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LYSOPHOSPHOLIPID RECEPTORS

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■ **Abstract** Lysophospholipids (LPs), including lysophosphatidic acid and sphingosine 1-phosphate, produce many cellular effects. However, the prolonged absence of any cloned and identified LP receptor has left open the question of how these lipids actually bring about these effects. The cloning and functional identification of the first LP receptor, *lp_{A1}/vzg-1*, has led rapidly to the identification and classification of multiple orphan receptors/expression sequence tags known by many names (e.g. *edg, mrec1.3, gpcr26, H218, AGR16, nrg-1*) as members of a common cognate G protein-coupled receptor family. We review features of the LP receptor family, including molecular characteristics, genomics, signaling properties, and gene expression. A major question for which only partial answers are available concerns the biological significance of receptor-mediated LP signaling. Recent studies that demonstrate the role of receptor-mediated LP signaling in the nervous system, cardiovascular system, and other organ systems indicate the importance of this signaling in development, function, and pathophysiology and portend an exciting time ahead for this growing field.

INTRODUCTION

Lysophospholipids (LPs) have been long considered to be metabolites in the biosynthesis of membrane phospholipids. Two prominent examples of LPs are lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) (see Figure 1 for chemical structures of LPs and related lipids). In addition to their metabolic roles, however, both of these structurally related LPs also have cell-signaling properties, producing similar types of responses in a range of cells. Some of the earliest examples of potential LP signaling were reported in the 1960s when LPA was found to induce intestinal contraction (1).

In early mechanistic studies, LPs were postulated to act primarily as intracellular second messengers, as suggested for LPA (2–5) or based upon intrinsic chemical

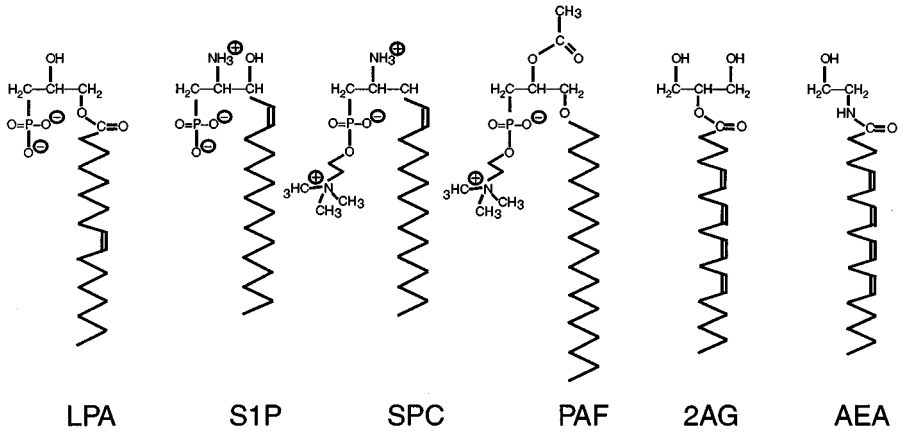

Figure 1 Chemical structures of signaling lysophospholipids and related lipids. LPA, lysophosphatidic acid; S1P, sphingosine 1-phosphate; SPC, sphingosylphosphorylcholine; PAF, platelet-activating factor; 2AG, 2-arachidonyl glycerol; AEA, arachidonyl ethanolamide.

properties (e.g. Ca^{2+}-binding activity of LPA) (2, 6). Just as with LPA, a mechanism postulated to be responsible for S1P effects included second-messenger roles (5, 7).

However, a seminal study by van Corven et al demonstrated that the proliferative effects of LPA in fibroblasts were mediated by G proteins (8). This finding raised the probability that a cognate G protein-coupled receptor (GPCR) could exist, which would account for many of the diverse cellular effects induced by LPA in many types of cells (9, 10). Similarly, exogenous application of S1P also was shown to result in various cellular responses (11–15) that implicated GPCR-based mechanisms (13, 14). In addition, the similarity of the cellular responses documented for both LPA and S1P suggested that these two LPs might even share the same GPCRs (16–18).

However, despite circumstantial data supporting the existence of LPA or S1P GPCRs (17, 19, 20), cloned LP receptors defied identification. This failure was in part a reflection of the lipophilic nature of the ligands, which complicated standard approaches such as expression cloning. The absence of cloned receptors was compounded by an absence of any specific competitive antagonists to block LP-dependent responses, leaving open alternative possibilities to explain these responses.

Identification of the first LP receptor gene, *ventricular zone gene 1* (*vzg-1*), was reported in 1996 during studies on mammalian neurogenesis in which a cloned orphan receptor was isolated by virtue of its expression in the neurogenic zone of the embryonic cerebral cortex (21–23). *vzg-1* encoded a GPCR that had the properties of a high-affinity LPA receptor. Based on the nucleotide sequence of *vzg-1*, it immediately became apparent that several orphan receptor genes or expression sequence tags (ESTs) in DNA sequence databases most certainly

interacted with LPA or similar LP ligands such as S1P—particularly in view of the above-mentioned studies suggesting a common receptor for these two lipids. Following identification of the first LPA receptor, the first S1P receptor was identified independently by both Zondag et al (24) and Lee et al (25) in 1998. Subsequent studies on homologous genes by many groups (noted below) have revealed the existence of a gene family of LP receptors with a high degree of predicted amino acid similarity (22, 23). Based on sequence similarity and genomic structures, this GPCR gene family consists of at least two major subgroups (22, 23). One group consists of the LPA receptor subfamily (commonly referred to by an orphan receptor gene nomenclature: *vzg-1*/endothelial differentiation gene, edg (*edg-2/mrec1.3*, *edg-4*, and *edg-7*), whereas the other group (*edg-1, edg-3, H218/AGR16/edg-5*, and *nrg-1/edg-8*) represents the receptor subfamily for S1P (Figure 2, Table 1). We currently use a nomenclature based on receptor function in which LPA receptors are termed LP receptors of an "A" group consisting of three known members (LP_{A1}, LP_{A2}, and LP_{A3}) and a "B" group consisting of S1P receptors (LP_{B1}, LP_{B2}, LP_{B3}, and LP_{B4}; Figure 2, Table 1). The subscript numbers indicate the order in which function was reported. (22, 23, 26). Another related receptor gene, *edg-6*, also has been shown to mediate S1P responses (27, 28). However, based on its

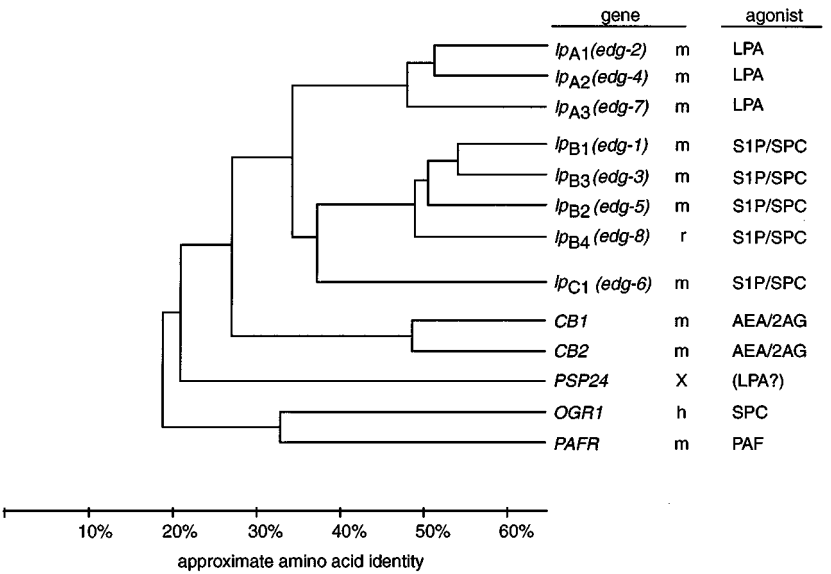

Figure 2 Divergence of the lysophospholipid and related lipid receptor gene families. *CB*, cannabinoid receptor; *PAFR*, PAF receptor; m, mouse; r, rat; X, *Xenopus*; h, human. The *edg-4* sequences are nonidentical with lp_{A2}, and sequence similarities were calculated based on lp_{A2} sequences (see text for details). The closest branch point between two genes is located at the point on the horizontal axis corresponding to the approximate percentage of amino acid identity between the encoded proteins.

