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Review

Lysophospholipid receptors: Signalling, pharmacology and regulation by lysophospholipid metabolism

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Abstract

The lysophospholipids, sphingosine-1-phosphate (S1P), lysophosphatidic acid (LPA), sphingosylphosphorylcholine (SPC) and lysophosphatidylcholine (LPC), activate diverse groups of G-protein-coupled receptors that are widely expressed and regulate decisive cellular functions. Receptors of the endothelial differentiation gene family are activated by S1P ($S1P_{1-5}$) or LPA (LPA_{1-3}); two more distantly related receptors are activated by LPA ($LPA_{4/5}$); the $GPR_{3/6/12}$ receptors have a high constitutive activity but are further activated by S1P and/or SPC; and receptors of the OGR1 cluster (OGR1, GPR4, G2A, TDAG8) appear to be activated by SPC, LPC, psychosine and/or protons. G-protein-coupled lysophospholipid receptors regulate cellular Ca^{2+} homoeostasis and the cytoskeleton, proliferation and survival, migration and adhesion. They have been implicated in development, regulation of the cardiovascular, immune and nervous systems, inflammation, arteriosclerosis and cancer. The availability of S1P and LPA at their G-protein-coupled receptors is regulated by enzymes that generate or metabolize these lysophospholipids, and localization plays an important role in this process. Besides FTY720, which is phosphorylated by sphingosine kinase-2 and then acts on four of the five S1P receptors of the endothelial differentiation gene family, other compounds have been identified that interact with more ore less selectivity with lysophospholipid receptors.

Keywords: G-protein-coupled receptor; Lysophospholipid; Sphingosine-1-phosphate; Lysophosphatidic acid; Sphingosine kinase; Autotaxin

Contents

Abbreviations: $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; DGPP 8:0, dioctanoylglycerol pyrophosphate; EDG, endothelial differentiation gene; ERK, extracellular signal-regulated kinase; FAP, fatty alcohol phosphate; GPCR, G-protein-coupled receptor; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPP, lipid phosphate phosphatase; NGF, nerve growth factor; PDGF, platelet-derived growth factor; PLA, phospholipase A; PLC, phospholipase C; PLD, phospholipase D; PPARγ, peroxisome proliferator-activated receptor-γ; PTX, pertussis toxin; S1P, sphingosine-1-phosphate; SPC, sphingosylphosphoryl choline; SPP, sphingosine-1-phosphate phosphatase; THI, 2-acetyl-4-tetrahydroxybutylimidazole

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1. Introduction

Ligands at G-protein-coupled receptors (GPCR) are structurally as diverse as biogenic amines, amino acids, nucleotides, photons, peptides, glycoproteins, odorants, ions, and lipids (see historic review by [1]). Many classes of lipid mediators are now recognized to act as agonists at GPCRs, among them the cyclooxygenase and lipoxygenase products of arachidonic acid, the cannabinoids, platelet-activating factor, and the lysophospholipids (for overview and nomenclature, see [2,3]). Lysophospholipid mediators can have a glycerol or sphingoid backbone and are characterized by having a single carbon chain and a polar headgroup. These structural features render them more hydrophilic and versatile than their corresponding phospholipids. Among several lysophospholipids for which a biological action has been demonstrated, lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) have been characterized in greatest detail so far. The last decade has seen an enormous increase in knowledge about lysophospholipid receptors and metabolism, as well as biological functions of S1P and LPA. A family of five S1P-GPCRs and three related LPA-GPCRs (for review, see [4–8]), three not related GPCRs with high constitutive activity that are nevertheless further activated by S1P and sphingosylphosphorylcholine (SPC) [9], and two more LPA-GPCRs [10,11] have been identified so far. Furthermore, a group of GPCRs that are differentially activated by protons and by the lysophospholipids, SPC, lysophosphatidylcholine (LPC) and psychosine have been described (reviewed in [12–14]). It cannot be excluded that there might be other lysophospholipid receptors among the remaining ~150 orphan GPCRs of the human genome.

Lysophospholipid GPCRs are widely expressed and it has been suggested that all cells in mammals respond in one way or another to LPA and S1P [5]. Lysophospholipid GPCRs regulate a broad range of cellular functions: cell proliferation and survival, migration and chemotaxis, cytoskeletal architecture, cell-cell-contacts and adhesion, Ca^{2+} homoeostasis and Ca^{2+}-dependent functions. This results in a particular impact of lysophospholipids on angiogenesis and lymphocyte trafficking, development of the nervous system, cancer growth and metastasis, as well as inflammation and arteriosclerosis [6,15–18].

Understandably, lysophospholipid GPCRs are promising pharmacological targets [19,20]. The first drug interacting with S1P-GPCRs, FTY720 [21], also named fingolimod, is undergoing clinical trials for prevention of kidney graft rejection and multiple sclerosis. Other, receptor subtype-selective drugs for S1P- and LPA-GPCRs are emerging, increasing the potential medicinal importance of this research field.

This review will provide a general overview on established and still controversial lysophospholipid GPCRs, their signal transduction pathways, cellular and biological actions, with a focus on recent findings. Furthermore, our understanding of how S1P and LPA are delivered to their respective receptors, and how their action is terminated, has been substantially increased and is reviewed here in detail. Finally, the emerging lysophospholipid pharmacology will be presented and discussed. We apologize that important work will not be mentioned here because of space limitations.

2. Established and controversial lysophospholipid receptors and their signalling pathways

2.1. S1P- and LPA-GPCRs of the endothelial differentiation gene family

In 1996, a GPCR, named ventricular zone gene-1, that was expressed in cortical neurogenic regions of the developing mouse brain, was identified and shown to be a LPA receptor [22]. Soon thereafter, a related orphan GPCR, discovered in 1990 as a transcript induced during differentiation of endothelial cells and named endothelial differentiation gene-1 (EDG-1) [23], was shown to be activated by S1P [24–26]. These groundbreaking discoveries led to rapid deorphanization of altogether eight homologous GPCRs, five activated by S1P and three activated by LPA, of the so-called EDG receptor family that had been named after EDG-1. These GPCRs are now named according to IUPHAR nomenclature after their main physiological ligand and numbered in the order of discovery [2].

$S1P_{1-3}$ and LPA_{1-3} are ubiquitously expressed. There are comprehensive recent reviews on these receptors available [4–6,8,20,27–29]. Therefore, signalling of $S1P_{1-3}$ and LPA_{1-3} is summarized here only in brief, see also Table 1. The $S1P_1$ receptor is unique because it is apparently coupled only to G_i proteins, by which it nevertheless regulates many cellular functions, e.g., extracellular signal-regulated kinase (ERK) activation and proliferation, Akt activation and survival, and, most important, Rac activation and migration. Crucial functions of $S1P_1$ such as stimulation of angiogenesis and lymphocyte trafficking are apparently based on its ability to stimulate migration [30,31]. $S1P_2$ is coupled to G_i, G_q and $G_{12/13}$ proteins and activates phospholipase C (PLC), mediates increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and stimulates ERK.

