Effects of LPA and S1P on the Nervous System and Implications for Their Involvement in Disease

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Abstract: Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are two well-studied lysophospholipids that are known to be important regulators of cellular events. Their actions are mediated by activating a family of G-protein coupled receptors present in many cell types and tissues. These receptors have diverse biological roles owing to the heterogeneity of their signal transduction pathways. Many of these receptors are expressed in subsets of cells in the developing and mature mammalian nervous system and are thought to have important functions in its formation and maintenance. They are also widely expressed within other organ systems such as the immune system. Growing interest in the field has stimulated the development of a number of molecules that act as agonists or antagonists to LPA and S1P receptors. These molecules may lead to the development of new therapeutic compounds. Indeed, one such compound (FTY720) is currently in clinical trials for use in preventing transplant rejection and treating multiple sclerosis. The purpose of this manuscript is to: 1) review effects elicited by LPA and S1P on cells and tissues with a particular emphasis on the nervous system, 2) examine possible roles of these lipids in the development of disease, and 3) summarize the existing literature describing their agonists/antagonists.

Key Words: lysophosphatidic acid, sphingosine 1-phosphate, nervous system, agonists, antagonists, immune regulation, multiple sclerosis, pain.

INTRODUCTION

Lysophospholipids (LPs) are a large family of molecules that are derived from phospholipids of the plasma membrane. Once thought to be simply inert structural components, it is now clear that a number of LP species have essential signaling properties that regulate many developmental and cellular processes. These effects are mediated by a family of G-protein coupled receptors (GPCRs) (described below). LP receptors are present in most tissues, but are heterogeneous in their expression. They may couple to different G-proteins such as G_i, G_q, and $G_{12/13}$ depending on receptor species and cell type. This allows LPs to exploit a variety of downstream mediators such as protein kinase C, adenylate cyclase, and Rho to elicit such responses as proliferation, migration, differentiation, and cell death. The complexity of LP signaling will be simplified in this review by focusing on two well-characterized and physiologically important molecules: lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P). It should be noted, however, that other related lipids, such as sphingosylphosphorylcholine and lysophosphatidylcholine, are also known to have signaling properties.

LPA and S1P are ubiquitous membrane components found in most cell types and in all eukaryotic organisms. The metabolism of LPA involved in signaling is complex and poorly understood. LPA can be generated rapidly *via* the cleavage of phosphatidic acid by cPLA2 as well as *via* cleavage of lysophosphatidylcholine by autotaxin, a lysophospholipase D isoform [1]. Interestingly, before its lysophospholipid-related biochemical activity was understood, autotaxin had been identified as a tumor cell motility factor, although at the time it was studied for a distinct substrate preference, 5'- nucleotide phosphodiesterase/ATP pyrophosphatase and ATPase activities [2]. Its effect on cancer cell motility is dependent on generation of LPA and subsequent stimulation of LPA_1 [3]. By contrast, S1P metabolism is better characterized and the enzymes that mediate its synthesis and degradation are conserved in yeast, *Dictyostelium*, nematodes, flies, mice, and humans (reviewed in: [4]). Disruption of S1P metabolism in model genetic systems has provided evidence for its involvement in reproduction, muscle integrity, cell motility, and regulation of cell fate [5-10]. In contrast to the well-conserved metabolism of S1P, high-affinity receptors for LPs have been identified only in vertebrates. This suggests that the emergence of receptor-mediated LP signaling is a relatively recent evolutionary phenomenon and effects seen in invertebrates reflect intracellular roles of LPs. Alternatively, structural divergence of the receptors themselves may be complicating their identification, as suggested by report of a fourth LPA receptor with divergent sequence homology [11].

Although there is evidence for some second-messenger roles of S1P in mammalian cells [12], to date the vast majority of responses elicited by LPs at physiological concentrations can be explained by a growing family of GPCRs. The first of such receptors to be identified was a high-affinity LPA receptor originally named ventricular zone gene-1 (vzg-1) [13] because of its expression and functional activity in proliferating neuroblasts of the cortical ventricular zone (VZ) (see below). Since then, four additional LPA receptors and five S1P receptors have been characterized [14-16]. A standard nomenclature has been adopted for these receptors, now referred to as $LPA_{1\text{-}5}$, and $S1P_{1\text{-}5}$.