TABLE 1 Characteristics of lysophospholipid receptors

Name (gene)	Synonyms	Chromosomal location	Coupled G proteins	Cellular function
LP_{A1} (*lp_{A1}*)	VZG-1 MREC1.3 GPCR26 EDG-2	Mouse 4, close to *vc* Human 9q31.3-32	$G_{i/o}$ $G_{q/11/14}$ $G_{12/13}$	AC inhibition SRE activation DNA synthesis PLC activation Actomyosin stimulation
LP_{A2} (*lp_{A2}*)	Nonmutated EDG-4	Mouse 8, close to *kat* Human 19p12	$G_{i/o}$ $G_{q/11/14}$ $G_{12/13}$	AC inhibition MAP kinase activation SRE activation PLC activation Actomyosin stimulation
LP_{A3} (*lp_{A3}*)	EDG-7 (EDG-5)	Mouse 3, close to *va* Human 1	$G_{i/o}$ $G_{q/11/14}$	AC stimulation or inhibition MAP kinase activation PLC activation
LP_{B1} (*lp_{B1}*)	EDG-1	Mouse 3	$G_{i/o}$	AC inhibition MAP kinase activation PLC activation
LP_{B2} (*lp_{B2}*)	AGR16 H218 EDG-5	Mouse 9 Rat 8, close to *lp_{B4}*	$G_{i/o}$ $G_{q/11/14}$ $G_{12/13}$	AC stimulation MAP kinase activation SRE activation PLC activation Actomyosin stimulation
LP_{B3} (*lp_{B3}*)	EDG-3	Mouse 13 Human 9q22.1	$G_{i/o}$ $G_{q/11/14}$ $G_{12/13}$	AC inhibition/ stimulation MAP kinase activation SRE activation PLC activation
LP_{B4} (*lp_{B4}*)	NRG-1 EDG-8	Rat 8, close to *lp_{B2}*	$G_{i/o}$	AC inhibition
LP_{C1} (*lp_{C1}*)	EDG-6	Mouse 10 Human 19p13.3	$G_{i/o}$	PLC activation MAP kinase activation

AC, adenylyl cyclase; SRE, serum response element; PLC, phospholipase C.

distinct amino acid residues, this gene may warrant classification as a member of a third C group (LP_{C1}) or as a dissimilar member of the B group. This nomenclature also avoids confusion over names shared by nonreceptor molecules [e.g. *edg-3*, (29)].

In addition to the LP receptor family, two other receptors are notable. A reported *Xenopus* LPA receptor called PSP24 has low sequence similarity to LP

receptors (30; Figure 2). It also has low sequence similarity to another, distinct receptor that binds another phospholipid-signaling molecule, platelet-activating-factor (PAF), despite use of cloning strategies based on PAF receptor homology (30). It currently remains unclear whether PSP24 is an LPA receptor (30a), and for this reason it will not be discussed further in this review. Of more interest to this discussion of LP receptors is a receptor with some sequence similarity to the PAF receptor that recently has been identified and referred to as OGR1 (31). OGR1 appears to be a cognate GPCR for sphingosylphosphorylcholine [SPC (32; Figure 1)]. Here we review what is known about the LP receptor family. Aspects of their predicted amino acid sequence, genomic structure, expression patterns, and signaling properties are considered. Biological roles of receptor-mediated LP signaling are also discussed. Glimpses of these roles have come from more recent studies, and pertinent examples will be noted. The historical background for LPA-, S1P-, and SPC-induced cellular responses has been extensively examined in many excellent reviews (5, 9, 10, 33, 34) and will not be covered here.

LYSOPHOSPHATIDIC ACID RECEPTORS

lp_{A1}/vzg-1/mrec1.3/edg-2

The first LPA receptor gene was identified during developmental studies of the mouse cerebral cortex in which experiments were designed to identify novel GPCR genes associated with the production of neurons (21–23). The search for novel GPCRs associated with neurogenesis was based on the existence of G proteins found during embryonic development and the clear role of such receptors in cell proliferation and differentiation in other organisms (35, 36). GPCR gene fragments were amplified by degenerate polymerase chain reaction (PCR) of cDNAs from immortalized mouse cortical neuroblast cell lines (37). The resulting products were used for in situ hybridization screening to identify genes with enriched expression within the embryonic cortex, particularly within neurogenic regions [ventricular zone or VZ (Figure 3*A*)]. This analysis led to the cloning of a then novel orphan receptor gene, *vzg-1*, so named because of its enriched expression pattern within the VZ of the embryonic cerebral cortex (21; reviewed in 22, 23). This name has since been replaced by its functional nomenclature name, *lp_{A1}*.

Analyses of the obtained mouse cDNA revealed that it encoded a 41-kDa protein consisting of 364 amino acids with seven transmembrane domains. It shared homology with two known receptors, the cannabinoid and melanocortin receptors (30% and 32% amino acid identity, respectively). The greatest similarity was shared with an orphan receptor cloned from human endothelial cells called *edg-1* [37% amino acid identity (Figure 2)] (38).

Subsequent analyses of mouse lp_{A1} genomic structure revealed that the gene spans >45 kilobases (kb) and contains five exons with one intron located within the middle of transmembrane domain VI, which, to date, appears to be a feature unique to these LPA receptor genes (23, 39). Alternative use of an exon also produces

