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Lysophospholipid Receptors in the Nervous System*

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The lysophospholipid mediators, lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), are responsible for cell signaling in diverse pathways including survival, proliferation, motility, and differentiation. Most of this signaling occurs through an eight-member family of G-protein coupled receptors once known as the endothelial differentiation gene (EDG) family. More recently, the EDG receptors have been divided into two subfamilies: the lysophosphatidic acid subfamily, which includes LPA_1, (EDG-2/VZG-1), LPA_2 (EDG-4), and LPA_3 (EDG-7), and the sphingosine-1-phosphate receptor subfamily, which includes $S1P_1$ (EDG-1), $S1P_2$ (EDG-5/ H218/AGR16), $S1P_3$ (EDG-3), $S1P_4$ (EDG-6), and $S1P_5$ (EDG-8/NRG-1). The ubiquitous expression of these receptors across species, coupled with their diverse cellular functions, has made lysophospholipid receptors an important focus of signal transduction research. Neuroscientists have recently begun to explore the role of lysophospholipid receptors in a number of cell types; this research has implicated these receptors in the survival, migration, and differentiation of cells in the mammalian nervous system.

KEY WORDS: G-protein–coupled receptor; lysophosphatidic acid; nervous system; sphingosine-1-phosphate.

INTRODUCTION

Lysophospholipids, including lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), are metabolites of glycerophospholipids and sphingolipids, lipid components of cell membranes (Fig. 1). These lysophospholipids have both cell signaling properties and diverse functions, including the stimulation of mitogenesis, suppression of apoptosis, reorganization of the cytoskeleton, and regulation of cell movement (reviewed in references 1–7). All of these roles may be important for the development of the nervous system (8,9). Their diverse actions, ubiquitous expression, and evolutionary conservation suggest that they play a critical role in a number of fundamental processes; they have been implicated in tumorigenesis, angiogenesis, immunity, and atherosclerosis, as well as, recently, in neuronal survival, differentiation, and neurotransmission.

LPA and S1P Metabolism

Lysophospholipids are produced by *de novo* synthesis and during metabolism of plasma membrane lipids, as well as at different subcellular sites (reviewed in references 1, 10, and 11). Surprisingly, although LPA is one of the simplest natural phospholipids and is considered to be a key intermediate in *de novo* lipid synthesis, little is known about its biosynthesis. Among the best-characterized cellular sources of bioactive

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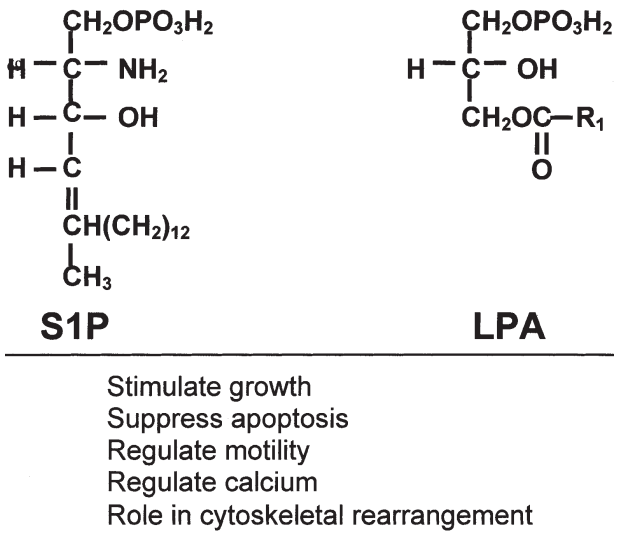

Fig. 1. Lysophospholipid structures.

LPA and S1P are blood platelets, which release these mediators following their activation (3). It has been reported that platelets release LPA when stimulated by thrombin or alpha-2-receptor agonists (12,13). Precisely how LPA is released into the extracellular environment remains unknown. It is believed that platelet-derived LPA is produced from newly generated phosphatidic acid by the action of phospholipase D followed by phospholipase A_2–mediated deacylation (3,14–16). However, recent results suggest that LPA is not released from activated platelets (17), suggesting that the source of extracellular LPA has not yet been discovered. Moreover, the extensive distribution of LPA receptors suggests that there may be more diverse sources. Other phospholipases including lysophospholipase D and phospholipase A_1 may also contribute to LPA production (14–16).