LP receptors are ubiquitously expressed in mammalian tissues, in that they are present in some combination in most cell types. Their expression patterns are often dynamically regulated by developmental and physiological stimuli. In addition, each isoform is able to couple with different G-proteins. (For detailed reviews of this aspect of lysophospholipid signaling see: [14, 15].) This heterogeneity is responsible for the diverse and sometimes antagonistic responses that are known to be elicited by LPs. For example, $LPA_{1\&2}$ induce cell rounding in rat neuroblastoma cells whereas LPA_3 induces neurite extension [17]. An additional level of complexity is produced by the availability of the cognate ligands.

ACTIONS OF LPA/S1P IN THE NERVOUS SYSTEM

Since both LP receptors and their cognate ligands are enriched in the central nervous system (CNS) it is not surprising that a number of investigators have identified effects of LPA and S1P in the regulation of cellular events in many nervous system cell types. (Summarized in Fig. (**1**).) Most studies of LP signaling mechanisms

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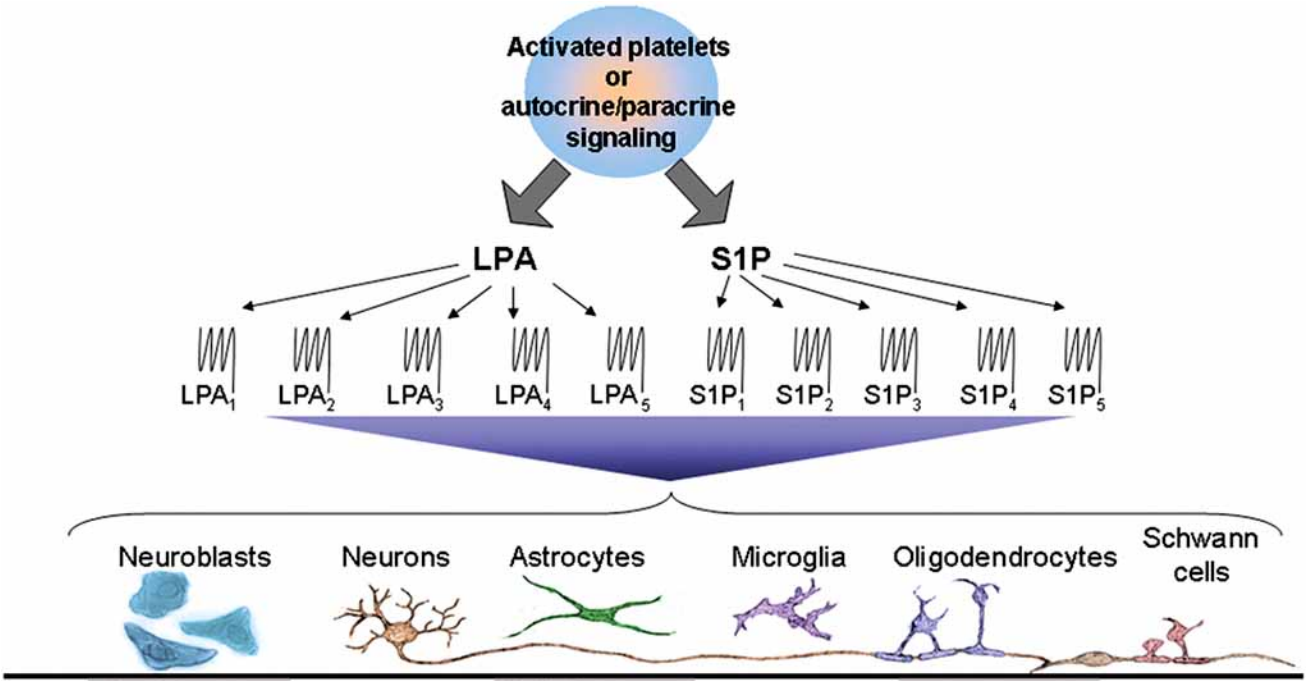

	Neuroblasts	Neurons	Astrocytes	Microglia	Oligodendrocytes	Schwann cells
LPA	IP production Actin polymerization Ca++ mobilization Early conductance changes Clonal expansion	Ca++ mobilization Apoptosis Growth cone collapse Conductance changes	Proliferation Ca++ mobilization Metabolic changes Actin rearrangement AA release cAMP inhibition	Ca++ mobilization chemotaxis	Proliferation Process retraction Ca++ mobilization	Survival Cytoskeletal changes Cell clustering Cell junction formation Migration
S1P	Proliferation ERK phosphorylation Cell aggregation	NGF-mediated neurite extension Increased synaptic transmission	Proliferation Ca++ mobilization AA release IP production GF production	LPA_3 upregulation TNF-α release	Proliferation Process retraction Survival	Cytoskeletal changes Migration