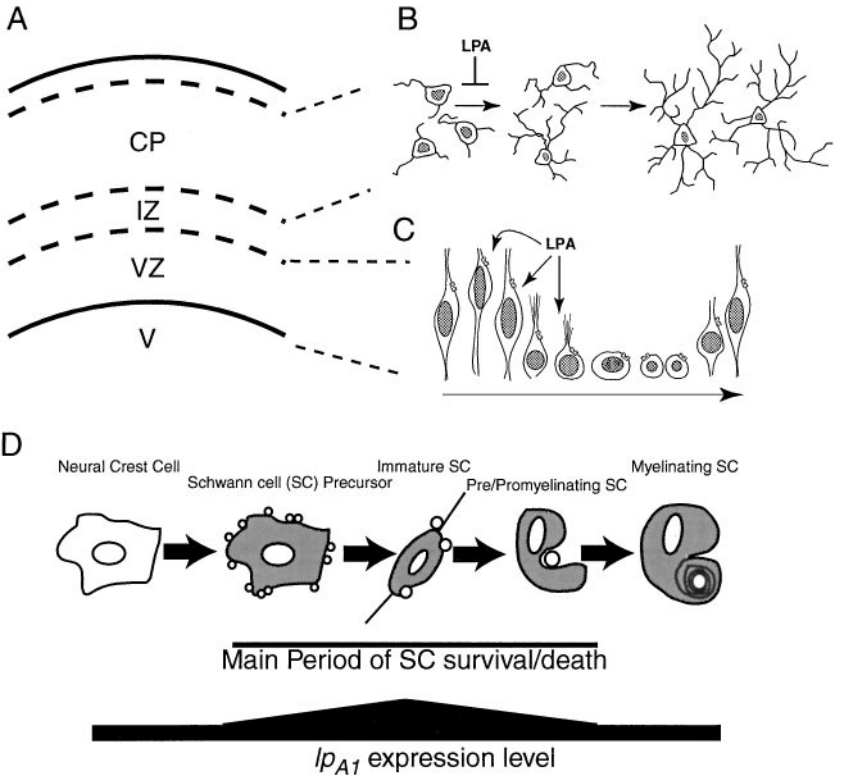

Figure 3 Background on development of cerebral cortical neuroblasts and Schwann cells. (*A*) Anatomical zones in the embryonic cerebral cortex. CP, cortical plate; IZ, intermediate zone; VZ, ventricular zone; V, lateral ventricle. (*B*) Differentiating neurons undergoing process outgrowth in the CP. LPA may alter process formation and/or growth. (*C*) Neuroblasts undergoing morphological changes associated with cell- cycle progression (known as "interkinetic nuclear migration") in the VZ. LPA actions may be involved in this phenomenon. (*D*) Schwann cell development. As Schwann cells differentiate to form myelin contacts with axons, supernumerary cells are removed by apoptotis. Temporal expression of lp_{A1} corresponds with this period.

another variant of this receptor, *mrec1.3*; the name reflects its first report as an orphan GPCR gene (40). This isotype lacks 18 amino acids in the amino-terminal region. The lp_{A1} gene is located on mouse chromosome 4 for most of the mouse strains examined, with an additional, partial duplication of the locus present in *Mus spretus* on chromosome 6 (39). The location of lp_{A1} on chromosome 4 maps near to a gene called *vacillans* (*vc*). This extinct mutant mouse showed nervous system defects (41; for more about this relationship, see below).

In our initial studies using gene overexpression in cortical neuroblast cell lines, from which lp_{A1} was isolated, we concluded that LP_{A1} was a functional LPA

receptor that mediated the inhibition of cyclic AMP (cAMP) production and the stimulation of cell rounding (21). These overexpression studies included analyses of endogenous receptor protein expression and also utilized an extensive repertoire of independent analyses. Although somewhat surprising skepticism over this identity persisted (42, 43), further analyses have eliminated the few remaining doubts.

A key part of such analyses of LPA receptors was the development of a heterologous expression system based on identification of two mammalian cell lines that did not express lp_{A1} or respond to LPA (44). These cell lines were derived from two distinct cellular lineages—hepatoma (RH7777) and neuroblastoma cells (B103)—and are now in common use in this field (45, 46). Because of the lipophilic nature of LPA, which precludes detailed approaches to receptor–ligand-binding studies, several alternative strategies were developed that reliably assessed LPA-dependent receptor activation. These strategies were based on rapidly occurring, cell biological changes (induction of cell rounding in B103 and stress fiber formation in RH7777) and [^{35}S]GTPγS binding to plasma membranes (44, 47). The use of an epitope-tagged LP_{A1} combined with these assays for LPA responses shows that lp_{A1} expression is sufficient to produce LPA-dependent signaling, including G protein activation, Rho-mediated actin rearrangement, increased cell proliferation, and stimulation of serum response elements (SREs) in these cells (Figure 4) (44). These data have confirmed that LP_{A1} couples to $G_{i/o}$, which leads to cell proliferation. In addition, LP_{A1} is also coupled to the Rho pathway, leading to actin rearrangement and cell proliferation. Both G_i and Rho activation are also observed in Schwann cells, with the major cell survival pathway, rather than proliferation, apparently involving G_i-mediated activation of phosphatidylinositol 3-kinase and Akt (48) (JA Weiner, N Fukushima, J Chun, in preparation; Figure 4). Recently, our data with heterologous expression of lp_{A1} in B103 neuroblastoma cells have indicated that LP_{A1} induces phospholipase C (PLC) activation through a pertussis toxin (PTX)-insensitive pathway, implicating the interaction of LP_{A1} with G_q family members (48a). These data demonstrate that LP_{A1} acts as a multifunctional LPA receptor that couples to $G_{i/o}$ and PTX-insensitive G proteins ($G_{12/13}$ and $G_{q/11/14}$) in distinct cell types (Figure 4). This finding is supported in part by the demonstration of coupling between LP_{A1} and $G_{i/o}$ or G_{11} (49). It should be noted that coupling may vary depending on receptor expression levels or amounts of available G protein during the assay, which might explain the differing results that have been obtained using different cell lines or expression systems (46, 50, 51).

lp_{A1} in situ hybridization and Northern blot analyses first detected lp_{A1} expression within the embryonic nervous system (21). During embryonic life, lp_{A1} is expressed prominently in the VZ of the developing cerebral cortex in the mouse (Figure 3*A*). This locus of expression developmentally follows neurogenesis within the cerebral cortex. The expression gradually diminishes with disappearance of the VZ by the end of cortical neurogenesis, which occurs just before birth (21, 52). During this embryonic period, a second locus of lp_{A1} expression is present near the pial (outer) surface of the embryonic cerebral cortex, although the precise cell type responsible for this expression has not been determined.

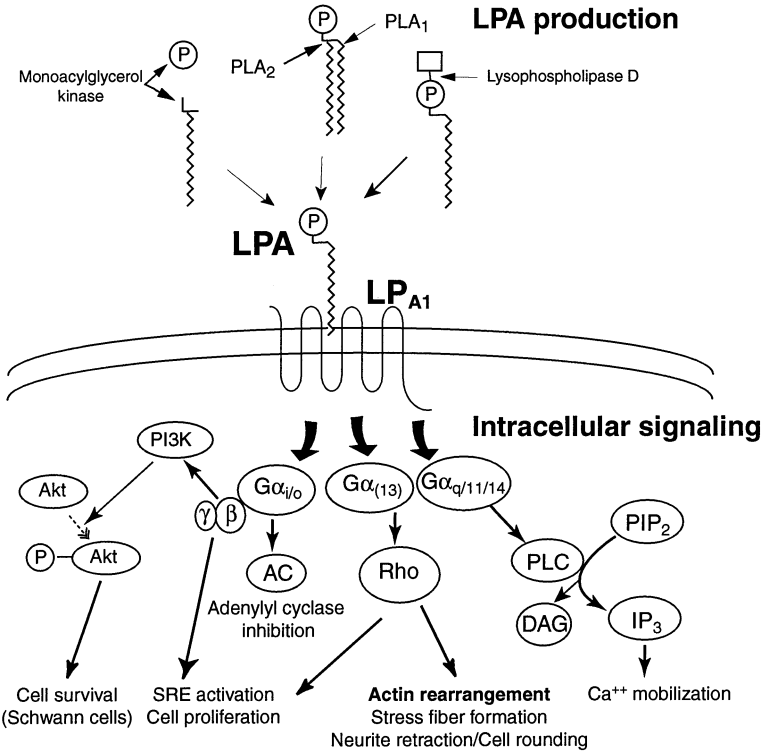

Figure 4 LP_{A1}-mediated signaling. LPA production, receptor binding, and resultant activation of diverse cellular responses.