S1P is produced by the sphingosine kinase–dependent phosphorylation of sphingosine (reviewed in reference 10). Cellular levels of S1P are low and tightly controlled by the balance between its synthesis and degradation. Sphingosine kinase is activated by numerous external stimuli, including many growth and survival factors, leading to transient increases in S1P levels. This growing list includes PDGF (18), NGF (19), muscarinic acetylcholine agonists (20), cytokines such as TNF-α (21) and IL-1β (22), and the crosslinking of immunoglobulin receptors (23,24). S1P turnover is mediated either by dephosphorylation back to sphingosine catalyzed by specific S1P phosphatases (25,26) or irreversible cleavage by S1P lyase to hexadecenal and ethanolamine phosphate (27).

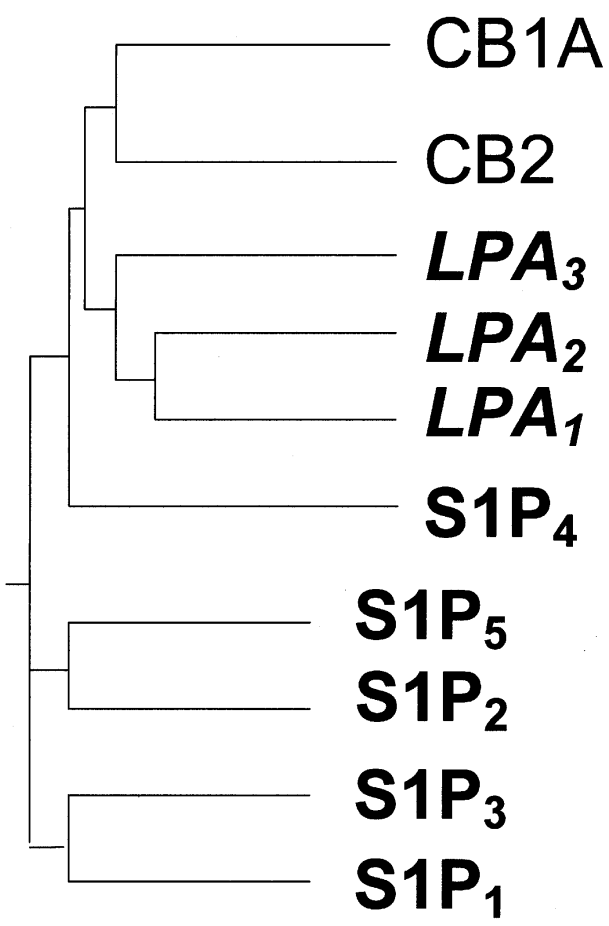

Fig. 2. Phylogenetic tree of lysophospholipid receptors.

Platelets have sphingosine kinase but lack S1P lyase activity and thus contain high levels of S1P. A recent study suggests that S1P, rather than LPA, is the major bioactive lysophospholipid that is released from platelets (17).

LPA and S1P Receptors

Once formed, LPA and S1P can act as both intracellular second messengers and extracellular mediators. Whereas specific targets for their intracellular actions have not yet been conclusively identified, their extracellular effects are mediated via a family of specific plasma membrane G-protein–coupled receptors, lysophospholipid receptors, previously known as EDG family receptors (1,7,10,11). As is characteristic of G-protein–coupled receptors, these lysophospholipid receptors have seven transmembrane spanning domains, are closely related to the cannabinoid receptors CB1A and CB2, and have been divided into two sub-