Table 1
G-protein-coupled lysophospholipid receptors

Receptor	Endogenous ligand(s) and activators	Main tissue distribution	Main signal transduction pathways and cellular effects			Ref.
$S1P_1$	S1P, dihydro-S1P >SPC	Ubiquitous	$G_{i/o}$	ERK↑, PI3K/Akt↑, Rac↑, AC↓	Migration, proliferation, survival, cell–cell-contacts, angiogenesis, lymphocyte trafficking	Reviews: [4–6,8,20,27–29]
$S1P_2$		Ubiquitous	$G_{i/o}$, G_q, $G_{12/13}$	PLC↑, $[Ca^{2+}]_i$↑, ERK↑, Rho↑, Rac↓, Cdc42	Migration ↓, contribution to vascular development, differentiation of SMC	
$S1P_3$		Ubiquitous	$G_{i/o}$, G_q, $G_{12/13}$	PLC↑, $[Ca^{2+}]_i$↑, Rho↑, Rac↑, ERK↑, Akt↑	Heart rate ↓, contribution to vascular development, NO-dependent vasorelaxation	
$S1P_4$	Phyto-S1P, dihydro-S1P >S1P>SPC	Lymphoid and haematopoietic tissue	$G_{i/o}$, $G_{12/13}$	PLC↑, $[Ca^{2+}]_i$↑, ERK↑, Rho↑↓, Cdc42↑	Migration ↑↓, proliferation and cytokine secretion in T cell lines ↓	[44,47,48]
$S1P_5$	S1P, dihydro-S1P	Brain, white matter tracts, oligodendrocytes; skin	$G_{i/o}$, $G_{12/13}$	AC↓, ERK↓, JNK↑	Proliferation ↓,cell rounding, process retraction in OPC, survival of mature ODC	[53,56,57]
LPA_1	LPA	Ubiquitous	$G_{i/o}$, G_q, $G_{12/13}$	AC↓, ERK↑, Akt↑, Rho↑, Rac↑, PLC↑, $[Ca^{2+}]_i$↑	Proliferation ↑, survival ↑ (e.g. of Schwann cells), neurite retraction, brain development, olfaction	Reviews: [4–6,8,27,226]
LPA_2	LPA	Ubiquitous	$G_{i/o}$, G_q, $G_{12/13}$	Rho ↑, PLC↑, $[Ca^{2+}]_i$↑, ERK↑, Akt↑, AC↓	Proliferation, survival	
LPA_3	LPA; preference for unsaturated acyl chains	Ubiquitous	$G_{i/o}$, G_q	PLC↑, $[Ca^{2+}]_i$↑, ERK↑, AC↑ ↓	Implantation (via COX-2) and embryo spacing in mice	
P2Y$_9$/GPR23 (LPA_4)	LPA	Low level in many tissues, high levels in ovary		AC↑, $[Ca^{2+}]_i$↑		[10]
GPR63 (LPA_5)	LPA	Low level in many tissues, high levels in small intestine and DRG	G_q, $G_{12/13}$	AC↑, $[Ca^{2+}]_i$↑	Stress fibre formation, neurite retraction	[11]
GPR3	Const. activity, S1P	Rodent oocytes, diverse EC and VSMC	G_s, $G_{i/o}$	AC↑, $[Ca^{2+}]_i$↑	Meiotic arrest of rodent oocytes	[9,60–64]
GPR6	Const. activity, S1P	Mouse brain, diverse EC and VSMC	G_s, $G_{i/o}$	AC↑, $[Ca^{2+}]_i$↑		
GPR12	Const. activity, S1P or SPC	Mouse brain, diverse EC and VSMC, rodent oocytes	G_s, $G_{i/o}$	AC↑, $[Ca^{2+}]_i$↑	Increase in synaptic contacts in rat cortical neurons, meiotic arrest of rodent oocytes	
OGR1	SPC, proton	Widely; e.g. bone	$G_{i/o}$, other	L: $[Ca^{2+}]_i$↑, ERK↑ P: IP↑, AC↑	Inhibition of proliferation, osteoclastogenesis	Reviews: [13,14]
GPR4	SPC, LPC, proton	Widely; e.g., endothelial cells; overexpressed in cancer cells	$G_{i/o}$, other	L: $[Ca^{2+}]_i$↑, ERK↑ C: ERK↓ P: AC↑	Migration, angiogenesis, impairment of endothelial barrier function	Articles: [80,82–89,91,227]
G2A	LPC, SPC, proton (?)	Lymphoid tissues, lymphocytes, macrophages	$G_{i/o}$, G_q, G_s, G_{13}	L: $[Ca^{2+}]_i$↑, ERK↑ C: IP↑, AC↑, Rho↑, stress fibre formation ↑ P: IP↑ (?)	Migration, apoptosis, suppression of autoimmunity	
TDAG8	Psychosine, gluco-psychosine, lysosulfatide, proton	Lymphoid tissues, T cells; overexpressed in cancer cells	$G_{i/o}$, other	L: $[Ca^{2+}]_i$↑, AC↓ P: AC↑, Rho↑, stress fibre formation ↑	Formation of multinuclear cells (?), apoptosis	

Abbreviations used in the table: AC, adenylyl cyclase; C, constitutive signalling by transfected receptors; const. activity, constitutive activity; COX, cyclooxygenase; IP, inositol phosphates; L, activities induced by lipid ligand; ODC, oligodendrocytes; OPC, oligodendrocyte precursors; P, activities induced by protons; SMC, smooth muscle cells. For further references, see text.

Interestingly, $S1P_2$ strongly activates Rho and inhibits Rac, thereby promoting cellular stress fibre formation and inhibiting migration of many cell types, including melanoma cells [32,33]. Similar to $S1P_2$, $S1P_3$ is coupled to G_i, G_q and $G_{12/13}$ proteins. This receptor strongly activates PLC and induces $[Ca^{2+}]_i$ increases, ERK, Rho and Rac activation. $S1P_3$, via $[Ca^{2+}]_i$ increases and Akt stimulation, induces NO synthase activation and vasorelaxation [34]. $S1P_3$ furthermore via G_i activates G-protein-regulated inward rectifier potassium channels, thereby slowing the heart rate [35–37]. Looking at signalling by S1P-GPCRs in general, there appears to be redundancy as well as functional antagonism (see discussion in [29]). Redundancy probably prevents major symptoms in mice in which either $S1P_2$ or $S1P_3$ has been deleted [38,39]. Functional antagonism is observed for example with $S1P_1$ and $S1P_2$, which strongly stimulate or inhibit cell migration, respectively (reviewed in [16]), or with $S1P_5$ and the other S1P-GPCRs, because $S1P_5$ uniquely inhibits ERK and cell proliferation.

The LPA-GPCRs, LPA_1, LPA_2 and LPA_3, are similarly able to couple via G_i, G_q and $G_{12/13}$ proteins to many signalling pathways [17]. These receptors stimulate PLC and induce $[Ca^{2+}]_i$ increases, activate Ras and ERK and stimulate proliferation, and via Akt promote cell survival. LPA-GPCRs have furthermore a strong impact on cytoskeleton and migration by coupling to both Rho and Rac. In heterologous expression systems, GPCRs might be able to couple to pathways which are not preferred by endogenous receptors. Considering knockout approaches, it was suggested that the main, but not only, signalling pathways of endogenous LPA-GPCR might be the following: LPA_1, G_i-dependent signalling; LPA_2, G_{12}/Rho-regulated cytoskeleton rearrangements and cell rounding; LPA_3, G_q-mediated PLC activation and $[Ca^{2+}]_i$ increases [27]. Further studies are needed for assigning endogenous LPA receptor subtypes to specific signal transduction pathways.

Much less is known about the $S1P_4$ and $S1P_5$ receptors. $S1P_4$ was isolated from dendritic cells and is expressed in lymphoid and haematopoietic cells and tissues [40]. This receptor differs from the other S1P-GPCRs of the EDG family with respect to its binding pocket [41]. This is reflected by a higher affinity for phyto-S1P and dihydro-S1P than S1P [42,43]. $S1P_4$ couples to G_i and $G_{12/13}$ proteins, but not to G_q and $G_{15/16}$, at least as tested with a GTP photoaffinity label [44]. $S1P_4$ via G_i stimulated ERK, PLC and $[Ca^{2+}]_i$ increases [45,46]. It activated Rho and rearranged the cytoskeleton in one study [44], while it did not activate Rho or Rac in another study, but stimulated Cdc42 and cell migration [47], both studies in transfected CHO cells. These inconsistencies could probably be due to problems in trafficking of overexpressed $S1P_4$ to the plasma membrane [47]. When transfected into T cell lines devoid of endogenous S1P-GPCRs, $S1P_4$ had no influence on migration and chemotaxis, but inhibited cell growth and secretion of proinflammatory cytokines [48]. Interestingly, FTY720, which caused internalization of $S1P_1$, did not internalize $S1P_4$ [49], although phosphorylated FTY720 activates both receptors [50,51].

$S1P_5$ was originally cloned from PC12 cells, where its mRNA was downregulated by nerve growth factor (NGF) [52]. $S1P_5$ expressed in CHO cells activated $G\alpha_{i/o}$ and $G\alpha_{12}$ proteins, but not $G\alpha_s$ or $G\alpha_q$, as shown by GTPγS binding to immunoprecipitated G-proteins [53]. $S1P_5$ furthermore mediated inhibition of adenylyl cyclase [53,54], and interestingly had a negative impact on ERK and cell growth, whereas it activated JNK [53]. Neither activation of PLC nor increases in $[Ca^{2+}]_i$ were observed with $S1P_5$ [53]. Overexpressed $S1P_5$ inhibited ERK and induced cell rounding even in the absence of exogenous S1P, indicating constitutive activity [55]. In situ hybridization studies and histochemical analysis with a specific antibody revealed its abundant expression in white matter tracts of rodent brain [54,56,57]. The $S1P_5$ antibody and mRNA probes specifically stained all developmental stages of oligodendrocytes [56,57]. In differentiated oligodendrocytes which predominantly express the two lysophospholipid receptors, LPA_1 and $S1P_5$, both LPA and S1P induced activation of ERK in a pertussis toxin (PTX)-insensitive manner [58], indicating that signalling by endogenous $S1P_5$ in its natural environment might be different from that observed with the transfected receptor. Furthermore, $S1P_5$ via Rho kinase mediated process retraction in pre-oligodendrocytes but not in mature oligodendrocytes, while $S1P_5$ via G_i and Akt enhanced survival of mature oligodendrocytes but not of pre-oligodendrocytes [57]. These results suggest that the functional role of the receptor is dependent on the cellular differentiation status, and that $S1P_5$ plays a role in brain myelinization. However, mice in which $S1P_5$ was genetically deleted had no apparent behavioural deficits, and their neuropathological examination did not reveal an apparent myelin deficiency [57].