During postnatal life, lp_{A1} expression reemerges in a different cellular locus in the central nervous system (43, 52). The lp_{A1} expression first reappears in the hindbrain around postnatal day 3 and subsequently expands from caudal to rostral regions with peak expression around postnatal day 18. The expression is concentrated in and around white matter tracts in which oligodendrocytes, the myelinating cells in the central nervous system, are prevalent. Studies using double-label in situ hybridization with lp_{A1}, combined with either the proteolipid protein gene (an oligodendrocyte marker) or glial fibrillary acidic protein gene (an astrocyte marker), confirmed the lp_{A1}-expressing cells as oligodendrocytes but not astrocytes. In addition, Schwann cells, the myelinating cells in the peripheral nervous system, also express lp_{A1}, with the highest levels of expression being observed in the first postnatal week (48). Thus, it is likely that lp_{A1} expression correlates both temporally and spatially with the development of oligodendrocytes and Schwann cells, implicating LPA signaling as a regulator of some aspect of the biology of these myelinating cells (see below for details).

The expression of lp_{A1} is widespread outside the nervous system (21). Northern blot analyses revealed that lp_{A1} was expressed prominently in testis and

intestine and modestly in heart, lung, kidney, spleen, thymus, muscle, and stomach (21, 40, 52a). However, detailed cellular loci remain to be determined, and no immunohistochemical data for endogenous receptor expression are currently available. In addition to this expression in normal organs, lp_{A1} is also expressed in several cancers (53), which suggests pathological roles for receptor-mediated LPA signaling (see below).

lp_{A2}/*edg-4* (Nonmutant Form)

GenBank searches revealed a novel human genomic sequence that encoded a GPCR with ~60% amino acid similarity to lp_{A1} (Figure 2, Table 1) (22, 39). Our characterization of an isolated mouse homolog (cDNA and genomic DNA) (54) for this gene, lp_{A2}, demonstrated a predicted amino acid sequence of 348 amino residues, yielding a calculated molecular mass of 39 kDa. Analyses reveal that the lp_{A2} gene structure consists of three exons with the previously noted intron inserted within putative transmembrane domain VI, as observed for lp_{A1}, indicating that lp_{A1} and lp_{A2} were derived from a common ancestral gene. The lp_{A2} gene is located on chromosome 8, near loci that account for myodystrophy (*myd*) and "kidney-anemia-testis" (*kat*), although it is likely that neither phenotype is caused by the lp_{A2} mutation. Another intriguing feature of the lp_{A2} gene is the existence of several human variants observed in cancer cells. This feature was most obvious in a large number of nucleotide variations in 3′ untranslated regions (mostly by single nucleotide replacement). The prevalence of mutations within this gene likely accounts for the inadvertent use of a carboxyl-terminal mutant, which was used in a study designed to analyze human lp_{A2} (named *edg-4*) (51, 55). This aberrant clone was obtained from an ovarian tumor cell line, and subsequent analyses demonstrated that the clone contained a G deletion that produced a frame-shift mutation. This mutation results in a predicted mutant protein with 4 replaced and 31 additional amino acids (382 amino acids as compared with native lp_{A2} which has 351 amino acids) (54). Because C-terminal regions of GPCRs are generally essential for appropriate coupling to G proteins, this mutated LP_{A2} may produce different cellular responses compared with wild-type LP_{A2}, such as those associated with cellular transformation.

Despite initial examination of a mutant LP_{A2}, the properties of both mutant and wild-type LP_{A2} support its identity as a second LPA receptor (46, 48a, 51, 55). LP_{A2} couples to G_i and G_q, which mediates LPA-induced PLC activation and leads to intracellular Ca^{2+} increases and inositol phosphate production (48a, 50, 51). The estimated 50% effective concentrations to achieve a variety of responses are also, as with LP_{A1}, in the low nanomolar range. These properties, taken together, demonstrate that LP_{A2} is a multifunctional LPA receptor and thus functionally is similar to LP_{A1} (Table 1). As with LP_{A1}, the coupling of LP_{A2} to specific G proteins also appears to depend on the assay system used.

Northern blot analyses showed that embryonic and neonatal mouse brains expressed two lp_{A2} transcripts of distinct size (2.8 kb and 7 kb); however, neither transcript was detectable in adult brain tissue (52a). This temporal expression profile was also confirmed by our in situ hybridization studies, which suggest

expression of lp_{A2} in postmitotic neuronal regions (e.g. developing cortical plate, Figure 3*A*) as well as the VZ of embryonic mice (C McGiffert & J Chun, in preparation). These findings suggest a role for lp_{A2} in brain development. Two species of transcripts were also observed in human lymphoid cell lines, although these transcripts differed in size (1.8 kb and 8 kb) from those found in the mouse (55). The generation of these two species may be caused by distinct polyadenylation sites (54). Outside the nervous system, the smaller transcript was abundant in testis, kidney, and lung in adult mice (52a).

lp_{A3}/edg-7 (edg-5)

In the course of studies on lp_{A1} and lp_{A2}, we identified a third related gene, lp_{A3}, (filed 12/1997 as *edg-5* in U.S. patent 6057126). lp_{A3} encodes a 40-kDa GPCR of 354 amino acids with ~60% amino acid similarity to mouse lp_{A1} and lp_{A2} (Figure 2). Independent isolation by reverse transcriptase-polymerase chain reaction, with degenerate primers and Jurkat T-cell RNA, also identified the same human gene (called *edg-7*) (51). Mouse lp_{A3} is present on chromosome 3 near the varitint waddler (*va*) locus and consists of three exons along with an identically placed intron located within transmembrane domain VI, just as is found for lp_{A1} and lp_{A2} (52a). Based on the similarity in the conserved intron insertion and predicted amino acid sequence, this gene is clearly a third member of the same LP_A family of receptors.

One anticipated function of LP_{A3}, LPA-dependent increases in intracellular Ca^{2+} concentration, was confirmed by studies of mammalian (RH7777 cell line) and insect (Sf9 cell line) cells overexpressing lp_{A3} (46, 51). This LP_{A3}-mediated Ca^{2+} increase was not blocked by pretreatment with PTX. Our studies also confirmed that LP_{A3} activated PLC in a PTX-independent manner (48a). These results suggest coupling of LP_{A3} to a member of the G_q family. Less certain is the possible LP_{A3} coupling to G_i, where studies have yielded contradictory or equivocal results. LP_{A3} activation leads to stimulatory pathways for adenylyl cyclase in insect cells (51); whereas in mammalian cells, there is no (46) or significant coupling (48a). Thus, the involvement of $G_{i/o}$, as well as $G_{12/13}$, has not yet been fully determined, although it would be somewhat surprising if these pathways were not activated, and leaves open the question of actual signaling properties for this particular receptor.

lp_{A3} expression was most abundant in human testis, prostate, heart, and frontal regions of cerebral cortex (46, 51), although it was detected prominently in mouse lung, kidney, and testis (52a).

BIOLOGICAL FUNCTIONS OF LYSOPHOSPHATIDIC ACID

Essential questions about receptor-mediated LPA signaling have moved from the identification of receptors to understanding the in vivo roles of receptor-mediated signals.

