus, there is not only a heterogeneity of sphingolipid receptors, but these receptors can obviously also couple to distinct G proteins, i.e. PTX-sensitive and -insensitive G proteins, the exact nature of these coupling proteins needs to be determined.

In human platelets, SPP and LPA apparently share a com-

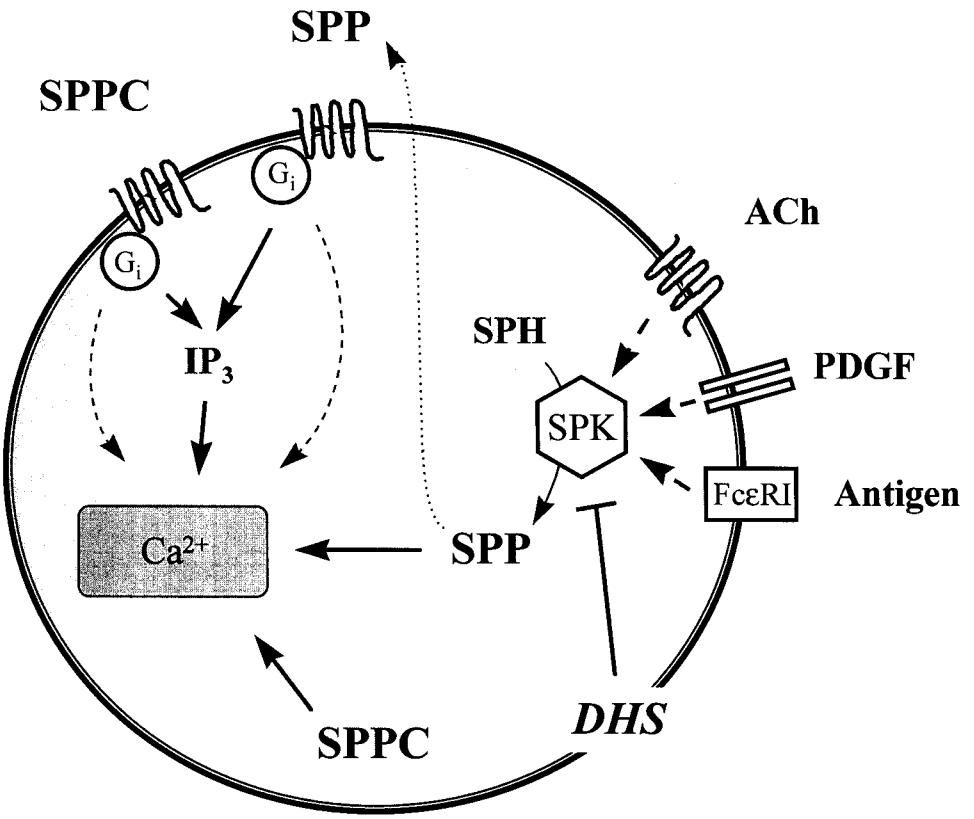

Fig. 2. Intracellular versus receptor-mediated Ca^{2+} signalling by sphingolipids. PDGF, FcεRI antigen and muscarinic acetylcholine (ACh) receptors activate sphingosine kinase (SPK), thus increasing intracellular SPP production. Inhibition of SPK with DHS attenuates Ca^{2+} release induced by each of the receptors indicating involvement of SPK in Ca^{2+} signalling. In some cell types, SPP may directly release Ca^{2+} from intracellular stores, while in others only SPPC, but not SPP, is capable to induce Ca^{2+} release. SPP and SPPC also act at G protein-coupled plasma membrane receptors. Ca^{2+} release induced by the sphingolipid receptors is mainly mediated by G_i proteins and involves either phospholipase C stimulation or an as yet unidentified pathway. Intracellularly produced SPP may also be released to activate plasma membrane receptors in an autocrine or paracrine manner.

mon binding site, and platelet activation in response to LPA was desensitized by prior application of SPP [34]. Since cross-desensitization between SPP and LPA was not observed in many other cell types [14,17], and cells even missing LPA receptors potently responded to SPP [16], it might be speculated that there is a specific SPP/LPA receptor subtype in platelets.

3. Intracellular action versus receptor-mediated signalling

Apart from ceramide formation, relatively little is known about intracellular sphingolipid generation. SPP can be formed in intact cells by phosphorylation of sphingosine by sphingosine kinase (Fig. 2) [43], and is degraded, alternatively, by SPP lyase [44] or a phosphatase [45,46]. An extracellular stimulus reported to increase SPP formation is PDGF [36,47], but activation of sphingosine kinase may not be restricted to growth factor receptors. Recently, SPP formation was reported to be involved in Ca^{2+} mobilization by high-affinity IgE receptors (FcεRI) as well [18]. Preliminary data point towards sphingosine kinase also playing a role in Ca^{2+} signalling by G protein-coupled receptors such as muscarinic acetylcholine and bradykinin receptors [48,49]. To examine the role of sphingosine kinase, the two enzyme inhibitors DL-*threo*-dihydrosphingosine (DHS) and *N,N*-dimethylsphingosine [50,51] are usually applied. However, inhibition of sphingosine kinase may lead to enhanced levels of sphingosine [51], which in turn may be active on its own or be metabolized, e.g. to ceramide.

Since SPP formation is apparently involved in Ca^{2+} mobilization, sphingosine kinase has recently been ascribed a similar role as phospholipase C [52]. Indeed, SPP has been shown to cause release of Ca^{2+} from intracellular stores [53,54]. Others, however, reported SPPC being the sphingolipid ligand leading to Ca^{2+} release from the endoplasmic reticulum [19,20,55]. Finally, by intracellularly forming SPP, which is subsequently released extracellularly, as demonstrated for human platelets [33,34], sphingosine kinase may also be indirectly responsible for activation of G protein-coupled membrane receptors for SPP, acting in an autocrine or paracrine manner. Thus, besides the plasma membrane-located G protein-coupled sphingolipid receptors inducing Ca^{2+} mobilization indirectly, by stimulating phospholipase C with subsequent inositol 1,4,5-trisphosphate generation or other not yet defined mechanisms, SPP and/or SPPC can apparently also directly mobilize Ca^{2+} from intracellular stores. Future work will have to clarify the exact mechanisms involved in this putative Ca^{2+} signalling pathway.

4. Future directions

There are several open questions and urgent demands for

future research. First, the sources of extracellular SPP and other spingolipids apparently acting via specific plasma membrane receptors have to be identified. Second, the mechanisms controlling the cellular release and, thus, the levels of extracellular sphingolipids exhibiting a wide spectrum of biological functions need to be characterized. Third, molecular cloning of the sphingolipid receptors is urgently needed for exact characterization of receptor subtypes, tissue distribution, signal transduction and cellular functions. Finally, non-lipid agonists and/or antagonists have to be synthesized to activate and/or to block the sphingolipid receptors, possibly even suited for therapeutic intervention. Thus, the next decade promises to be an exciting period for sphingolipid research.

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