2.2. GPR3, GPR6, GPR12

These GPCRs form a cluster with high similarity to each other and ~40% similarity to EDG, cannabinoid and melatonin receptors [59]. Heterologous expression of GPR3, GPR6 and GPR12 led to strong activation of adenylyl cyclase in the absence of exogenous ligand. However, members of this GPCR cluster were also shown to respond to S1P and/or SPC [9,60–62]. Transfection of human GPR3, GPR6 and GPR12 enhanced S1P-stimulated $[Ca^{2+}]_i$ increases in HEK-293 cells; these responses were caused by nanomolar S1P and fully inhibited by PTX. S1P furthermore induced internalization of GPR6 [9]. Other authors expressed mouse GPR12 with a signal peptide for improved membrane insertion, and observed that it enhanced the background $[Ca^{2+}]_i$ increases induced by SPC with an EC_{50} of 66 nM, while S1P was clearly less potent [60]. Mouse GPR6, however, responded preferentially to S1P like the human receptor [61]. Furthermore, both GPR6 and GPR12 via G_i mediated activation of G-protein-gated inwardly rectifying K^+ currents in transfected Xenopus oocytes by S1P and SPC, respectively, and GPR6 mediated ERK activation by S1P [60,61]. GPR6 and GPR12 are strongly expressed in mouse brain. GPR12 mRNA transcripts were detected in all areas of the developing mouse central nervous system, especially the cerebral cortex, and expression of this receptor was upregulated at the stage in which neurons start to migrate and differentiate. Other than LPA_1, GPR12 is apparently not expressed in the ventricular zone where proliferation of neuronal precursors takes place. In adult mouse brain, GPR12 was predominantly detected in the forebrain region where major constituents of the limbic system, e.g., hippocampus, were labelled [60]. A functional analysis of endogenously expressed GPR12 was performed in the hippocampal cell line HT22 and in embryonic cerebral cortical neurons, both of which do not express the high-affinity SPC-GPCRs, GPR4 and OGR1. SPC stimulated proliferation and cell clustering of the hippocampal cells, and increased synaptic contacts and synaptophysin expression in the embryonic cortical neurons [60].

There are also reports showing that GPR3, GPR6 and GPR12 play important roles outside the nervous system. For example, all three receptors are abundantly expressed in diverse endothelial and vascular smooth muscle cells, and fluid shear

stress upregulated GPR3 and GPR12 proteins in human umbilical vein endothelial cells [62]. Interestingly, GPR3 and GPR12 are highly expressed in mouse oocytes, but not in the surrounding somatic cells, and these receptors mediate a signal that maintains the oocytes in meiotic arrest [63,64]. Mammalian oocytes stay in prophase arrest until ovulation is triggered by luteotropin. This depends on an unknown signal from the surrounding somatic cells, since removal of oocytes from antral follicles resumes meiosis. Furthermore, various lines of evidence show that high cAMP levels are essential for meiotic arrest (for review, see [65]). Spontaneous oocyte maturation in vitro was inhibited by injection of GPR3 and GPR12 mRNA, while antisense oligonucleotides caused meiotic resumption [64]. Furthermore, oocytes from GPR3 knockout mice spontaneously resume meiosis within antral follicles in vivo, independently of an increase in luteotropin, and this phenotype was reversed by injection of GPR3 mRNA into the oocytes [63]. Unexpectedly, GPR3-deficient mice were fertile, but they displayed progressive reduction in litter size, indicating premature ovarian failure due to early oocyte aging [66]. These data support previous findings, namely that oocyte loss in adult female mice induced by radiation was completely prevented by in vivo therapy with S1P [67,68]. Finally, a role for lipid ligands in meiotic arrest was shown by demonstrating that in vitro maturation of oocytes was delayed by SPC and S1P [64].

2.3. LPA_4 and LPA_5

In 2003, a novel LPA-GPCR, $P2Y_9$/GPR23, now named LPA_4, was detected that was structurally distant from LPA receptors of the EDG family [10]. Very recently, it was demonstrated that the orphan GPR92 is activated by LPA, and it was named LPA_5 [11]. These two receptors, LPA_4 and LPA_5, have ~35% amino acid identity with each other and are thus more related to each other than to the LPA-EDG receptors. LPA_4 shares only 20-24% amino acids with LPA_1, LPA_2 and LPA_3, which share 50–57% of their amino acids [6,10]. When transfected into no- or low-background cells, LPA_4 preferentially bound 1-oleoyl-LPA with a K_D of ~50 nM, and this LPA species also preferentially activated a reporter gene in LPA_4 expressing cells, while LPA species with saturated or shorter acyl chains were less affine and active, and other lysophospholipids did not compete with LPA binding. Functionally, the receptor mediated increases in cAMP and $[Ca^{2+}]_i$ [10]. The GPR92/LPA_5 receptor mediated stress fibre formation in RH7777 rat hepatoma cells, neurite retraction via $G\alpha_{12}$, $G\alpha_{13}$ and Rho kinase in B193 neuroblastoma cells, and furthermore stimulated increases in cAMP and, via $G\alpha_q$, elevated $[Ca^{2+}]_i$ [11]. Although both LPA_4 and LPA_5 elevated cAMP levels, their coupling to G_s proteins remains to be proven. LPA_5 was internalized by LPA but not by S1P, however, its precise ligand specificity remains to be determined. Low levels of mRNA transcripts of both LPA_4 and LPA_5 are abundantly expressed; high levels of LPA_4 were found in ovary, while LPA_5 was strongly expressed in small intestine, spleen, dorsal root ganglion cells and embryonic stem cells [10,11]. It is tempting to speculate that these two receptors allow the lysophospholipid LPA to couple to G_s pathways which are not among the preferred signalling pathways of LPA-EDG receptors.

2.4. Lysophospholipid- and proton-regulated GPCRs

Many cells respond in diverse ways to SPC, LPC, psychosine and glucopsychosine, and GPCR-dependent as well as GPCR-independent signalling of these lipids has been suggested. OGR1, GPR4, G2A and TDAG8 form a cluster of homologous GPCRs that are candidate high-affinity receptors for SPC (OGR1, GPR4, G2A), LPC (GPR4, G2A) and psychosine (TDAG8). Shortly after their first description as lipid receptors [69–72], crucial binding data could apparently not be reproduced, thus leading to the retraction of papers concerning GPR4 and G2A [73,74]. A major proof for SPC as a ligand at OGR1 and GPR4 had been the observations that transfection of these receptors enabled SPC to induce $[Ca^{2+}]_i$ increases in otherwise non-responsive cells, and that SPC caused internalization of plasma membrane localized ORG1- and GPR4-GFP fusion proteins [69,70]. However, other authors did not observe internalization of GPR4 upon stimulation with SPC, although internalization of OGR1 by SPC was confirmed [75]. Transfected GPR4 was furthermore shown to be constitutively active, inhibiting ERK in a ligand-independent manner [75]. Interestingly, in 2003, OGR1 and GPR4 were described as proton-sensing receptors [76]. Expression of these receptors rendered otherwise non-responsive cells sensitive to acidification, with protons stimulating inositol phosphate production and cAMP accumulation in OGR1- and GPR4-transfected cells, respectively. The receptors were inactive or only slightly active at pH 7.6 to 7.8, and strongly activated at pH 7.2 to 7.0. By homology modelling with rhodopsin and mutational analysis, five histidine residues were identified that were conserved in ORG1 and GPR4 and required for proton sensing [76]. It was suggested that hydrogen bonding between histidines stabilizes the receptors in the inactive state, while protonation elicits the conformational change into the active state [76]. Interestingly, proton-induced inositol phosphate accumulation in OGR1-expressing cells was insensitive to PTX, while $[Ca^{2+}]_i$ increases in response to SPC were PTX-sensitive [69], suggesting that differential G-protein coupling could be caused by the two activation mechanisms. Later, proton sensing was not only confirmed for OGR1 [77], but also shown for G2A [78] and TDAG8 [79]. Acidification induced internalization of TDAG8 and stimulated Rho and actin rearrangement [80]. Comparing the proton sensitivity of the receptors of the OGR1 cluster, it appeared that G2A was not as sensitive as OGR1, GPR4, and TDAG8 [81], which could be due to lack of critical histidines [13,14]. Most importantly, the putative lipid agonists failed to activate the GPCRs of the OGR1 cluster in these studies. In contrast, proton-induced signalling was inhibited by SPC [77], LPC [78] and psychosine [79]. For this effect, at least 1 μM of LPC was required to inhibit proton sensing of G2A, whereas 10 μM were required for clear inhibition at OGR1 and TDAG8. Psychosine did not activate TDAG8 or one of the other receptors, but 10 μM psychosine