Nervous System

A major locus of expression for LPA receptors is the nervous system. Analyses of lp_{A1} expression by in situ hybridization identified three major cellular loci in the nervous system: cortical neuroblasts of the neuroproliferative VZ, oligodendrocytes, and Schwann cells (21, 43, 48, 52). Preliminary studies suggest that lp_{A2} may be expressed in both overlapping and complementary populations of cells, such as postmitotic or differentiating neurons during development (C McGiffert & J Chun, in preparation). We have used the nervous system as a model for exploring the function of receptor-based LPA signaling, and we will now discuss the possible roles for LPA signaling in the nervous system.

Cortical Neuroblasts In mammals, most cerebral cortical neurons are generated by neuroblasts that proliferate in the VZ, a pseudostratified zone overlying the lateral ventricle (56) (Figure 3*A*). As the cell cycle proceeds during neurogenesis, neuroblast nuclei show a to-and-fro movement along the radial (apico-basal) axis. This movement has been termed "interkinetic nuclear migration" (57) (Figure 3*C*). During the S phase of the cell cycle, neuroblasts are fusiform with nuclei positioned at the superficial border of the VZ. During the G2 phase, the nucleus descends to the ventricular surface, the pial process is retracted, and the cell rounds up. The cell then divides at the M phase, after which daughter cells either migrate away as postmitotic neurons, undergo programmed cell death (58, 59), or reextend their bipolar processes to enter the cell cycle again (57, 60). Several peptidergic growth factors or neurotransmitters have been shown to influence neuroblast proliferation by activating receptor tyrosine kinase pathways or modulating ionic conductance (61). However, the molecular mechanisms underlying neuroblast morphological changes and cell cycle progression remain unclear.

To determine whether exogenous LPA signaling could affect cerebral cortical neuroblasts from the VZ on a short time scale, LPA-dependent ionic conductance changes were examined by using whole-cell recordings on acutely dissociated VZ neuroblasts (62). Exogenous application of LPA induces two clear types of ionic changes within the majority of VZ neuroblasts, changes that occur within 30 to 60 s. These changes consist of increases in either chloride or nonselective cation conductances and result in depolarization of the cells, based on the resting potential. It is interesting that the ionic responses developmentally precede or coexist in a neuroblast population with the previously identified γ-aminobutyric acid and/or L-glutamate ionotropic conductance changes (62, 63). The LPA-dependent ionic changes might modulate DNA synthesis or cell proliferation, which is consistent with recent studies showing proliferative effects of LPA in VZ neuroblasts (63a).

In addition to inducing electrophysiolgical changes, LPA induces morphological changes (cellular and nuclear migration, cell rounding, and the formation of fine retraction fibers) of VZ neuroblast in cluster cultures and whole brain explant cultures (63b). The intracellular signaling pathways (Rho and actomyosin pathways) activated in response to LPA are similar to those mediated by LP_{A1} in neuroblastoma

cell lines (44). The LPA-induced morphological changes in neuroblasts are similar to those observed during interkinetic nuclear migration (60), suggesting the involvement of LPA signaling in this classical feature of VZ neuroblasts (Figure 3*A*).

Collectively, these multiple functions for LPA in proliferative cortical neuroblasts implicate receptor-mediated LPA signaling as a biologically relevant mechanism during cortical neurogenesis. Many peptide growth factors or transmitters, as well as their receptors, are also present during the same embryonic period (61), suggesting that these components are likely to function harmoniously with one another. Indeed, it has been documented that LPA-induced activation of intracellular signaling pathways can cross-talk with those pathways activated by other stimuli via receptor tyrosine kinases [e.g. epidermal growth factor receptor (64)]. The cooperative interaction between LPA and other neurogenic signals is an area for future investigation.

Differentiating Neurons Cerebral cortical neuroblasts exit the cell cycle to become postmitotic neurons and leave the VZ by migrating toward the subplate, intermediate zone, and cortical plate. As these cells reach their final destination in the cortical plate, they continue differentiation that includes axonal elongation and dendritic branching (Figure 3*B*). Both cell migration and neurite formation require cytoskeletal rearrangement. Extracellular signaling factors, such as chemorepellants or chemoattractants, have been demonstrated to play guidance roles in migration and neurite formation through cytoskeletal rearrangement (65, 66). LPA may have pertinent signaling roles based on several observations. First, LPA induces cytoskeletal rearrangement in neuroblastoma cells (67, 68) or primary neurons (69, 70). Second, LPA has regulatory effects on cell migration in some cell lines (17, 71, 72). Third, lp_{A2} expression appears to occur in these differentiating neurons. Preliminary analyses in support of this relationship suggest that LPA signaling influences neurite formation of differentiating neurons (N Fukushima & J Chun, in preparation).

Myelinating Cells: Oligodendrocytes and Schwann Cells lp_{A1} is expressed in myelinating cells of both the peripheral and central nervous systems (48, 52). This finding implicates LPA signaling in the biology of these cells. In addition, two observations relate to possible lp_{A1} expression in previously described mutant mice that show dysfunction of myelinating cells. In the *jimpy* mouse, increased oligodendrocyte apoptosis is associated with decreased expression of lp_{A1} (52). As mentioned above, another mutant, vacillans, mapped to the same proximal region of chromosome 4 as lp_{A1} (39), and this extinct mutant mouse exhibited signs of peripheral neuropathy consistent with a defect in nerve function that can accompany demyelination (41). These data suggest that LP_{A1}-mediated signaling could influence the basic neurobiology of myelinating cells, including their ability to survive.

To address this possibility in an experimentally tractable culture system, Schwann cells were examined because of their ease of growth in culture compared

with oligodendrocytes. Primary cultures of rat Schwann cells are usually grown in the presence of serum but can be induced into apoptosis via serum withdrawal (73, 74). Part of this apoptotic response may reflect the loss of LPA signaling because LPA is present in serum at micromolar concentrations (75). To assess potential survival roles, the effects of LPA were examined in a well-defined culture model of Schwann cell survival. Exogenously added LPA in serum-free medium protects these cells from apoptosis following serum withdrawal without affecting cell proliferation (48). The potency is equivalent to that of neuregulins, which are peptidergic survival factors for Schwann cells that signal through cognate receptor tyrosine kinases (73). Of particular note is that LPA-induced survival effects are mediated by LP_{A1}-activated phosphatidylinositol 3 kinase/Akt pathways (Figure 4) that are also activated by neuregulins. These results indicate that divergent extracellular signaling can produce convergent signaling, which, in theory, might also compensate should disruption of one signal occur. It is interesting that S1P showed no effect on Schwann cell survival, in spite of the relatively abundant expression of its cognate GPCR, LP_{B3}, which indicates a nonredundant role for LP receptors in this system.

As Schwann cells differentiate and myelinate axons during postnatal development, Schwann cell survival is regulated via apoptotic mechanisms (Figure 3*D*) (48, 73). During this period, lp_{A1} expression is upregulated (48), which suggests a physiological role for LP_{A1}-mediated LPA signaling in peripheral nerve development. Because similar intracellular signaling pathways (e.g. phosphatidylinositol 3 kinase/Akt) are involved in oligodendrocyte survival (76), LP_{A1}-mediated signaling may play a similar role in the survival of central myelinating cells.