Fig. (1). Lysophospholipid signaling in cells of the nervous system.

in the nervous system exploit tractable cell lines such as PC12, C6 glioma, NG108-15, or N1E-115 cells. While a great deal of information may be gathered from these studies regarding basic cellular processes, these immortalized cell lines often manifest markedly different characteristics compared to acutely-isolated neural tissue. To better relate experimental data to possible therapeutic potential, we will focus on data derived from the use of primary cell preparations and *in vivo* studies.

Neuroblasts (Mitotic Neuronal Precursor Cells)

LPA

The first studies involving the roles of LPA in neuroblasts were performed after LPA_1 was cloned from a neuroblast cell line (TR) and identified as a bona fide LPA receptor [13]. It was found to be highly expressed in the proliferative ventricular zone of the murine cortex in a pattern that spatially and temporally parallels cortical neurogenesis (i.e. high in the VZ at embryonic day 12 (E12), declining until its absence at E18). Addition of LPA to the media of TR neuroblast cell cultures stimulates inositol phosphate (IP) production and pertussis toxin (PTX)-insensitive cell-rounding [17, 18]. This morphological change is mediated by an initial loss of membrane ruffling due to actin depolymerization followed by process retraction that is mediated by actin polymerization. Initial depolymerization is dependent on mobilization of intracellular Ca^{++} and α-actinin, whereas subsequent polymerization is Rho-dependent. Overexpression of LPA_1 or LPA_2 (both of which are endogenously expressed) increased this response [17]. Interestingly, cell rounding was *inhibited* by overexpression of LPA_3 which is normally not expressed in these cells. This is indicative of the divergent and non-redundant actions of LPA. In concordance with these studies, it was found that LPA induces neurite retraction in another mouse neuroblast cell line (NB2a) [19].

In addition to these responses in *cultured* neuroblasts, *primary* neuroblasts respond to LPA with electrophysiological and morphological changes. In acutely isolated neural progenitor cells, LPA can stimulate ionic conductance changes [20]. Notably, these cells respond to LPA before similar responses can be elicited by either GABA or L-glutamate. This is significant in that it identifies LPA as one of the earliest electrophysiological extracellular effectors of neurogenic processes. In addition, studies of cortical explant cultures demonstrate that LPA induces the Rho-mediated formation of f-actin retraction fibers and causes the nuclei to migrate and alter their morphology from fusiform to round [21]. These effects are consistent with morphological changes associated with proliferation and differentiation of neuroblasts, further implicating the involvement of LPA in cortical neurogenesis.

Further evidence for the role of LPA in mediating neural development comes from a study of chick neural retina [22]. It was found that these cells respond to LPA with the release of intracellular Ca^{++}

which is PLC-dependent, and partially inhibited by PTX, indicating the involvement of G_i-coupled GPCRs. Interestingly, this response decreased as neurogenesis progressed in that early progenitors were most responsive while differentiated neurons did not respond.

Additionally, murine embryonic stem cells proliferate rapidly in culture and form free-floating spheres. Formation of these "neurospheres" was shown to be dependent on the presence of epidermal growth factor (EGF) in the media [23]. Recently, it was demonstrated that LPA is able to stimulate this clonal expansion of neural stem cells even in the absence of EGF [24], suggesting that LPA contributes to stem cell proliferation *in vivo*.

S1P

Although a previous demonstration reveals that $S1P_1$ is specifically expressed in the neurogenic regions of the developing murine cerebral cortex [25], only one study to date specifically examined the role of S1P in neuroblasts [26]. Harada, *et al*. demonstrated that S1P stimulation causes a marked increase of G-protein activation in neurogenic regions of the embryonic rat brain. Primary cultures of rat hippocampal neural progenitor cells are shown to express four of the five known S1P receptors by RT-PCR. Addition of nanomolar concentrations of S1P to the culture media induced ERK phosphorylation and cellular proliferation, both of which could be inhibited by PTX. In addition, at a higher concentration (3μM), S1P induced Rho-dependent cell aggregation; a response that was also elicited by 1μM LPA.