caused a rightward shift of pH-dependent signalling effects of TDAG8, OGR1 and GPR4 [79]. To explain the diverse effects of protons and lipids at GPCRs of the ORG1 cluster, a model was proposed in which the receptors have two regulatory sites, one for protons and the other for lipids. The lipids were suggested to interact with both sites, as agonist and antagonist, respectively [13,14]. However, this model does not explain why the putative lipid agonists were sometimes able and sometimes not to activate GPCRs of the OGR1 cluster. Furthermore, it remains unclear whether the inhibitory action of lipids on proton signalling was mediated at all by the GPCRs of question.

The reported controversy could be due at least in part to artificial coupling of overexpressed receptors, incomplete plasma membrane insertion of transfected receptors, GPCR heterodimerization, or artificial cellular backgrounds (see also discussion in [59]). These difficulties are circumvented when endogenously expressed receptors are studied in their natural environment. Such studies indeed suggest a role for lipids in signalling by GPCRs of the OGR1 cluster. For example, GPR4, but not OGR1, G2A or TDAG8, was found to be expressed in diverse endothelial cells, and siRNA knockdown of GPR4 abrogated endothelial tube formation by SPC, but not by S1P or vascular endothelial growth factor [82]. GPR4 was also required for SPC-stimulated migration of endothelial cells. Interestingly, while GPR4-transfected HEK-293 cells responded to pH 7.0 with increase in cAMP, this effect was not observed in endothelial cells, even if GPR4 was additionally transfected [82]. Another group showed that siRNA knockdown of GPR4 in endothelial cells inhibited LPC-stimulated decrease in monolayer resistance, while pH < 7.4 did not alter baseline or LPC-stimulated resistances [83]. On the other hand, OGR1 and GPR4 were found to be expressed in human aortic smooth muscle cells, in which acidic pH induced inositol phosphate production, cAMP formation and prostaglandin I_2 secretion. These effects were inhibited by OGR1-siRNA, but not by GPR4-siRNA [84]. While these data demonstrate a role for endogenous OGR1 in proton sensing, it has to be noted that at least pH 6.8 was required for clear effects, while maximal stimulation required pH 6.4–6.0. The pH dependence of inositol phosphate production by endogenous OGR1 in smooth muscle cells was highly different from that reported by Ludwig et al., which was biphasic with a maximum at pH 7.0. The reported acidic conditions that were required raise the question whether physiological proton sensing might take place by internalized receptors in endosomes, especially in the light of confocal data showing a remarkable amount of receptors in intracellular compartments [76,81,85]. Accordingly, G2A overexpressed in T cells or in Swiss 3T3 cells was strongly retained in endosomes, and LPC specifically induced transport of this GPCR to the plasma membrane [85]. Furthermore, the recycling blocker, monensin, blocked LPC-stimulated G2A translocation, migration and ERK activation, raising the questions whether LPC acted as an antagonist, stabilizing the plasma membrane localization, or as an agonist, signalling through membrane G2A, and what was the signal for translocation in the latter case.

Another study with endogenous OGR1 demonstrated that this receptor was strongly induced during differentiation of bone marrow mononuclear cells into osteoclasts, and that inhibition of OGR1 by antibody or siRNA attenuated osteoclastogenesis [86]. A possible role of auto- or paracrine lysophospholipids, however, was not investigated. Endogenous G2A receptor was studied in a T cell line and in mouse peritoneal macrophages, and deletion of G2A impaired migration towards LPC [87,88]. TDAG8 (T cell death-associated gene-8) was upregulated in glucocorticoid-treated lymphoma cell lines and primary thymocytes, and psychosine and glucopsychosine, but not S1P, SPC, LPA or LPC, enhanced glucocorticoid-induced apoptosis in a manner dependent on TDAG8 expression [89]. Other authors found that deletion of TDAG8 fully abrogated proton-induced cAMP formation in thymocytes, while deletion of G2A had no effect [81].

Taken together, the present data support the hypothesis that GPCRs of the OGR1 cluster can be regulated by both specific lipid agonists and protons. However, the precise molecular mechanisms, by which dual regulation of these receptors takes place, remain to be determined. G2A and TDAG8 appear to play a functional role in migration and apoptosis of immune cells, while OGR1 and GPR4 are wider expressed and for example contribute to osteoclastogenesis and angiogenesis, respectively. Mice in which G2A was genetically deleted were described in 2001, they suffered from a late-onset autoimmune syndrome that was attributed to insufficient T cell apoptosis [90]. Recently, it was reported that TDAG8 knockout mice are normal in appearance, size and mating, and their immune functions, including glucocorticoid-induced apoptosis of thymocytes, were not obviously impaired [91]. It was speculated that TDAG8 could be substituted by G2A in immune cells of TDAG8 knockout mice.

2.5. Interaction of LPA with peroxisome proliferator-activated receptor-γ

GPCR independent, intracellular actions have been suggested primarily for S1P [92]. However, in 2003, an intracellular target for LPA was identified, which was the nuclear transcription factor, peroxisome proliferator-activated receptor-γ (PPARγ) [93]. PPARγ controls transcription of genes that are involved in glucose and fatty acid metabolism, adipocyte differentiation and inflammation processes in the vasculature, and is activated by the antidiabetic thiazolidinediones [94,95]. As endogenous ligands, anionic fatty acids and their oxidised derivatives have been described. LPA, but not phosphatidic acid, competed for binding of the thiazolidinedione, rosiglitazone, to PPARγ, and stimulated expression of PPARγ-controlled genes [93]. Furthermore, LPA-induced progressive formation of neointima in a rat carotid artery model was inhibited by a PPARγ antagonist and mimicked by rosiglitazone [96]. The structure–activity relationship for neointima formation by LPA analogues in vivo was identical to that of PPARγ activation in vitro and differed from that described for LPA-GPCRs. LPA 20:4 upregulated the CD36 scavenger receptor and caused dedifferentiation of cultured vascular smooth muscle cells that was prevented by PPARγ

antagonist [96]. Extracellularly applied LPA was apparently able to enter the cells and reach the intracellular receptor. The interaction of LPA with PPARγ was furthermore analysed in preadipocytes, which endogenously express high levels of that transcription factor [97]. Interestingly, LPA did not increase transcription of two PPARγ-sensitive genes in a mouse preadipocyte cell line, but, in contrast, decreased PPARγ2 expression, inhibited the action of rosiglitazone on gene transcription, and reduced triglyceride accumulation. These anti-adipogenic actions were not observed in cells from LPA_1 knockout mice, indicating that LPA_1 can mediate down-regulation of PPARγ2 [97]. These obvious differences to the previous studies were ascribed to high expression of ecto-lipid phosphate phosphatases activity in preadipocytes, preventing LPA to enter the cells in sufficient concentrations for PPARγ activation [98].

