Minireview

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Lysophospholipid G Protein-coupled Receptors*

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The many biological responses documented for lysophospholipids that include lysophosphatidic acid and sphingosine 1-phosphate can be mechanistically attributed to signaling through specific G protein-coupled receptors. At least nine receptors have now been identified, and the total number is likely to be larger. In this brief review, we note cogent features of lysophospholipid receptors, including the current nomenclature, signaling properties, development of agonists and antagonists, and physiological functions.

The increasingly well studied lysophospholipids (LPs)[1] known as lysophosphatidic acid or LPA (1–5) and sphingosine 1-phosphate or S1P (5, 6) (Fig. 1) have garnered interest for their extracellular signaling properties. It is now clear that a majority of the responses documented for extracellular LPs is attributable to the activation of specific, seven-transmembrane domain G protein-coupled receptors (GPCRs). There are currently nine distinct LP receptors, four of which mediate effects of LPA and five that mediate effects of S1P (Table I). These receptors have been known by many different orphan receptor names, which recently led to a consensus, receptor renaming, based upon the identity of high affinity ligands (7): the LPA receptors consisting of LPA_{1-4} and S1P receptors consisting of $S1P_{1-5}$ (5, 8, 9). Genetic nulls (Table II) have driven a number of recent analyses toward understanding physiological functions (see Fig. 3).

In addition to these proven receptors, an enlarging number of orphan receptors have been provisionally identified as LP receptors; however, in many cases conflicting data exist on their identity. In particular, some putative receptors for sphingosylphosphorylcholine (SPC) and lysophosphatidylcholine (LPC) (10) may in fact be proton sensors, unrelated to LP signaling (11); these and other orphan/putative LP receptors are reviewed elsewhere (5). Similarly, no attempt is made to cover the important developments in understanding LP biochemistry and metabolism, which have been the subject of many excellent reviews (3, 5, 12–21). In this minireview, with apologies to many colleagues for citation limits, we highlight major features of LPA and S1P GPCRs.

LPA GPCRs

There are four identified LPA receptors in mammals (5). A distinct gene encodes each receptor that activates downstream signaling pathways mediated by one or more G proteins (Tables I and II; Figs. 2 and 3). The first three, LPA_{1-3}, share sequence homology with one another, whereas LPA_4 is divergent in sequence. LPA_1 represents the first LP receptor identified. In mice, a multi-exon gene structure was reported, with the coding region characterized by conservation of a single intron separating two coding regions at the sixth transmembrane domain. This intronic structure is shared with lpa_2 and lpa_3. LPA_1 contains 364 amino acids (aa) in a seven-transmembrane receptor structure, with an apparent molecular mass of ~41 kDa. LPA_1 couples to multiple G proteins (Fig. 2). In both humans and mouse, adult expression is widespread and includes most major tissues. However, within a single tissue, heterogeneity of cell types expressing lpa_1 also exists. Targeted deletion of lpa_1 revealed ~50% perinatal lethality in a mixed background strain (Table II). Remaining survivors showed reduced body mass and head/facial deformity and increased cell death of Schwann cells. Postnatal lethality was in part related to suckling problems associated with olfactory defects, whereas exencephaly and frontal brain hemorrhage likely contributed to a small proportion of embryonic loss. LPA signaling was lost or vastly decreased in mouse embryonic fibroblasts (MEFs) and cerebral cortical neuroprogenitor cells. Independent deletion of LPA_1 in mice has been associated with behavioral changes reminiscent of psychiatric disorders (22). Key roles in cell migration have been recently described (23) as well as surprising effects on the formation of the central nervous system (Fig. 3) (24).

LPA_2 was the second LPA receptor identified. A mutant variant named EDG-4 is absent from wild-type genomes and is therefore not synonymous with LPA_2. Gene structure analyses reveal the conserved intron in transmembrane domain 6. LPA_2 contains 351 aa (human) or 348 aa (mouse) with a predicted molecular mass of ~39 kDa. LPA_2 also couples with multiple forms of G proteins (Fig. 2) and shows widespread adult tissue expression in humans and mouse. It has been detected in various cancer cell lines, and variants within the 3′-untranslated region exist. Targeted genetic nulls of lpa_2 do not have blatant phenotypes yet do show defects and/or loss of wild-type LPA signaling in MEFs (Table II). Double mutants of $lpa_1^{(-/-)}$ and $lpa_2^{(-/-)}$ show MEF defects in most LPA-related signaling (*e.g.* AC inhibition, c-Jun N-terminal kinase and Akt activation, PLC activation, Ca^{2+} mobilization, stress fiber formation, and cell proliferation). The dual elimination of both receptors has also revealed involvement in central nervous system development (24).