Table I. Properties of Lysophospholipid Receptors

Name	Coupled G-proteins	Downstream pathways
LPA_1	$G\alpha_i$, $G\alpha_o$	Adenylyl cyclase inhibition
	$G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$	PLC activation
	$G\alpha_{12}$, $G\alpha_{13}$	PI3K activation
		Akt activation
		Rho activation
		DNA synthesis
LPA_2	$G\alpha_i$, $G\alpha_o$	Adenylyl cyclase inhibition
	$G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$	MAPK activation
	$G\alpha_{12}$, $G\alpha_{13}$	PLC activation
		Arachadonic acid release
		Calcium mobilization
LPA_3	$G\alpha_i$, $G\alpha_o$	Adenylyl cyclase stimulation or
	$G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$	inhibition
		MAPK activation
		PLC activation
		Arachadonic acid release
		Calcium mobilization
$S1P_1$	$G\alpha_i$, $G\alpha_o$	Adenylyl cyclase inhibition
		MAPK activation
		PLC activation
		Rho activation
		Calcium mobilization
$S1P_2$	$G\alpha_i$, $G\alpha_o$	Adenylyl cyclase stimulation
	$G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$	MAPK activation
	$G\alpha_{12}$, $G\alpha_{13}$	PLC activation
		Rho activation
		Calcium mobilization
$S1P_3$	$G\alpha_i$, $G\alpha_o$	Adenylyl cyclase stimulation
	$G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$	MAPK activation
	$G\alpha_{12}$, $G\alpha_{13}$	PLC activation
		Rho activation
		Calcium mobilization
$S1P_4$	$G\alpha_i$, $G\alpha_o$	MAPK activation
		PLC activation
$S1P_5$	$G\alpha_i$, $G\alpha_o$	Adenylyl cyclase inhibition
	$G\alpha_{12}$, $G\alpha_{13}$	MAPK inhibition
		PLC activation
		Calcium mobilization

families based on their ligand specificity. The LPA receptor subfamily includes LPA_1 (EDG-2/VZG-1), LPA_2 (EDG-4), and LPA_3 (EDG-7), and the S1P receptor subfamily includes $S1P_1$ (EDG-1), $S1P_2$ (EDG-5/H218/AGR16), $S1P_3$ (EDG-3), $S1P_4$ (EDG-6), and $S1P_5$ (EDG-8/NRG-1). This article is focused on the role of these lysophospholipid receptors in the nervous system where they are differentially expressed.

LPA_1

The first LPA receptor (LPA_1) was discovered in studies of gene expression during development of mouse cerebral cortex. While performing experiments to identify novel GPCRs associated with neuronal production, Chun and colleagues (28) identified an orphan GPCR with an enriched expression pattern in the ventricular zone that they named VZG-1. Northern blot analysis first revealed that a 3.8-kb *vzg-1/lpa*$_1$ transcript was expressed within the developing brain and later, in situ hybridization clearly demonstrated its expression in the cortical neuroblasts of the neuroproliferative ventricular zone (28,29). Membrane binding studies indicated that VZG-1 overexpression increased LPA specific binding. These analyses identified the *vzg*-1 gene product as a receptor for LPA and suggested that LPA signaling mechanisms were important in cortical neurogenesis (28). Postnatally, LPA_1 is present in oligodendrocytes, the myelinating cells of the central nervous system; it is not expressed in astrocytes. Its expression is highest during the first several postnatal weeks and decreases to low levels in adulthood. Additionally, Schwann cells, the myelinating cells of the peripheral nervous system, maintain high levels of LPA_1 expression throughout life, suggesting a possible role for LPA_1 in myelination of the nervous system (30).

Although ligation of LPA_1 has been shown to stimulate cell proliferation, the extent and timing of this increase suggest that it may not be a biologically relevant role for LPA_1 in the nervous system (31). However, both morphological changes, including cell rounding and process retraction (28,32), and ionic changes, including increased chloride and nonselective cation conductances, in primary cortical neuroblasts and neuroblast cell lines have been attributed to activated LPA_1 (33). Such changes in conductance have previously been shown to influence DNA synthesis (34). Activated LPA_1 in primary Schwann cells enhances survival through a $G\alpha_i$-dependent process (31,35). In addition, LPA_1 can couple to other G proteins, including $G\alpha_{11}$, $G\alpha_{12}$, $G\alpha_{13}$, $G\alpha_{14}$, and $G\alpha_q$, in different cell types (36,37). LPA_1 also mediates activation of phosphatidylinositol 3-kinase, Akt and the Rho pathway (35), and inhibits cAMP production (28), important for regulation of cell survival and motility.

The importance of LPA_1 in the nervous system was emphasized by the phenotype of mice with a targeted deletion of LPA_1 that results in a high degree of neonatal lethality. Surviving newborns have impaired suckling ability, cerebral cortical neurons of reduced size, craniofacial dysmorphism, and increased apoptosis of the sciatic nerve (38). Taken together, these findings

suggest that LPA_1 plays a critical role in neuronal development and behavior, as well as in the maintenance and functioning of mature myelinating cells.