3. Regulation of lysophospholipid availability at lysophospholipid receptors

3.1. S1P metabolism: kinases, phosphatases, lyase

The enzymes that generate and degrade S1P are evolutionary highly conserved among eukaryotes from yeast and plant to mammals [18,99]. S1P is formed by phosphorylation of sphingosine by sphingosine kinases, and dephosphorylated

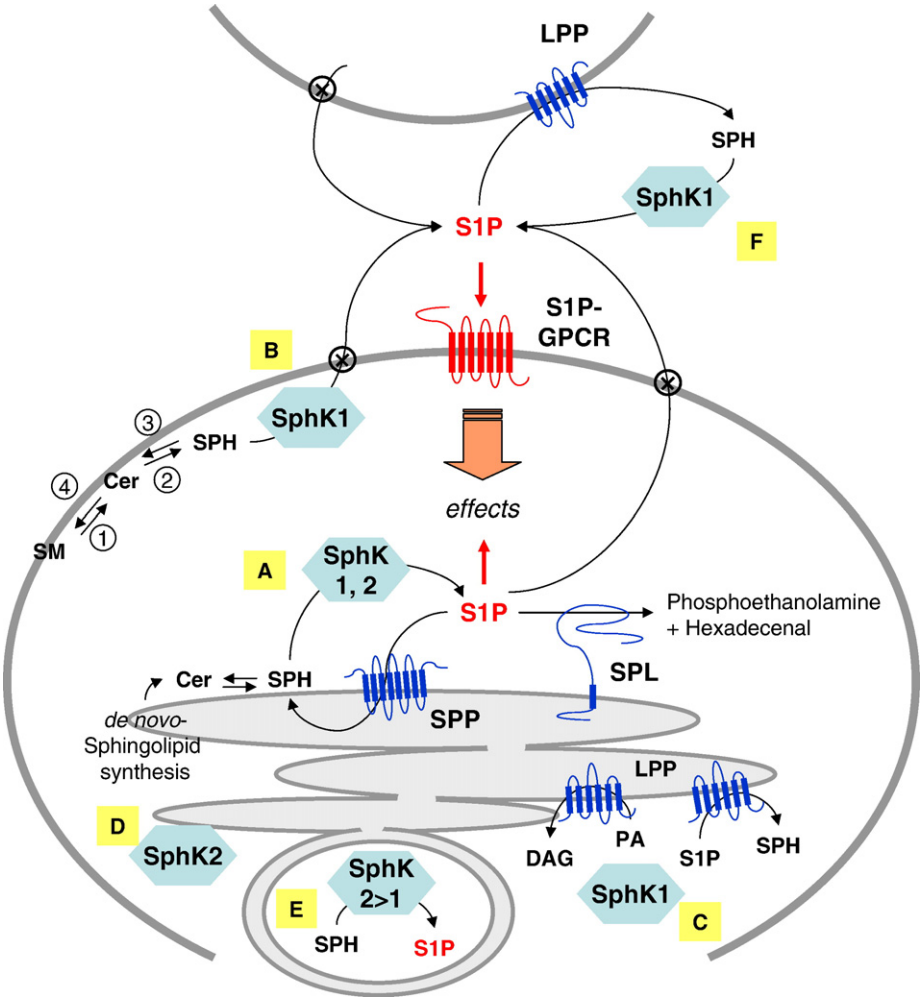

Fig. 1. Regulation of S1P availability at G-protein-coupled S1P receptors by localized generation and metabolism of S1P. S1P is formed from sphingosine (SPH) by sphingosine kinases (SphK) and either dephosphorylated by lipid phosphate phosphatases (LPP) and S1P phosphatases (SPP), or irreversibly cleaved by S1P lyase (SPL). SphK1 is a cytosolic enzyme (A) that, upon cellular stimulation, can translocate to the plasma membrane (B) or to intracellular sites such as sites of phosphatidic acid (PA) production (C) [134, 228]. SphK2 is also cytosolic and was observed at the endoplasmic reticulum in serum-depleted cells (D) [124], but so far has not been found at the plasma membrane. Predominantly SphK2, but also SphK1, can be found in the nucleus (E). S1P, depending on the localization where it is generated, can act on so far unknown intracellular target sites, or it can be excreted, probably by one or more transport mechanisms (⊗) [157]. Extracellular S1P acting on S1P-GPCRs can thus be derived from auto- or paracrine secretion. In addition, it may be produced by extracellular SphK1 (F) [159]. Termination of extra- and intracellular S1P signals is caused by LPPs, SPPs and S1P lyase. LPPs are plasma membrane-bound enzymes with their catalytic activity directed to the extracellular space, regulating extracellular levels of LPA and S1P. LPPs, predominantly LPP2 and LPP3, can also be found at the endoplasmic reticulum or other intracellular membranes [134]. SPPs and S1P lyase are endoplasmic reticulum proteins. The catalytic site of S1P lyase is directed towards the cytosol [141], while that of SPPs has been predicted to be directed towards the lumen of endoplasmic reticulum. Both SPPs and S1P lyase contribute to regulation of extracellular S1P levels [129,132,161]. ①–④ Enzymes of the sphingomyelin pathway [102]: ① sphingomyelinase, ② ceramidase, ③ ceramide synthase, ④ sphingomyelin synthase. Further abbreviations used in the figure: SM, sphingomyelin; Cer, ceramide; DAG, diacylglycerol. For additional references, see text.

back to sphingosine by non-specific LPPs or specific S1P phosphatases (SPPs) (Fig. 1). S1P and sphingosine are in equilibrium with ceramide, which is a membrane-bound intracellular mediator with functional roles mostly opposite to S1P (Fig. 1). An irreversible cleavage of S1P is catalysed by S1P lyase. For review on sphingolipid metabolism in general and its subcellular localization, see [18,100–102].

Two mammalian isoforms of sphingosine kinase, SphK1 and SphK2, with molecular weights of 43 and 65 kDa, respectively, and a number of alternatively spliced isoforms of SphK1 and SphK2 that differ at their N-termini have been identified [103–108] (for review, see [109–111]). The two sphingosine kinase isoenzymes have distinct kinetic properties and are differentially expressed during development as well as in adult tissues, which indicates distinct biological functions. Indeed, SphK1 promotes cell growth in many cell types, is upregulated in tumour cells, protects from apoptosis, and has features of an oncogene [112–121], whereas SphK2 mediates apoptosis [108,122–124]. In contrast, mice deficient in either SphK1 or SphK2 are viable and fertile, while double knockout mice died during embryogenesis and had no measurable tissue levels of S1P, suggesting that the two sphingosine kinase isoenzymes can substitute for each other [125–128].

Particularly SphK1 contributes to extracellular S1P acting on S1P-GPCRs, and therefore regulation of this enzyme will be discussed in greater detail below.

The enzymes that dephosphorylate S1P and LPA belong to a superfamily of lipid phosphatases/phosphotransferases with a common structural motif and specialized functions, for review see [129–132]. LPP isoenzymes non-specifically dephosphorylate phosphatidic acid, LPA, ceramide-1-phosphate and S1P to generate diacylglycerol, monoacylglycerol, ceramide and sphingosine, respectively. Three mammalian LPP isoforms have been identified, termed LPP1, LPP2 and LPP3. In addition, there is a family of four LPP-related proteins, termed LPR or plasticity-related genes (PRG), the functions of which are not entirely clear. LPP1 and LPP3 are widely expressed while expression of LPP2 is more restricted [132]. LPPs are integral membrane proteins with six predicted transmembrane domains, localized to the plasma membrane, endoplasmic reticulum and other endomembranes [132]. Their catalytic centres face the extracellular side of the plasma membrane or the luminal side of organelles. Two functions have been attributed to these enzymes: (1) regulation of extracellular levels of lysophospholipids such as LPA and S1P by their ecto-phosphatase activity, and (2) degradation of intracellular phospholipid second messengers, e.g., phosphatidic acid, and thereby intracellular signal termination [132–134]. The two known SPPs are structurally related to LPPs, but hydropathy analysis suggests the presence of 8–10 transmembrane domains rather than 6 [135–138]. These enzymes have predicted molecular masses of 46 and 49 kDa, respectively, and broad and partially overlapping expression patterns [136–138]. Interestingly, SPPs are inhibited by detergents such as Triton X-100 and show a marked preference for substrates attached to protein carriers. Both SPP1 and SPP2 specifically dephosphorylate S1P and localize to the endoplasmic reticulum, their catalytic site is predicted to face the luminal site of the endoplasmic reticulum [136–138]. Cellular depletion of SPP1 by siRNA caused accumulation of S1P within the cells and also in the medium, indicating that SPP1 plays a role in regulating extracellular S1P levels [137].

S1P lyase is a pyridoxal-5′-phosphate-dependent enzyme of 63 kDa with one transmembrane domain close to its N-terminus [139,140]. The enzyme is located at the endoplasmic reticulum, and the pyridoxal-5′-phosphate binding domain as well as the catalytic site are exposed to the cytosol [141]. Expression of S1P lyase mRNA transcripts was widespread in mouse tissues, but did not always fully parallel enzyme activity. S1P lyase expression and activity was very low in mouse brain and, in agreement with previous reports, absent in platelets [141]. Obviously, S1P lyase contributes to the balance between proapoptotic ceramide and antiapoptotic S1P, since overexpression of the enzyme enhanced ceramide levels and caused ceramide-dependent apoptosis [142]. Accordingly, *Drosophila* lacking S1P lyase displayed increased apoptosis in developing embryos and diminished egg-laying [143]. Most interestingly, these flies had specific abnormalities in dorsal longitudinal flight muscles and a severe flight defect, which reveals the importance of S1P lyase in metazoan development [143]. Reports about the phenotype of S1P lyase deficient mice are awaited for the near future. Recent data demonstrate that S1P lyase influences extracellular S1P levels, contributing to the formation of S1P tissue gradients; this is reported in greater detail below.