Other Nervous System Loci Little is known about the role for LP receptor signaling in neurotransmission. However, a recent report has demonstrated that local injection in vivo of antisense oligonucleotides against lp_{A1} partially attenuates LPA-induced nociception (77). This study suggests the involvement of LP_{A1} in pain transmission in the peripheral nervous system.

Tumor Cells

LPA has been shown to influence cell motility in cancer cells by means of cytoskeletal rearrangement via Rho and/or Rac signaling pathways, although other types of signaling [e.g. PLC, protein kinase C, or Ca^{2+} signaling] are also important (17, 70, 78–80). These changes lead to the stimulation or inhibition of cell migration or invasion of cancer cells, depending on cell types. Given the presence of LPA in ascites from ovarian cancer patients (81, 82), the effects of LPA on cancer cells are an important issue that needs to be addressed. Although it is still unclear how individual LPA receptors are involved in LPA-dependent invasion of cancer cells, accumulating evidence suggests roles for certain types of LPA receptors in tumor biology. For example, in ovarian cancer cells, lp_{A1} has been suggested to be a negative regulator for cell growth, although this effect is independent of

LPA (53). lp_{A2} has also been suggested to be involved in the LPA-dependent cell proliferation (83).

Recent Studies on Genetic Nulls

A major impediment to assessing the roles for any LP in a biological setting is the absence of specific competitive antagonists for any receptor. An alternative strategy that is being pursued by many groups is the creation of genetic nulls for these receptors. Our analyses of lp_{A1}-null mice have revealed that many of these mice have impaired suckling behavior, and all have reduced total body mass and craniofacial defects (63a). These findings implicate a nonredundant role for single LP receptors, and further demonstrate the requirement for members of this gene family in mediating LP effects in vivo.

PRODUCTION AND DEGRADATION OF EXTRACELLULAR LYSOPHOSPHATIDIC ACID

To better understand the biology of signaling LPA, the cells of origin and metabolic and biosynthetic pathways need to be determined. Major cellular sources of LPA include platelets and adipocytes (75, 84). Both release LPA when stimulated by thrombin or alpha-receptor agonists, respectively. Platelet-derived LPA is present in serum at micromolar levels and is bound to albumin and/or low-density lipoprotein, which provides stabilization against degradation by phospholipases (85, 86). This LPA has been considered to play an important role in pathological conditions, such as wound healing. Adipocyte-derived LPA appears to mediate the proliferation of preadipocytes, suggesting paracrine mechanisms for the growth of adipose tissue. In addition to these LPA sources, LPA has also been detected in ascites from ovarian cancer patients (81, 82). Recently we have demonstrated that postmitotic cortical neruons are capable of producing LPA (63b). Secreted LPA may be a signaling component regulating cortical neurogenic events. This list is surely a partial one considering the wide distribution of LPA receptors. The major difficulty in addressing the site of synthesis for signaling LPA is that the biochemical pathways of production are incompletely defined (87), especially within primary cells from specific anatomical regions such as the many cell types within the central nervous system. Secreted PLA_2 is thought to be involved in LPA production (88). However, there are other types of phospholipases or lipid kinases that may be involved in LPA production, including lysophospholipase D, PLA_1, and monoacylglycerol kinase; the latter two produce arachidonyl-LPA (Figure 4) (87, 89).

Several enzymes that are potentially involved in the degradation of LPA have been documented, including lysophospholipases, lipid phosphate phosphohydrolases, and endophilin (90–92). However, lysophospholipases are considered to be intracellular enzymes that can also cleave other nonsignaling LPs, such as lysophosphatidylcholine (91). Whether these enzymes physiologically function in

the degradation of extracellular LPA remains to be determined. Lipid phosphate phosphohydrolases are hydrophobic enzymes that have four potential membrane-spanning regions (90). Lipid phosphate phosphohydrolases can degrade both LPA and S1P. Recently, another role for LPA in synaptic vesicle recycling has been demonstrated in studies of endophilin, which has acyl-CoA transferase activity that produces phosphatidic acid from LPA that is present within synaptic vesicle membranes (92). Its relationship to extracellular signaling, if it exists, remains to be determined.

SPHINGOSINE 1-PHOSPHATE RECEPTORS

With the identification of LP_{A1} as the first LP receptor (21), comparative analyses of ESTs and orphan receptor genes indicated the existence of a closely related group, the lp_B family. Although the identity of the high-affinity ligand for LP_B receptors was not known at the time, the high degree of sequence similarity made it most likely that the ligand would have similarities to LPA, particularly in view of prior functional studies that postulated a shared receptor for LPA and S1P (16–18). Two groups independently reported LP_{B1} as the first S1P receptor in 1998 (24, 25), and a wealth of subsequent studies from many groups has supported the identification of other LP_B members as high-affinity S1P receptors (10, 22, 23).

lp_{B1}/edg-1

lp_{B1} was originally isolated by Hla and Maciag as an immediate early gene induced by phorbol ester in human endothelial cells, and its orphan receptor name, *edg-1*, reflects a postulated role for the gene in endothelial differentiation (38). Analyses of the human lp_{B1} gene revealed that it encoded a GPCR consisting of 381 amino acids with a molecular mass of 43 kDa. Mouse lp_{B1} consists of two exons; the second exon includes the entire open reading frame and is localized to chromosome 3 (93; J Contos & J Chun, unpublished data). This genomic organization is common to all lp_B family members but is distinct from lp_A family members (see above).

Formal mammalian heterologous expression studies for LP_{B1}, unlike LP_{A1-3}, have not been possible because of a continued low level of expression of *lp_{B1}* or related receptor genes in all examined cell lines. Nevertheless, based on the concordance of data obtained for LP_A receptors using both overexpression and heterologous expression, it is very likely that essentially similar data will be obtained using heterologous expression approaches (see below). On the basis of LP_{B1} overexpression studies, two independent groups identified S1P as a high-affinity ligand for LP_{B1} ($K_d = 8$ nM) (24, 25). Studies from these and other groups also demonstrate that LP_{B1} activates multiple cellular responses, all mediated by a PTX-sensitive pathway (Table 1). These responses include PLC activation, intracellular Ca^{2+} mobilization, MAP kinase activation, and inhibition of forskolin-stimulated cAMP production (24, 25, 94–96). Consistent with these findings, coupling of LP_{B1}

to other types of G proteins—including G_q, G_{12}, G_{13}, and G_s—is not detectable in an overexpression system using insect cells (97), and thus far, LP_{B1} has been shown to couple only with $G_{i/o}$. However, this finding is somewhat surprising in view of LP receptors that couple with multiple G proteins; indeed, morphogenetic differentiation in LP_{B1}-overexpressing cells is not blocked by PTX but is inhibited by C3 exoenzyme (25), indicating activation of PTX-insensitive signaling mechanisms such as those present for LP_A receptors. The determination of which G proteins are involved in this process, assuming that these are not secondary effects, remains for future studies.

Northern blot analyses have shown wide expression of lp_{B1} in adult mice (38, 47). In addition to vascular endothelial cells, from which lp_{B1} was isolated, the major cellular loci identified are murine cerebellar Purkinje cells and hippocampal granule cells (93).

lp_{B2}/*AGR16/H218/edg-5*

lp_{B2} was independently cloned by two groups and given the orphan GPCR gene names of *AGR16*, for a rat gene isolated from cardiovascular system (98), or *H218*, for a rat gene from the nervous system (Table 1) (99). Our independent isolation of mouse lp_{B2} gene revealed that it encodes a 39-kDa protein of 352 amino acids with ~58% amino acid similarity to lp_{B1} (47). It is localized on chromosome 9 (G Zhang, J Contos & J Chun, in preparation) and has a genomic organization similar to that found in other members of the lp_B family.