LPA_2

LPA_2 shares approximately 60% sequence similarity with LPA_1 (2,29). Intriguingly, the *lpa*$_2$ gene is expressed in several variant forms in human cancer cells, one of which was used for the original characterization (36). However, Northern blot analysis using a wild-type LPA_2 probe revealed both 2.8- and 7-kb transcripts in embryonic and neonatal mouse brain, although neither transcript was detectable in adult brain tissue. By *in situ* hybridization, the 7-kb transcript was also shown to be temporally expressed, located in postmitotic neuronal regions and the ventricular zone of embryonic mice (39). The smaller LPA_2 transcript was also abundantly expressed in the adult testis, kidney, and lung (40).

LPA_2 couples to both $G\alpha_i$ and $G\alpha_q$. Ligation of LPA_2 has been associated with activation of phospholipase C and extracellular signal regulated kinase (ERK), increases in intracellular calcium, release of arachadonic acid, production of inositol phosphate, and inhibition of cAMP formation (36,41). LPA_2 is also responsible for LPA-induced cell rounding of B103 rat neuroblastoma cells (41).

LPA_3

Like LPA_2, LPA_3 also shares approximately 60% sequence similarity with LPA_1. Its expression appears to be species specific, and it is abundantly expressed in human testis, prostate, heart, and frontal cortex (42,43) and in mouse lung, kidney, and testis (40). In contrast to LPA induced cell rounding in LPA_1- and LPA_2-expressing cells, overexpression of LPA_3 resulted in neurite extension in B103 cells and inhibited LPA-dependent cell rounding of primary mouse neuroblast cells that endogenously express LPA_1 and LPA_2 but not LPA_3 (41). LPA_3 mediates pertussis toxin (PTX)-insensitive intracellular calcium increases (42,43) and phospholipase C activation (41), indicating coupling to G proteins other than $G\alpha_i$. Ligation of LPA_3 also activates ERK1/2, releases arachidonic acid, and inhibits cAMP accumulation (41). Contradictory findings have been reported regarding the coupling of LPA_3 to $G\alpha_i$. Activation of LPA_3 stimulates adenylyl cyclase in insect cells (42), yet has no apparent effect on cAMP levels in certain types of mammalian cells (43).

$S1P_1$

The LPA and S1P receptor subfamilies share approximately 35% sequence identity, yet the two families are notably distinguished by the presence of an intron in the coding region of the sixth transmembrane domain of the LPA subfamily (29). The first of the S1P receptors to be identified, EDG-1/$S1P_1$ was cloned as an immediate early gene induced during endothelial differentiation (44). While a 3.2-kb $S1P_1$ transcript is ubiquitously expressed throughout rat tissues, expression of a 4.9-kb $S1P_1$ transcript is restricted to the brain, the sympathetic neuronal-like PC12 cell line, and C6 glioma cells (45,46). $S1P_1$ expression is developmentally regulated; in the rat brain, $S1P_1$ mRNA is detected on embryonic day 15 and steadily increases, reaching its highest level in adulthood (45). Similar developmental expression patterns were reported for the mouse (47). Given that overexpression of $S1P_1$ in PC12 cells does not influence proliferation (45) and that $S1P_1$ is primarily expressed in the adult brain, it is more likely that $S1P_1$ plays a role in the maintenance of mature neurons rather than in the development of the nervous system. The recent finding that $S1P_1$ is expressed in the Zebrafish embryonic brain suggests that S1P signaling plays an evolutionarily conserved role in the central nervous system (48).

The role of $S1P_1$ signaling in the central nervous system is not yet well characterized and most of the available clues stem from studies in non-neuronal cells. For example, $S1P_1$ activation inhibits forskolin-stimulated intracellular cAMP accumulation in a PTX sensitive manner (49–51). In cells overexpressing $S1P_1$, sustained activation of ERK1/2, as well as S1P-stimulated phospholipase C, is also PTX sensitive. In CHO cells, but not in COS-7 or HEK 293 cells, $S1P_1$ has been associated with PTX-sensitive calcium mobilization (49–51). Since PTX ADP ribosylates and inactivates $G\alpha_{i/o}$ subunits, these findings indicate the tendency of $S1P_1$ to couple to these G proteins (49–54). Importantly, some $S1P_1$ actions are PTX insensitive and involve signaling pathways mediated by the small GTPases of the Rho family. These include morphological changes, as well as increased cadherin expression and formation of adherin junctions (53–55). These findings suggest $S1P_1$ may play a role in Rho-dependent pathways, such as neuritogenesis, in the developing nervous system.