3.2. Role of SphK1 in generating extracellular S1P: signalling inside-out

SphK activity is regulated both transcriptionally and post-transcriptionally by a large number of agonists, among them diverse GPCRs (e.g. muscarinic, formyl peptide, lysophospholipid, bradykinin), receptor tyrosine kinases (PDGF, EGF), cytokine and antigen receptors (for review, see [109,110,144]). However, SphK1 has a substantial basal activity, and post-transcriptional activation often leads to only moderate increases in its enzymatic activity (discussed in [111]). It is becoming increasingly clear that translocation to distinct subcellular regions is an important mechanism by which SphK1 is regulated [110,111,124] (Fig. 1). Artificial targeting of SphK1 to the endoplasmic reticulum or to the plasma membrane had a major impact on its function [124,145]. Phorbol ester, tumour necrosis factor-α, NGF or activation of muscarinic acetylcholine receptors induced a rapid translocation of SphK1 from the cytosol to the plasma membrane [146–149]. Several reports have shown that signalling via SphK1 can lead to S1P export and activation of S1P-GPCRs. For example, SphK1 overexpression in NIH 3T3 fibroblasts induced formation of stress fibres and focal adhesions, inhibited migration, stimulated proliferation and protected from apoptosis, and thus mimicked actions of extracellular S1P [150]. Accordingly, stress fibre formation induced by SphK1 overexpression required signalling via $G_{12/13}$, while proliferation and survival could not be

attributed to G-protein signalling [150]. Furthermore, NGF via its TrkA receptor induced translocation of SphK1 to the plasma membrane and internalization of $S1P_1$ and $S1P_5$ [149]. Knockdown of SphK1 by siRNA inhibited neurite extension induced by NGF in dorsal root ganglion cells, while extracellular S1P accelerated neurite extension by NGF in these cells, demonstrating the importance of S1P-GPCR cross-activation in this process [149]. SphK1 is known to be crucial for antigen receptor signalling in mast cells [151], and it was shown that the $S1P_2$ receptor was required for degranulation [152]. Furthermore, platelet-derived growth factor (PDGF) induced SphK1 translocation to the plasma membrane at the leading edge of migrating cells, and $S1P_1$ was required for PDGF-stimulated migration [153]. Although there are other examples for S1P signalling inside-out, there are also data demonstrating an interaction of $S1P_1$ and PDGF receptors independently of autocrine S1P secretion. Even $S1P_1$ mutants defective in S1P binding enhanced PDGF receptor signalling [154]. This cross-talk is considered to be based on a receptor signalling platform and sharing of G-protein subunits [155,156]. The mechanism by which S1P can be exported remains largely unknown, although the involvement of ABC transporters has been suggested at least in platelets [157]. On the other hand, SphK1 can also be found extracellularly, it was constitutively secreted by vascular endothelial cells and found in mouse and human plasma [158,159]. It should be noted that SphK1 in addition to signalling inside-out also plays an intracellular role, like SphK2, since it can be translocated to intracellular sites such as sites of phosphatidic acid production [134], has a nuclear export sequence that contributes to its cytosolic localization [160], and several of its effects are not imitated by exogenous S1P (e.g., [150]), see Fig. 1.

3.3. Role of S1P lyase in controlling extracellular S1P gradients

Recently, it was shown that S1P lyase plays an important role in maintaining a steep S1P concentration gradient between blood and tissues, which is required for lymphocyte trafficking [161] (commentary in [162]). The food colorant, 2-acetyl-4-tetrahydroxybutylimidazole (THI), known for its immunosuppressive action, was shown to inhibit S1P lyase. In mice treated with THI, S1P levels in thymus, spleen and lymph nodes were greatly enhanced, while those in plasma, which under control conditions were higher than those in lymphoid tissues, were not much changed [161]. As a consequence, the $S1P_1$ receptor was internalized by interstitial fluid extracts from lymphoid tissue or lymph of THI-treated mice. In addition, $S1P_1$ was downregulated by plasma, but not by lymphoid tissue extracts of control animals [161]. The $S1P_1$ receptor mediates lymphocyte egress and is internalized by the novel immunosuppressant, FTY720 (see below). S1P lyase inhibition by THI as well as siRNA downregulation of S1P lyase imitated this phenotype. A model has been developed in which S1P lyase contributes to the usually low S1P levels in lymphoid tissue, while the higher plasma levels of S1P act as a chemoattractant for emigration of lymphocytes [162]. Interestingly, FTY720, besides being a substrate for sphingosine kinases, inhibited S1P lyase in vitro and in vivo and slightly increased tissue S1P levels [163]. It was suggested that this activity of FTY720, besides downregulation of $S1P_1$ by phosphorylated FTY720, may contribute to its immunosuppressive action [163].

3.4. LPA metabolism: diverse pathways

There are several pathways that contribute to production of LPA. For generation of bioactive LPA acting on GPCR, three pathways, catalysed by extracellular enzymes, appear to play a role: (1) deacylation of phosphatidic acid by PLA_1 and PLA_2, (2) cleavage of lysophospholipids by lyso-PLD, and (3) mild oxidation of low-density lipoproteins (for comprehensive review, see [164,165]). Secretory type-II PLA_2 and phosphatidic acid-selective PLA_1 cleave surface-exposed phosphatidic acid, which for example occurs in microvesicles shed from activated inflammatory cells. The main source of extracellular LPA, however, appears to be lyso-PLD-catalysed cleavage of lysophospholipids such as LPC [166,167]. The lyso-PLD that generates LPA has recently been found to be identical with the cell motility-stimulating factor, autotaxin [168,169] (see below). Another possibility for LPA production is phosphorylation of monoacylglycerol. Recently, an acylglycerol kinase was described that had a diacylglycerol kinase catalytic domain but differed from the known diacylglycerol kinases, and phosphorylated monoacylglycerol (preferentially 1-oleoyl-glycerol, but also 2-arachidonyl-glycerol, an endocannabinoid) and diacylglycerol to form LPA and phosphatidic acid, respectively [170]. This enzyme was located at the mitochondria, and its overexpression promoted formation and secretion of LPA. However, data from autotaxin deficient mice illustrate the prevailing dominance of this enzyme in production of bioactive LPA, and therefore autotaxin is discussed in more detail below. Degradation of extracellular LPA can be attributed to the ectophosphatase activity of plasma membrane LPPs or acylation by LPA acyl transferases [129–132] (Fig. 2).

3.5. Production of extracellular LPA by autotaxin

Autotaxin was originally isolated as an autocrine chemotactic factor from melanoma cells and was considered to be an ecto-nucleotide pyrophosphatase/phosphodiesterase (reviewed in [165]). Autotaxin occurs as a membrane-bound protein of ~125 kDa with a single transmembrane domain close to the cytosolic N-terminus, and as a soluble enzyme that is generated from the former by proteolytic processing and secreted from cells (Fig. 2). In 2002, it was demonstrated that autotaxin was the long-searched lyso-PLD in plasma and serum that cleaved LPC to form LPA [168,169]. The decisive contribution of autotaxin to LPA production in plasma was recently demonstrated by mice deficient in autotaxin [166,167]. Because homozygous autotaxin-deficient mice died early during embryogenesis, plasma levels of LPA were determined in heterozygous mice, and found to be half as high as in control mice [166,167]. In contrast, plasma S1P levels were not affected, demonstrating that the in vitro observed ability of autotaxin to