Functional analyses of this receptor from many laboratories, including ours, confirmed that LP_{B2} also acts as a high-affinity S1P receptor (K_d = 20–27 nM) (47, 94, 97, 100–102). LP_{B2}-mediated activation of signaling pathways appears to be more divergent than those mediated by LP_{B1} (Table 1). Following S1P exposure to LP_{B2}-expressing cells, the activation of multiple effector pathways occurs, including the activation of PTX-sensitive and PTX-insensitive G proteins, which leads to PLC stimulation, Ca^{2+} mobilization, MAP and SAPK kinase activation, and actin rearrangement. However, unlike LP_{B1}, which mediates a decrease in cAMP accumulation stimulated by forskolin, the stimulation of LP_{B2} results in an increase in basal (no stimulation by forskolin) cAMP formation (94, 102).

lp_{B2} expression is abundant in the lung and heart, but less so in the brain in the adult mouse and rat (47, 98). However, it is prominent in the brain during embryonic development (99). Immunohistochemical studies revealed that LP_{B2} is possibly localized in neuronal cell bodies during differentiation and in the axon during the period of neurite outgrowth (103), which suggests a role for LP_{B2}-mediated signaling in neuronal development. This finding may be related to S1P-induced neurite retraction mediated by LP_{B2} in a neuronal cell line (100).

lp_{B3}/*edg-3*

lp_{B3} is synonymous with a third gene, *edg-3*, isolated from human genomic DNA (104). Human and mouse lp_{B3} were mapped on chromosome 9q22.1 and

chromosome 13, respectively (104, G Zhang, J Contos & J Chun, in preparation). Mouse lp_{B3} encodes a 42-kDa protein that consists of 378 amino acids with ~60% amino acid similarity to lp_{B1} (47). The binding affinity for S1P to LP_{B3} was comparable with that for other LP_B members (K_d = 23–26 nM) (94, 100). The intracellular signaling pathways activated through LP_{B3} appear similar to those activated by LP_{B2}, although they may have comparative differences in efficacy and potency in S1P stimulation between these two receptors (Table 1) (94, 97, 105).

The expression of human lp_{B3} is detected in heart and kidney tissues and at lower levels in lung tissue (104), whereas mouse lp_{B3} was highly expressed in lung, heart, and kidney tissues (47). In addition to expression in these tissues, lp_{B3} is prominently expressed in rat Schwann cells (48) and mouse embryonic brain tissue (J Contos & J Chun, unpublished data).

lp_{B4}/*nrg-1/edg-8*

The fourth member of the lp_B family was identified as a result of a screening for genes associated with differentiation of rat pheochromocytoma 12 (PC12) cells (106) and as an EST homologous to other lp_B genes. This gene was called *nrg-1* (nerve growth factor-regulated gene-1), and the gene encodes a protein consisting of 400 amino acids. Rat lp_{B4} gene maps to chromosome 8, near the locus of lp_{B2} (106). LP_{B4} has features of a high-affinity S1P receptor (K_d = 2 nM) (45), which couples to $G_{i/o}$ pathways (Table 1). Whether LP_{B4} stimulates other pathways has not yet been determined.

In PC12 cells, treatment with nerve growth factor or other differentiation-inducing reagents resulted in the repressed expression of lp_{B4}, which suggests an inverse relationship to differentiation (106). Rat lp_{B4} expression is abundant in brain, particularly in the midbrain, hindbrain, and spinal cord. Although the precise cellular loci in the nervous system remains uncertain, its apparent expression in white matter implicates oligodendrocytes as a locus (45), as was previously observed for other LP genes (52).

lp_{C1}/*edg-6*

This gene encodes a protein consisting of 384 (human) or 386 (mouse) amino acids (107). Comparison of the amino acid sequence to lp_A and lp_B family members revealed 30%–34% identity and 40%–44% identity, respectively, which is significantly less than the percentage of identity observed within lp_A or lp_B family members (48%–52%). This finding may warrant categorization of this gene as the first member of a distinct subfamily (Figure 2), although its genomic organization is similar to other lp_B family members (J Contos, V Sah & J Chun, submitted). Human lp_{C1} is located on chromosome 19p13.3, whereas the mouse gene maps to chromosome 10 (J Contos, V Sah & J Chun, submitted).

In spite of the relatively distal position of lp_{C1} from the lp_B family in the dendrogram (Figure 2), LP_{C1} has many attributes of a high-affinity S1P receptor (K_d = 13–63 nM) (27, 28). It mediates S1P-induced PLC activation, intracellular

Ca^{2+} mobilization, and MAP kinase activation, all of which are blocked by PTX treatment.

lp_{C1} is expressed in lymphoid and hematopoietic tissues as well as the lung. This expression pattern is more restricted when compared with other lp_B family members.

SPHINGOSYLPHOSPHORYLCHOLINE RECEPTOR

OGR1 was originally identified as an orphan GPCR from a human ovarian cancer cell line (31). It shares high homology with another orphan GPCR, GPR4, but it also has modest sequence homology (28% amino acid identity and 46% similarity) with a PAF receptor (31). It has low similarity to any LP receptor family member (e.g. 25% identity to lp_{B1} in a small stretch-containing transmembrane domain VII; Figure 2). It maps to human chromosome 14q31. Other information on genomic structure is not yet available. OGR1 binds SPC with high affinity ($K_d = 33$ nM), resulting in intracellular Ca^{2+} mobilization through a PTX-sensitive pathway and MAP kinase activation through a PTX-insensitive pathway, which suggests some similarity to LP receptor signaling (32). Neither S1P nor PAF activate OGR1. It is notable that all LP_B/LP_{C1} family members also can be activated by SPC, albeit at lower apparent affinities (~10- to 100-fold less efficiently than S1P) (27, 45, 95, 102, 105, 108). This finding may reflect different configurations and/or binding sites. *OGR1* is expressed in the placenta, lung, liver, spleen, testis, and brain but not in the thymus, ovary, or kidney, which suggests a pathological origin for its expression within ovarian cell lines (31). The dissimilar molecular appearance of this receptor may reflect a basic difference in how choline-containing ligands appear, based upon the dissimilarity of the PAF receptor and OGR1 to the LP receptor noted here. Alternatively, there may be a need to expand the LP receptor family to include less similar receptors. This need is not without precedence, in view of the comparatively low similarity observed among prostanoid receptors (109).

BIOLOGICAL FUNCTIONS OF SPHINGOSINE 1-PHOSPHATE AND SPHINGOSYLPHOSPHORYLCHOLINE

Cardiovascular System

S1P- and SPC-induced responses in atrial myocytes suggest biological actions for these lipids in the cardiovascular system (13, 110, 111). Both S1P and SPC potently activate G_i-regulated, inwardly rectifying potassium channels, which suggests physiological roles for these lipids in heart-rate regulation (Figure 5). A recent study has demonstrated that LP_{B3} is involved in these actions (111a).