LPA_3 also has a gene structure containing the conserved intron in transmembrane domain 6. It contains 353 aa (human) and 354 aa (mouse), with a predicted molecular mass of ~40 kDa. It differs from the other previous two LPA receptors by not coupling to $G_{12/13}$ (Fig. 2) and showing a preference for LPA molecules with unsaturated acyl chains. Although still expressed in many adult tissues, it shows somewhat more restricted expression (5). Its signaling properties are generally similar to LPA_1 and LPA_2 except for AC-related effects that vary with respect to analyzed cell lines. Targeted deletions have not yet been reported.

LPA_4 (25) was the first LPA receptor with a divergent sequence that shows greater similarity to the platelet-activating factor GPCR. Comparatively less is known about this receptor. It appears to be encoded on a single exon, and both human and mouse receptors contain 370 aa with a molecular mass of ~42 kDa. Gene expression is most marked in the ovaries but is also observed at lower levels in several other tissues. Biological roles, null mutations, and its relationship to the other LPA receptors have not been reported.

S1P GPCRs

There are five identified S1P receptors in mammals (Tables I and II; Figs. 2 and 3) (5, 9, 13, 26). The first receptor identified was $S1P_1$ (5, 8, 27, 28), and it is also the best characterized S1P receptor. Unlike most LPA receptors it is encoded within a single exon,

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[1] The abbreviations used are: LP, lysophospholipid; LPA, lysophosphatidic acid; S1P, sphingosine 1-phosphate; GPCR, G protein-coupled receptor; SPC, sphingosylphosphorylcholine; aa, amino acids; MEF, mouse embryonic fibroblast; AC, adenylyl cyclase; PLC, phospholipase C.

and this gene structure is shared by all five S1P receptors. Both human and mouse receptors contain 382 aa with an apparent molecular mass of ~43 kDa. As with the LPA receptors, it has wide adult tissue expression and interacts with G_i proteins (Fig. 2). It also shows responses that are related to platelet-derived growth factor signaling, because platelet-derived growth factor-induced effects are perturbed in $s1p_1^{(-/-)}$ MEFs. The null genotype of $s1p_1$ was embryonic lethal (29) with death attributable to incomplete vascular maturation (Table II). Conditional deletion studies demonstrate that vascular endothelial cells are the primary target for the actions of $S1P_1$ loss (30) (Fig. 3). Recent reports demonstrate specific roles for $S1P_1$ in lymphocyte recirculation/egress (31, 32).

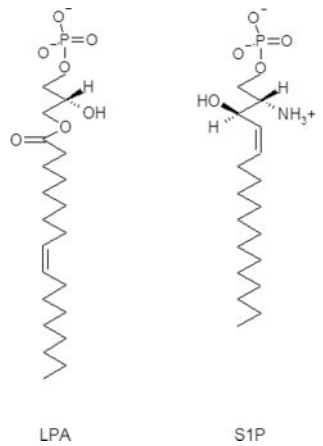

FIG. 1. **Chemical structures of the bioactive lysophospholipids LPA and S1P.**

$S1P_2$ is encoded on a single exon and contains 353 aa (human) and 352 aa (mouse) with an apparent molecular mass of ~39 kDa. It shows widespread tissue distribution and couples with multiple G proteins (Fig. 2). Genetic deletion of an apparent zebra fish $s1p_2$ orthologue (33) revealed developmental heart defects although an analogous phenotype was not observed in independent deletions of $s1p_2$ in mice (34, 35). In mice, $s1p_2^{(-/-)}$ genotype demonstrated MEF signaling defects for Rho activation (Table II). Although appearing grossly normal, some nulls revealed sporadic and at times lethal seizures in a neuroanatomically normal setting that may be related to increased excitability in neocortical pyramidal neurons (34). By comparison, other $s1p_2^{(-/-)}$ mice did not show seizure activity but did exhibit decreased litter size (35); the reasons for these differences may reflect background strain effects.