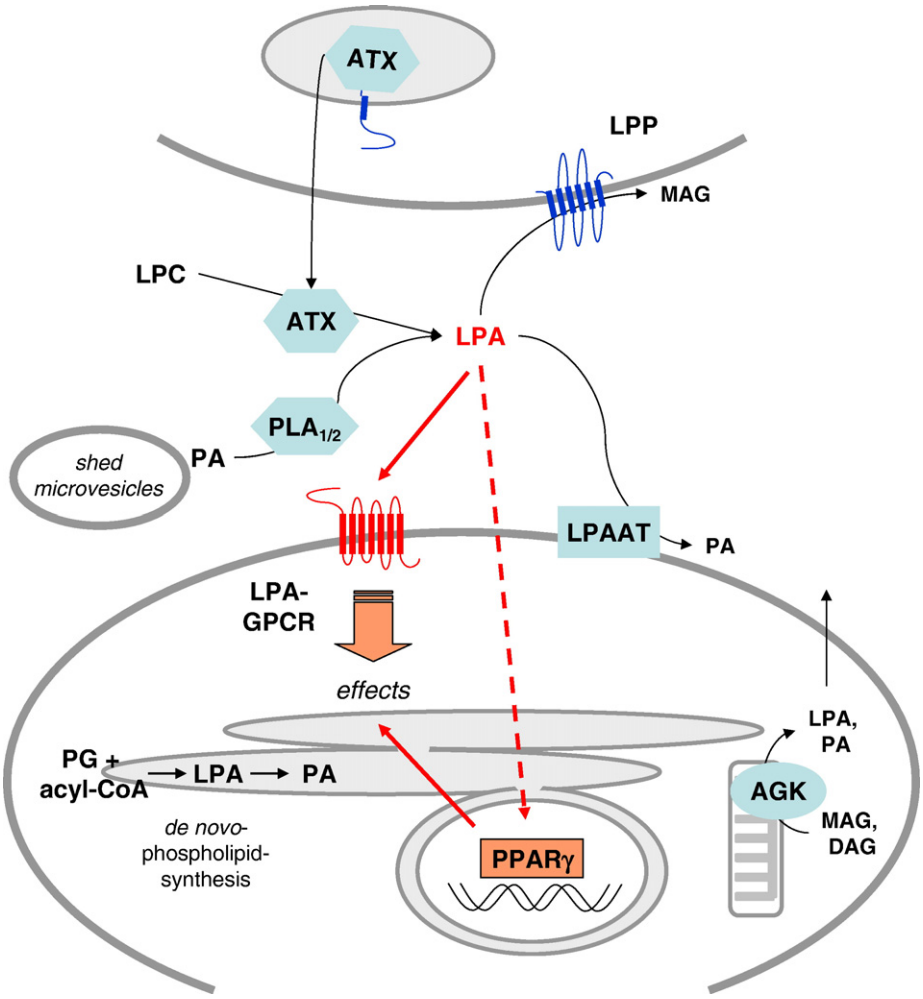

Fig. 2. Generation and degradation of bioactive LPA. In contrast to S1P, bioactive LPA seems not to be secreted, instead, it is formed extracellularly by diverse pathways (for review, see [164, 165]). LPA can be generated by deacylation of phosphatidic acid (PA), catalysed by phosphatidic acid-selective PLA_1 or secretory type-II PLA_2. For this, phosphatidic acid has to be transferred to the outer leaflet of the plasma membrane as it is the case in shed microvesicles. A major source of extracellular LPA is cleavage of lysophospholipids, predominantly LPC, by a lyso-PLD named autotaxin (ATX) [166–169]. This enzyme is generated as a membrane protein, further processed by proteolysis and secreted as a soluble protein. LPA is furthermore formed during de novo phospholipid synthesis at the endoplasmic reticulum by conjugation of phosphatidyl glycerol (PG) and acyl-CoA. Recently, an acylglycerol kinase expressed in mitochondria was described that phosphorylated monoacylglycerol (MAG) and diacylglycerol (DAG) and promoted LPA generation and secretion [170]. LPA is not only an agonist at G-protein-coupled receptors, but also activates the transcription factor, peroxisome proliferator-activated receptor-γ (PPARγ) [93]. Degradation of LPA occurs by dephosphorylation, catalysed by lipid phosphate phosphatases (LPP), or by acylation, catalysed by LPA acyl transferases (LPAAT) [129–132,164]. For additional references, see text.

cleave SPC (although with a high K_m) [171] indeed did not play a role in vivo. Mice with homozygous autotaxin deficiency died around embryonic day 10 with major vascular defects in yolk sac and embryo, and also had allantois malformation, neural tube defects and asymmetric headfolds [166,167]. This phenotype is much more severe than that of mice deficient in either LPA_1, LPA_2 or LPA_3 receptor [172–174]. LPA_1 deletion caused defects in craniofacial morphogenesis, brain development and olfaction, while LPA_2 knockout did not lead to obvious defects. Mice deficient in LPA_3 were recently reported to be born with ~50% reduced litter sizes, which could be traced back to downregulation of cyclooxygenase-2 in LPA_3-deficient uteri during preimplantation, leading to delayed implantation and altered positioning of embryos. However, the born mice were reported to be grossly normal [174]. In contrast, the severe phenotype of autotaxin deficiency demonstrates that this enzyme is decisive for LPA production during embryogenesis, thereby affecting the signalling of LPA in general [166]. Interestingly, autotaxin deficiency strongly resembled the phenotype of G_{13} knockout mice, showing the importance of this G-protein in signalling by LPA-GPCR [166]. Transcriptional regulation of autotaxin secretion was for example shown in adipocytes, in which autotaxin mRNA as well as autotaxin activity and LPA levels in culture supernatants were upregulated during differentiation [175]. Furthermore, autotaxin promoted the differentiation of preadipocytes and was substantially overexpressed in adipose tissue of obese diabetic (db/db) mice, but not in mice treated with high-fat diet or in mice with streptozotocin-induced diabetes [175,176]. However, autotaxin expression was downregulated by the insulin-sensitizing drug, rosiglitazone, and, most importantly, significantly upregulated in patients exhibiting both insulin resistence

and impaired glucose tolerance. These data suggest that autotaxin might play a role in adipocyte insulin resistance, rather than in obesity or hyperglycaemia [176]. Autotaxin was furthermore found to be overexpressed in frontal cortex of patients with Alzheimer-type dementia [177], however, its function in dementia is currently unknown.

4. Emerging lysophospholipid pharmacology

Since the early days of testing compounds in which the glycerol backbone of LPA was replaced by serine, tyrosine or ethanolamine for interaction with LPA receptors, the area of lysophospholipid pharmacology has seen much progress and interesting developments. The search for lysophospholipid receptor subtype-selective drugs is supported by homology modelling that greatly enhanced our understanding on how GPCRs recognize lipid ligands [41,178–182]. In 2002, it was reported by two groups that the novel immunosuppressive, FTY720, interacts with S1P-GPCRs [50,51]. It is now known that FTY720 is phosphorylated by sphingosine kinase in vivo and then acts as an agonist with low nanomolar affinity on $S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$, but not $S1P_2$ receptors. Phosphorylation of FTY720 was required for its immunosuppressive action, since it did not cause lymphopenia in SphK2 deficient mice [126,128], while interestingly SphK1 was not required [125]. Furthermore, the immunosuppressive action of FTY720 was mimicked by FTY720-phosphonate which cannot be dephosphorylated [50]. This indicates that the additional effects of non-phosphorylated FTY720, such as inhibition of S1P lyase [163] and antagonism at cannabinoid CB1 receptors [183], which both require low micromolar concentrations of FTY720, have only little impact on its immunosuppressive action.

The $S1P_1$ receptor was identified as the major target mediating FTY720-induced lymphopenia, since in mice whose haematopoietic cells lack $S1P_1$, T and B lymphocytes were unable to exit lymphoid tissues [31]. FTY720 treatment downregulated $S1P_1$, thereby mimicking $S1P_1$ deficiency [31,49]. Furthermore, also the $S1P_1$ selective agonist, SEW2871, inhibited lymphocyte recirculation in mice [37,184]. It is now clear that there is a S1P concentration gradient between plasma and extracellular fluid of lymphoid tissue that is created by differential actions of S1P lyase and sphingosine kinase, and that plasma S1P acts as a chemoattractant on lymphocytes in lymphoid tissues. The gradient is sensed by the $S1P_1$ receptor that mediates migration and is usually upregulated in lymphocytes within lymphoid tissue and downregulated in lymphocytes within blood [159,161,162,185]. Since FTY720 does not generally impair T- and B-cell proliferation and functions, it represents a novel mode of immunosuppressive action, which might be useful in transplantation as well as multiple sclerosis or autoimmune diabetes, leaving crucial functions of the immune system intact (reviewed in [21]). T lymphocytes furthermore contribute to ischemia–reperfusion injury, and FTY720 as well as SEW2871 reduced the damage caused by ischemia–reperfusion in liver and kidney [186–189]. Moreover, $S1P_1$ downregulation in other cell types may cause additional effects. Accordingly, FTY720 inhibited S1P- and vascular endothelial growth factor-induced angiogenesis, in which $S1P_1$ plays a role, and impeded primary and metastatic tumour growth in a murine model of melanoma [190].