Biological relevance of receptor-mediated S1P signaling in the vascular system is likely, based on several findings. First, treatment of vascular endothelial cells

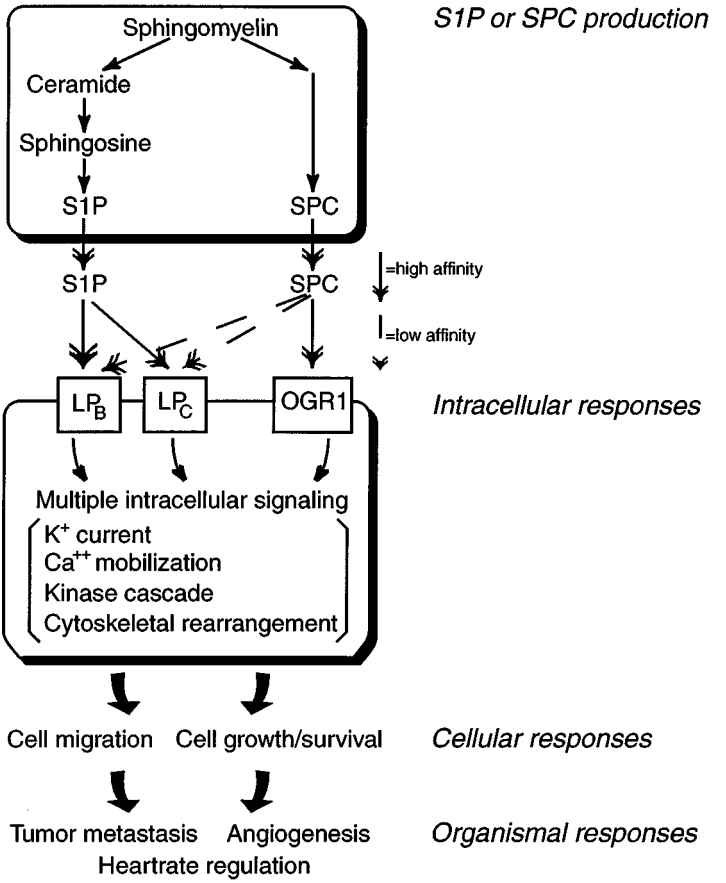

Figure 5 Sphingolipid signaling. Sphingosine 1-phosphate (S1P) or sphingosylphosphorylcholine (SPC) generated from sphigomyelin is released and can bind to LP_B, LP_C, or OGR1 receptors to elicit diverse cellular responses.

with phorbol esters results in morphological differentiation, accompanied by the acute expression of lp_{B1} (38). Second, application of S1P to these cells or vascular smooth-muscle cells increases intracellular Ca^{2+} concentrations (112, 113). Third, LP_{B1} overexpressed in fibroblasts mediates biological responses induced by S1P (25, 94, 95). Finally, endothelial cells also express lp_{B3}, whose receptor protein activates pathways distinct from those of lp_{B1} (114).

Support for such a role comes from studies that use lp_{B1} or lp_{B3} antisense oligonucleotide treatment of endothelial cells (114). This treatment results in the loss of S1P-induced adherens junction assembly and cortical actin fiber formation and affects cell survival, which results in inhibition of S1P-induced effects. S1P treatment enhances fibroblast growth factor-induced angiogenesis in vivo, and cotreatment of both antisense oligonucleotides blocks these effects. Lee and

colleagues conclude that both LP_{B1} and LP_{B3} cooperate in S1P-induced endothelial cell morphogenesis through activation of divergent signaling pathways and that both are required for S1P-induced angiogenesis (Figure 5). These findings implicate receptor-mediated S1P signaling in the formation of the endothelium. A recent report on generating null mice for lp_{B1} has shown the involvement of this gene in vascular maturation, but not angiongenesis and vasculogenesis (114a).

Tumor Cells

Like LPA, S1P has been shown to be capable of either promoting or inhibiting tumor cell migration, depending on cell type (17, 78). A common feature in S1P signaling in these cells is the involvement of the Rho pathway. Other pathways, including G_i or G_q signaling, have also been reported to mediate S1P actions on cell motility (78, 94), and G_i pathways activated by S1P also may contribute to tumor cell proliferation (115). Recent lp_B studies have supported the expression of multiple LP_B members in several types of cancer cells. lp_{B2} and lp_{B3} are expressed in breast cancer cells, where S1P stimulates SRE activation and cell proliferation (115). Human bladder carcinoma cells, whose motility is regulated by S1P, express lp_{B1} and lp_{B3} (116). On the other hand, SPC appears to inhibit cell growth of ovarian cancer cell lines that express *OGR1* (32). The actual roles for receptor-mediated S1P or SPC signaling in initiation, propagation or therapy of cancers remain open areas of research.

PRODUCTION AND DEGRADATION OF EXTRACELLULAR SPHINGOSINE 1-PHOSPHATE AND SPHINGOSYLPHOSPHORYLCHOLINE

Activated platelets have been demonstrated to be a major source of extracellular S1P (16, 117). When activated by thrombin or phorbol esters, platelets can release S1P into the bloodstream where it is present as an albumin- and/or lipoprotein-bound form at physiologically relevant concentrations (~500 nM) (118, 119). In platelets, S1P is thought to be produced exclusively through the phosphorylation of sphingosine by sphingosine kinase (117). An accumulation of generated S1P occurs through a lack of the major hydrolysing enzyme, S1P lyase, which is usually present in other types of cells (16). As with LPA, the wide distribution of LP_B members in both embryonic and adult tissues implicates other sources of extracellular S1P.

The hydrolysis of sphingomyelin by sphingolipid *N*-deacylase is the only known pathway for SPC generation (5, 34). Little is known about whether this pathway produces signaling SPC. An accumulation of SPC has been demonstrated in several types of diseases. For example, ascites fluid from ovarian cancer patients contains significant concentrations (50–190 nM) of SPC, which suggests a role for SPC signaling in ovarian cancer (32).

The degradation of extracellular S1P or SPC could theoretically regulate the concentration of these lipids relevant to receptor-mediated signaling. One potential enzyme in such regulation is lipid phosphate phosphohydrolases, as mentioned in the LPA section (90). No doubt further insights into degradative pathways relevant to the biology and pathology of these sphingolipids will emerge in the near future.

CONCLUSIONS AND FUTURE PROSPECTS

With the identification of the LP receptors, our understanding of extracellular LP signaling has moved rapidly from debate over the existence of receptors, to a clear understanding that many of the effects documented for LPs are caused by their activation of multiple, cognate GPCRs. The existence of numerous LP receptors raises the major question of the biological role for each receptor. Are these receptors simply redundant means of signaling the presence of extracellular LPs, or are there more specific roles for individual receptors? Given the current lack of receptor-subtype-specific agonists and antagonists, traditional pharmacological analyses of LPs, both in vitro and in vivo, are not feasible. On the other hand, molecular genetic strategies, particularly when used in vivo, can assess receptor function by misexpressing, overexpressing, or mutating receptors [e.g. the production of receptor-null mice (63a, 114a)]. This approach will provide clear opportunities to understanding new and important features of receptor-mediated LP signaling in the near future. Improved knowledge of these features, combined with further understanding of how extracellular LPs are synthesized, released, degraded, and regulated, should make this decade an exciting period for unraveling the complexities and intricacies for the receptor-mediated effects of these simple phospholipids. This new information will provide a foundation for understanding the biological roles for LPs as well as realizing their medicinal potential.

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