$S1P_3$ is also encoded on a single exon, and both human and mouse receptors contain 378 aa residues with an apparent molecular mass of ~42 kDa. It shows wide tissue distribution in humans and mouse. It also couples to multiple G proteins (Fig. 2). Gene targeting revealed no gross abnormalities aside from a slightly decreased litter size (Table II). By contrast, MEF S1P signaling was notably affected, particularly PLC activation and Ca^{2+} mobilization in contrast to normal Rho activation and inhibition of AC. Double null $s1p_2^{(-/-)}s1p_3^{(-/-)}$mice (35) have markedly reduced litter sizes and low survival beyond postnatal week 3. Loss of both receptors eliminates S1P-dependent Rho activation in MEFs.

TABLE I
Lysophospholipid receptors

The abbreviations used are: DGPP 8:0, diacylglycerol pyrophosphate 8:0; dh-S1P, dihydrosphingosine 1-phosphate; FAP-10, decyl fatty alcohol phosphate; FAP-12, dodecyl fatty alcohol phosphate; Ki16425, 3-(4-[4-([1-(2-chlorophenyl)ethoxy]carbonyl amino)-3-methyl-5-isoxazolyl] benzylsulfanyl) propanoic acid; NAEPA, *N*-acyl ethanolamide phosphate; OMPT, 1-oleoyl-2-*O*-methyl-*rac*-glycerophosphothionate, an ester-linked thiophosphate derivative of LPA; PA 8:0, dioctylphosphatidic acid 8:0; PhS1P, phytosphingosine 1-phosphate; SEW2871, 5-(4-phenyl-5-trifluoromethylthiophen-2-yl)-3-(3-trifluoromethylphenyl)-[1,2,4]oxadiazole; SPC, sphingosylphosphorylcholine; VPC12249, *N*-oleoylethanolamide phosphate substituted at the second carbon with a benzyl-4-oxybenzyl moiety.

Receptor[a]	Synonyms	Ligands	Agonists	Antagonists
LPA_1	VGZ-1 EDG-2 mrec1.3 GPCR 26 LP_{A1}	LPA (high affinity)	Several NAEPA derivatives	Suramin (low specificity); DGPP 8:0 and PA 8:0 (weak antagonists); Ki16425; FAP-12 (weak antagonist); VPC12249
LPA_2	$EDG\text{-}4_{(non\text{-}mutant)}$ LP_{A2}	LPA (K_d = 73.6 nM)	Several NAEPA derivatives; FAP-10; FAP-12	
LPA_3	EDG-7 LP_{A3}	LPA (K_d = 206 nM)	Several NAEPA derivatives; OMPT; a monofluorinated analog of LPA	DGPP 8:0; PA 8:0; Ki16425; FAP-12; VPC12249
LPA_4	$P2Y_9$ GPR23	LPA (K_d = 45 nM)		
$S1P_1$	EDG-1 LP_{B1}	S1P (K_d = 8–13 nM); dh-S1P; SPC (low affinity)	FTY720 and an analog, (R)-AAL, after phosphorylation to FTY720-P (Compound A) and (*R*)-AFD; SEW2871	
$S1P_2$	AGR16 H218 EDG-5 LP_{B2}	S1P (K_d = 20–27 nM); dh-S1P; SPC (low affinity)		Pyrozolopyridine derivative named JTE-013
$S1P_3$	EDG-3 LP_{B3}	S1P (K_d = 23–26 nM); dh-S1P; SPC (low affinity)	FTY720-P (Compound A) and (R)-AFD	Suramin
$S1P_4$	EDG-6	PhS1P (K_d = 1.6 nM)	FTY720-P (Compound A) and (R)-AFD	
	LP_{C1}	S1P (K_d = 13–63 nM); dh-S1P; SPC (low affinity)		
$S1P_5$	NRG-1 EDG-8 LP_{B4}	S1P (K_d = 2–10 nM); dh-S1P; SPC (low affinity)	FTY720-P (Compound A) and (R)-AFD	

[a] The current receptor nomenclature follows the guidelines of the International Union for Pharmacology (IUPHAR).

TABLE II
Phenotypes of reported LP receptor-null mice

The abbreviations used are: JNK, c-Jun N-terminal kinase; VSMCs, vascular smooth muscle cells.