Considering the molecular effect of FTY720-phosphate, and also that of SEW2871, it is obvious that $S1P_1$ activation is followed by downregulation, the latter being the clinically important effect. This could be interpreted as super-agonist activity of FTY720-phosphate and SEW2871. On the other hand, $S1P_1$ is obviously also downregulated in the high S1P level environment of plasma [161], suggesting that this receptor might be prone for up- and downregulation. Interestingly, the $S1P_1$ receptor has a high constitutive activity, and recently, an inverse agonist for $S1P_1$ has been described [154]. It is an intriguing question how this inverse agonist would affect lymphocyte trafficking. Interestingly, it was demonstrated very recently that a $S1P_1$ antagonist did not cause lymphopenia [191]. Obviously, there are still open questions with regard to regulation of lymphocyte trafficking by $S1P_1$.

Other actions of FTY720 correlate with activation but not downregulation of S1P-GPCRs. The undesired effect of bradycardia that is observed during the first days of FTY720 treatment is mediated by FTY720-phosphate's activation of $S1P_3$ [37]. In mouse aorta, FTY720 induced NO synthase activation and vasodilatation via $S1P_3$ [192]. Mimicking other effects of S1P on endothelial cells, FTY720 stimulated ERK and Akt, adherens junction assembly, survival and migration and reduced vascular permeability in vivo [193]. In contrast, the recently reported $S1P_1$ antagonist strongly enhanced capillary leakage in lung [191]. Regulation of the endothelial barrier function is of particular medicinal interest as its impairment plays a role in many pathological conditions.

SEW2871, the first compound highly specific for a S1P receptor subtype, was identified by high-throughput screening [37,184]. Interestingly, this compound lacks a phosphate group (for chemical structures, see [182]). Its EC_{50} for stimulating GTPγS binding by $S1P_1$ was ~13 nM, while that of S1P was ~0.4 nM. SEW2871 was a full agonist, stimulating Akt, ERK and cell migration. In vivo-application of SEW2871 induced lymphopenia in mice with a plasma EC_{50} of ~2 μM, but had no influence on heart rate, which is regulated by $S1P_3$ [37]. In ischemia–reperfusion damage in mouse kidney, a dose of 10 mg/kg SEW2871 was required for reduction of elevated plasma creatinine by ~70%; however, a nearly 50% reduction was already achieved with 0.1 mg/kg SEW2871. Nevertheless, FTY720 was much more potent [188]. A putative $S1P_3$ receptor antagonist, BML-241, was identified by searching a three-dimensional compound database with a pharmacophore model of S1P [194], however, BML-241 is poorly characterized. A specific $S1P_2$ receptor antagonist, JTE-013, was used to prove the inhibitory action of $S1P_2$ on cell migration [33,195], and to show that $S1P_2$ mediates contraction of coronary artery smooth muscle cells [196].

Recently, a series of aryl amide compounds was presented which were more or less receptor subtype-selective; the lead compound, VPC23019, was a competitive antagonist at $S1P_1$ and $S1P_3$ [197]. K_i values for VPC23019 in radioligand binding assays at $S1P_1$ and $S1P_3$ were in the low nanomolar range, and

VPC23019 inhibited S1P-induced migration and Ca^{2+} mobilization. Small structural changes converted the molecule into an agonist. Furthermore, VPC23019 as all compounds of this series behaved as agonist at $S1P_4$ and $S1P_5$, but had no activity at $S1P_2$ [197]. In mouse vascular endothelial cells expressing $S1P_2$ and $S1P_3$, but not $S1P_1$, VPC23019 inhibited S1P-stimulated Rac activation, migration and vascular tube formation, while JTE-013 enhanced these effects, indicating that $S1P_2$ and $S1P_3$ receptors have an opposing influence on these endothelial parameters [198]. For detailed discussion of ligand structure–activity relationships at S1P-GPCRs, see [182].

There are several compounds available that exhibit more or less selective antagonism at LPA-GPCRs. Dioctylglycerol pyrophosphate (DGPP 8:0) is a short-chain derivative of diacylglycerol pyrophosphate, which apparently is a signalling molecule in yeast and plants but not in higher animals (reviewed in [199]). It was demonstrated that DGPP 8:0 acts as a competitive antagonist at human LPA receptors, inhibiting preferentially LPA_3 (IC_{50} ~100 nM), and also LPA_1 (IC_{50} ~7 μM), but not LPA_2 [180,200]. The preference of DGPP 8:0 for LPA_3 was recently confirmed [201]. Platelet activation induced by LPA, but not by other platelet stimuli, was inhibited by DGPP 8:0, but not by DGPP 18:0, in a competitive manner with an IC_{50} of ~2.5 μM [202]. Although platelets express LPA_1, LPA_2 and LPA_3, the inhibitory action of DGPP implicates the involvement of LPA_3, and probably also LPA_1, in platelet shape change induced by LPA. DGPP 8:0 at 20 μM furthermore fully blocked platelet activation by mildly oxidated low density lipoproteins and by homogenates of lipid-rich core isolated from soft arteriosclerotic plaques, indicating that predominantly the LPA species, that were identified in this material, were involved in platelet activation by lipid-rich core [202].

A differential interaction with LPA-GPCRs was observed with fatty alcohol phosphates (FAP) with carbon chain lengths between 10 and 14 [203]. FAP-12 (dodecyl-FAP) activated LPA_2, thereby inducing $[Ca^{2+}]_i$ increases with an EC_{50} of ~700 nM, while it antagonized LPA_3 in a competitive manner, inhibiting LPA-induced $[Ca^{2+}]_i$ increases with an IC_{50} of ~90 nM. This compound was a weak antagonist at LPA_1 and did not interfere with S1P-GPCRs [203]. A compound rather non-selective for LPA receptor subtypes is Ki16425 (for structure, see [182], which was identified by high-throughput screening of 150,000 compounds for inhibition of LPA-induced $[Ca^{2+}]_i$ increases [201]. This compound inhibited LPA-stimulated inositol phosphate production and GTPγS binding with K_i values of 0.2–0.9 μM at LPA_1 and LPA_3, while its K_i values at LPA_2 were around 6 μM [201]. Ki16425 did not affect responses to S1P, platelet-activating factor, bradykinin or platelet-derived growth factor, and therefore might be useful as general LPA-GPCR inhibitor. A similar preference for LPA_1 and LPA_3 was observed with VPC12249 [204]. O-methylphosphothionate (OMPT), a LPA_3-selective agonist [205], aggravated ischemia–reperfusion injury in mouse kidney, while the $LPA_{1/3}$ antagonist, VPC12249, reduced tubular injury, ischemic necrosis in outer medulla, and leukocyte infiltration [206]. LPA itself inhibited renal injury at low concentrations, but aggravated the damage at higher concentrations, suggesting differential effects of LPA-GPCR subtypes in renal ischemia–reperfusion. The data observed with the inhibitors support the hypothesis that LPA_3 acts deleterious in this disease process [206]. For detailed discussion of structure–activity relationships of ligands at LPA-GPCRs, see [180,182]. Since most compounds that interact with LPA-GPCRs, except Ki16425, have a phosphate group that can be subject to dephosphorylation, a novel strategy focuses on metabolically stabilized LPA analogues such as phosphonates, phosphorothioates, phosphonothioates and fluorophosphonates [207]. These compounds affect LPA-GPCRs as well as LPA metabolizing enzymes [207].

Recently, a novel approach to pharmacological intervention at S1P-GPCRs, an anti-S1P antibody, was introduced [208] (commentary in [209]). Based on the concept that S1P promotes tumour proliferation, invasion and angiogenesis, the antibody was tested on transplanted mouse tumours. Proliferation of various tumour types, e.g., lung, breast, melanoma and ovarian cancers, was effectively retarded by the antibody. Furthermore, tumour-associated angiogenesis, plasma levels of interleukin-6, -8 and vascular endothelial growth factor, and the ability of S1P to protect tumour cells from apoptosis, was reduced by the anti-S1P antibody [208]. Other than sphingosine kinase inhibitors, which also successfully inhibit tumour cell growth [115,210], the anti-S1P antibody will attack S1P from all sources.

5. Concluding remarks

Research of the past decade has demonstrated that many important physiological and pathophysiological processes are regulated by lysophospholipids. As summarized in recent reviews, S1P and LPA play a role in the immune system [7,20,211–214], cardiovascular system [215–222], nervous system [223,224] and cancer [101,225]. G protein-coupled lysophospholipid receptors and the enzymes of lysophospholipid metabolism are therefore promising targets for medicinal interventions. Potential indications for drugs acting on lysophospholipid GPCRs have been suggested [8]. It remains a challenge to understand the complex interplay of lysophospholipid formation and degradation and receptor downregulation, recycling and cross-talk.

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