Interestingly, disruption of *s1p*$_1$ causes an embryonic lethal deficit in vascular smooth muscle cell and pericyte migration to reinforce nascent blood vessels (56); similar results were obtained by disrupting the PDGF-BB or PDGFR-β genes (57,58). Recent studies have revealed that the binding of PDGF to its receptor activates and recruits sphingosine kinase to the cell's leading edge (59). In turn, ligation of $S1P_1$ results in activation and integration of downstream signals essential for cell motility, such as FAK, Src, and Rac (59,60). Moreover, PDGFR is also tethered to $S1P_1$, providing a platform for integrative signaling by these two types of receptors (61). These studies strongly implicate $S1P_1$ in the regulation of growth factor induced cell motility and cytoskeletal rearrangement, further suggesting that $S1P_1$ may play a role in neuronal migration and neuritogenesis.

$S1P_2$ and $S1P_3$

$S1P_2$ and $S1P_3$ are closely related and share 92% sequence identity. Thus, it is not surprising that their tissue distribution and the biological effects that they mediate are also very similar. These receptors are predominantly expressed in the lung, heart, kidney, liver, spleen, thymus, testis, and brain (62). $S1P_2$ and $S1P_3$ mediate both mitogenic and anti-apoptotic effects, enhancing cell survival of rat hepatoma cells. However, overexpression of these receptors in HEK 293 and PC12 cells has been associated with increased apoptosis, cell rounding, and neurite retraction (63). It has been suggested that $S1P_2$ and, to a lesser extent $S1P_3$, might be responsible for neurite retraction and soma rounding of neuroblastoma cells induced by S1P (63).

Both receptors mediate S1P-induced ERK1/2 activation through a $G\alpha_i$-dependent pathway and rapidly induce c-Jun and c-Fos, suggesting induction of immediate early genes (64). In COS-7 cells and HEK 293 cells, activated $S1P_2$ and $S1P_3$ mediate PTX-sensitive calcium mobilization (65). Unlike $S1P_1$, $S1P_2$ and $S1P_3$ couple not only to $G\alpha_{i/o}$ but also to $G\alpha_q$, $G\alpha_{12}$, and $G\alpha_{13}$ (36,66,67) and activate several PTX-insensitive signaling pathways, including c-jun N-terminal kinase and p38, as well as stress fiber formation (63,68). Neurite retraction has been shown to involve $G\alpha_{12}$, $G\alpha_{13}$, and $G\alpha_q$, as well as the Rho family of small G-proteins (69). Additionally, activation of Rho by these receptors may be mediated through $G\alpha_{12}$ and $G\alpha_{13}$ (R. E. Toman and N. H. Lee, unpublished data) facilitating the cytoskeletal changes noted after overexpression of $S1P_2$ and $S1P_3$. Regulation of intracellular cAMP levels by $S1P_2$ and $S1P_3$ occurs in both PTX-sensitive and -insensitive manners, depending on cell type; this may also be a result of the diversity in G protein coupling (68,70).

While $S1P_2$ has long been implicated as an essential regulator of development, recent findings indicated an unanticipated role in neuronal excitability (71). $S1P_2$ is expressed in embryonic peripheral axons (72) and has been shown to mediate S1P-induced retraction of neurites in culture (63,73). However, no defects in peripheral axons were noted in newborn $S1P_2$ null mice (71). Still, these mice had spontaneous, sporadic seizures, had abnormal EEG readings, and displayed significant increases in spontaneous excitatory postsynaptic currents, as determined by whole-cell patch-clamp techniques (71). These observations suggest that $S1P_2$ may play an active role in neurotransmission. Considering that $S1P_2$ mediates decreased neurite length in PC12 cells, it has been suggested that deletion of $S1P_2$ may cause inappropriate establishment of neuronal connections (71), resulting in hyperexcitability and seizure induction.