Receptor deleted	Viability and fertility	Phenotype	Cellular signaling
LPA_1	Semi-lethal, fertile	Impaired suckling behavior; decreased postnatal growth rate; reduced size; craniofacial dysmorphism; low incidence of frontal hematoma (2.5%); increased apoptosis of Schwann cells in the sciatic nerve	Impaired cluster compaction and decreased cell proliferation of dissociated embryonic $LPA_1^{-/-}$ neuroblasts in response to LPA; reduced PLC activation and Ca^{2+} mobilization and abolished AC inhibition in MEFs following LPA stimulation
LPA_2	Viable, fertile	No major phenotype	Reduced PLC activation and Ca^{2+} mobilization in MEFs after stimulation with LPA
LPA_1/LPA_2	Semi-lethal, fertile	Phenotype comparable with $LPA_1^{-/-}$ mice with a higher incidence of frontal hematoma (26%); no alterations in cell proliferation, histology, or thickness of cerebral cortices; apoptosis in sciatic nerve was not analyzed	Abolished PLC activation and Ca^{2+} mobilization, abolished AC inhibition, severely reduced stress fiber formation, abolished activation of JNK and Akt as well as abolished proliferative response of MEFs to LPA
$S1P_1$	Lethal	Embryonic hemorrhage; intrauterine death between E12.5 and E14.5; impaired recruitment of VSMCs to blood vessels; defective ensheathment and maturation of vessels	Severely reduced migratory response of MEFs to S1P
$S1P_2$	Viable, slightly reduced fertility	Apparently normal or seizures between 3 and 7 weeks of age on mixed genetic background; no anatomical defects; neuronal hyperexcitability	Significant decrease of S1P- induced Rho activation in MEFs
$S1P_3$	Viable, slightly reduced fertility	No major phenotype	Decreased PLC activation and slightly decreased AC inhibition in MEFs following S1P stimulation
$S1P_2/S1P_3$	Reduced viability, severely reduced fertility	Reduced fertility	Complete loss of Rho activation and decrease in PLC activation in MEFs stimulated with S1P

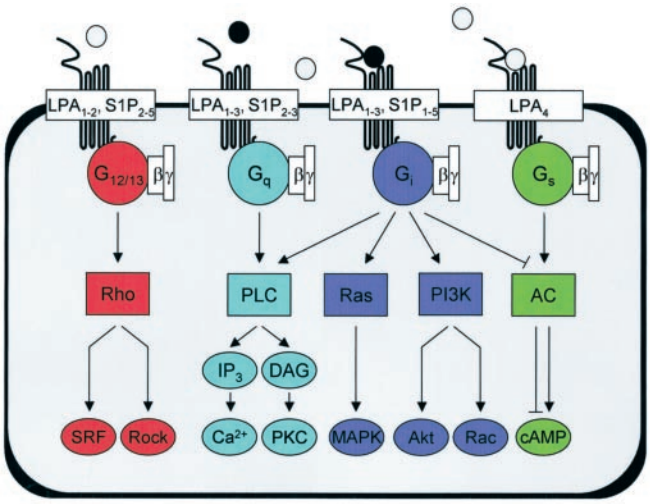

FIG. 2. **LPA and S1P signaling through G protein-coupled receptors.** Coupling of LPA and S1P receptors with different classes of G proteins, activation or inhibition of downstream second messenger molecules, and the most prominent resultant cellular effects are illustrated. *PI3K*, phosphoinositol 3-kinase; *DAG*, diacylglycerol; IP_3, inositol 1,4,5-trisphosphate; *MAPK*, mitogen-activated protein kinase; *PKC*, protein kinase C; *Rock*, Rho-associated kinase; *SRF*, serum response factor.