$S1P_5$

The newest addition to the S1P receptor family, $S1P_5$, shares an overall sequence identity of 40–44% and a transmembrane region homology of 53–55% with its subfamily members. In contrast to other S1P receptors, expression of two similar sized mRNA transcripts of 2.2- and 2.8-kb have been detected, both of which are expressed almost exclusively in the brain. Both Northern analysis and EST expression profiling reveal that $S1P_5$ expression is particularly abundant in lower brain regions, including the midbrain, pons, medulla, and spinal cord (74). *In situ* hybridization has further revealed $S1P_5$ to be associated with white matter tracts in the central nervous system (43). Importantly, $S1P_5$ mRNA levels are downregulated in response to NGF, fibroblast growth factor, and dibutyryl cAMP in PC12 cells (74). This is in direct contrast to $S1P_1$ expression, which is unaffected by NGF treatment, and $S1P_2$, which is only transiently repressed (45,75). Strikingly, $S1P_5$ is expressed in E7 and E17 mice embryos, but not in E11 or E15 embryos (76). These findings suggest that $S1P_5$ may be particularly important in the process of developmental neuronal differentiation.

Differentiation of PC12 cells requires sustained activation of the ERK pathway (77) which might be dependent on protein kinase A (PKA) (78). Further-

more, $S1P_5$ downregulation is also PKA dependent (74), suggesting that $S1P_5$ may be a negative regulator of differentiation. Overexpression of $S1P_5$ in CHO cells results in a marked decrease of proliferation (79), while in PC12 cells, it induces PTX-insensitive neurite retraction (R. E. Toman, unpublished data), further supporting the notion that $S1P_5$ may be important in terminal differentiation.

S1P inhibits cAMP accumulation in a PTX-sensitive manner, in both CHO and Rh7777 cells overexpressing $S1P_5$, suggesting that $S1P_5$ couples to $G\alpha_{i/o}$ (43,79). Additional studies in *Xenopus laevis* oocytes demonstrated that $S1P_5$ coupled to $G\alpha_{o/i}$ and $G\alpha_{q/o}$ to induce calcium mobilization, but did not activate $G\alpha_{q/s}$ (43). In CHO cells overexpressing $S1P_5$, S1P repressed serum-induced phosphorylation of ERK1/2 in a PTX-insensitive yet orthovanadate-and okadaic acid–sensitive fashion. This pattern of regulation is reminiscent of the angiotensin-2 (AT_2) receptor as in cultured neurons, AT_2 inhibits serum activation of ERK1/2 (80), and in N1E-115 neuroblastoma cells, AT_2 receptors act though a PTX-insensitive and orthovanadate-sensitive pathway, possibly involving SHP-1 (81). It has been proposed that the antagonistic modulation of ERK activity by the AT_1 and AT_2 receptors acts as a molecular counterbalance system (80). It is tempting to speculate that differential activation of S1P receptors may function in a manner similar to that of the AT_1/AT_2 system. *In vitro* analysis revealed that $S1P_5$ also couples to $G\alpha_{12}$. Remarkably, binding of S1P to $S1P_5$ also resulted in specific phosphorylation of the p54 isoform of JNK (79). Thus, $S1P_5$ may be an important regulator of proliferation and differentiation in the mammalian nervous system.

CONCLUSION

Current research on LPA and S1P receptors suggests that they signal primarily through direct coupling to GPCRs. However, the reciprocal interaction of $S1P_1$ and PDGF, as well as the ability of $S1P_5$ to inhibit ERK activation by regulation of tyrosine phosphatases, suggests that lysophospholipid receptors may regulate GPCRs in unique ways. This would not be the first novel signaling pathway to play in a role in neuronal development. Recently, Flanagan and colleagues characterized the regulation of the GPCR, CXCR4, via the reverse signaling of Eph-B activation (82). In this system, the C terminus of bound ephrin-B binds PDZ-RGS3, which can inhibit heterotrimeric G-protein signaling through the guanine nucleotide activating protein (GAP) activity of its RGS domain. It is tempting to speculate that PDZ-RGS3 might also regulate the function of lysophospholipid receptors.

It is known, however, that a major locus of expression for lysophospholipid receptors is the nervous system. Analysis has revealed S1P and LPA receptor expression in neuroblasts, neurons, and glial cells (28,30,43,74,83). The temporal expression of these receptors, together with their regulation of pathways crucial for survival, proliferation, migration, differentiation, and neurotransmission, suggests roles for LPA receptors and S1P receptors in both the developing and mature nervous system. Future analysis will certainly elucidate the regulatory roles of the lysophospholipid receptor signaling systems in these processes. Given that many biological functions are predominantly receptor driven, there is great potential of specific agonists and antagonists for these receptors for the treatment of developmental disorders as well as for neurodegenerative diseases.

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