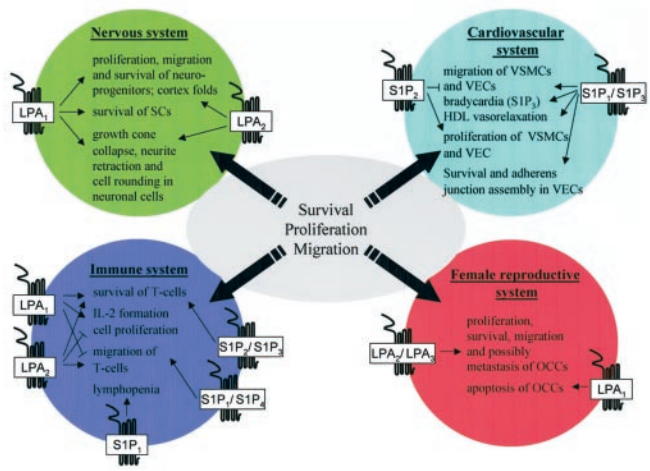

FIG. 3. **Biological roles of lysophospholipids in different systems.** Receptor-mediated cellular responses to LPA and S1P, such as survival, proliferation, and migration, exhibit biological significance particularly within the nervous system, the cardiovascular system, the immune system, and the female reproductive system. Indicated are physiological and pathophysiological functions of LPA and S1P and the involved receptors. *IL-2*, interleukin-2; *OCCs*, ovarian cancer cells; *SCs*, Schwann cells; *VEC*, vascular endothelial cells; *VSMCs*, vascular smooth muscle cells; *HDL*, high density lipoprotein.

Bradycardia that is mediated by this receptor has recently been reported (Fig. 3) (31).

$S1P_4$ is again found encoded on a single exon. It contains 384 aa (human) and 386 aa (mouse) with an apparent molecular mass of ~42 kDa. It has relatively low amino acid sequence similarity to the other S1P receptors suggesting that it might prefer a distinct ligand (8); indeed phytosphingosine 1-phosphate (4D-hydroxysphinganine 1-phosphate) appears to be such a ligand (36). Unlike other S1P receptors its expression pattern is predominantly in lymphoid compartments. $S1P_4$ couples with multiple G proteins (Fig. 2). Targeted deletion of this receptor has not been reported.

$S1P_5$ retains a single exon coding region (8). It contains 398 aa (human) and 400 aa (mouse) and has an apparent molecular mass of ~42 kDa. It couples to multiple G proteins (Fig. 2) and shows intermediate expression levels compared with the previously mentioned receptors having notable expression in rat brain where it is expressed in white matter tracts and oligodendrocytes. In contrast to other S1P receptors it appears to inhibit mitogen-activated protein kinase activation. Genetic nulls have not yet been reported.

Agonists and Antagonists for LP GPCRs

Important tools for the study of GPCRs are appropriate agonists and antagonists (5, 37). It is notable that many reported compounds have not been adequately validated in a range of assays or *in vivo*. Nevertheless, a number of promising compounds have entered the experimental literature (Table I). Examples of LPA-related compounds (37) include Ki16425, an LPA_1 and LPA_3 antagonist (38); an ethanolamide derivative (VPC12249) with LPA_1

and LPA_3 antagonist actions (37); decyl and dodecyl fatty alcohol phosphates referred to as FAP-10 and FAP-12 that can act as LPA_2 agonists (41); a phosphothionate analog of LPA (OMPT) that shows LPA_3 agonism (42); a monofluorinated analog of LPA also showing LPA_3 agonism (43); a diacylglycerol pyrophosphate (DGPP 8:0), which shows LPA_3 antagonism (40, 44); a fluoromethyl-phenyl oxadiazole (SEW2871) that shows $S1P_1$ selective agonism (31); and a pyrazolopyridine (JTE-013) showing $S1P_2$ antagonism (45). The best validated *in vivo* compound is the pro-drug FTY720 that shows non-selective agonism of several S1P receptors following its phosphorylation into an active species (46, 47).

Physiological Future for GPCR-mediated LP Signaling

LP signaling through GPCRs has major influences on multiple organ systems, and an increased understanding of the physiological and pathophysiological effects of LPs is perhaps the major growth area in this field (3, 5, 48, 49). Integration of data on individual receptors into organ system biology is providing a strategic focus for the field as it necessarily diversifies into more organ-specific topic areas. Major systems influenced by LPs include both the developing and adult cardiovascular system (12, 13, 50), reproductive system (5, 35, 51), immune system (52–56), and nervous system (Fig. 3) (5, 8, 50, 57–59); these represent only a partial list of influences considering the widespread expression of LP receptors viewed as a whole. Both LPA and S1P have been implicated in these influences, and the range of effects continues to increase. In addition to normal physiological processes, LP signaling has also been implicated in cancer (3, 60), wound healing (16), and atherosclerosis (39, 48). Joining the effects of LPA and S1P, it is certain that other chemical forms of LPs and their cognate GPCRs will also complement the many studies noted here. Elucidating both physiological and pathophysiological roles mediated by LP GPCRs will undoubtedly fuel continued growth of this exciting field.

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