***Ex Vivo* Tissues**

In order to study the roles of LPs in a system that more closely approximates the *in vivo* complexity of the developing CNS, our laboratory has developed a system in which intact brains may be excised from mouse embryos and cultured *ex vivo*. When appropriately treated, such preparations reproduce conditions that are indistinguishable from *in vivo* development for at least 24 hours in culture. In whole-brain preparations, we have demonstrated that LPA induces morphological changes and inhibits the migration of cortical neurons during critical stages of neurogenesis [27]. Further studies involving individually isolated and cultured cerebral cortices revealed additional effects of LPA that included an increase in cortical thickness and a striking change in gross anatomy [28]. LPA-treated cortices were characterized by dramatic folds that resemble sulci and gyri not normally present in rodent brains and were accompanied by a decrease in apoptotic cell death, an increase and displacement of mitotic figures away from the bottom of the VZ, and an increase in terminal mitosis. Surprisingly, there was no evidence for an increase in proliferation. These effects were completely abrogated in LPA_1/LPA_2 double knockout brains.

Neurons

LPA

In studies of primary rat hippocampal neuron cultures, LPA at 0.1 to 1.0 μM stimulated a reversible, sustained elevation of intracellular Ca^{++} [29] that was accompanied by an increase in the number of apoptotic cells [30]. The apoptosis could be abrogated by reducing reactive oxygen species with propyl gallate or nitric oxide synthase inhibitors [31]. Higher non-physiological concentrations of LPA (10-50 μM) caused necrotic cell death. In contrast, other studies suggest that LPA promotes cell survival in primary cultures of neonatal murine neurons, inhibiting apoptotic cell death caused by serum starvation [32] or β-amyloid peptide [33] at concentrations of 0.1-30 μM.

Application of LPA has been shown to inhibit NGF-mediated axon outgrowth from embryonic rat trigeminal ganglion [34] and cause the rapid collapse of growth cones in chick dorsal root ganglion (DRG) neurons [35] and *Xenopus* neural retina [36]. It was demonstrated in the latter system that this response is dependent upon activating proteasome-mediated protein degradation. This response was further shown to be dependent on p38 MAP kinase and involves the activation of caspase-3 [37], which distinguishes this process from growth cone collapse mediated by the axon guidance cue, Sema3a.

Similarly, LPA induces neurite retraction in murine embryonic cortical neurons and inhibits the migration of these cells from cortical explants [27]. The neurite retraction is accompanied by the formation of f-actin retraction fiber caps and rho-dependant tubulin rearrangement [38] and is likely to be mediated by LPA_1 and/or LPA_2. Neurite retraction was also observed in rat cerebellar granular neurons when stimulated with LPA [39]. This process is thought to involve the induction of glycogen synthase kinase-3, a regulator of a number of developmental processes in the CNS including cell fate decisions and axon growth [39]. In contrast, another study reports that LPA does not decrease the number of neurite-bearing cells in primary cultures of rat hippocampal neurons [40]. This difference may be due to the fact that this assay evaluates initiation rather than maintenance or extension of neurites.

A number of studies have implicated the involvement of LPA in regulating neuronal electrophysiology. Administration of LPA *in vivo* was shown to increase the duration and frequency of firing of rat spinal neurons, while *in vitro*, LPA raises the intracellular Ca^{++} concentration and stimulates G-protein activation [41]. In studies of transfected COS cells, LPA increases the conductance of TREK, a mechano-gated 2P-domain K^+ channel and elicits TREK-1-like currents in cultured mouse striatal neurons [42]. In addition, LPA inhibits tetrodotoxin (TTX)-sensitive Na^+ currents, but enhances TTX-resistant Na^+ currents in rat DRG neurons [43]. Since TTX-resistant currents are reportedly involved in nociception [44-46], the authors speculate that the observed increase of TTX-resistant currents may be related to a previous report of LPA-stimulated neuropathic pain [46]. (See discussion below.)

S1P

An elegant series of experiments was performed recently that describes the involvement of S1P in NGF-mediated neurite extension in rat DRG neurons [47]. This study, performed in the laboratory of Dr. Sarah Spiegel, demonstrates that NGF stimulation causes the production of S1P through the activation of sphingosine kinase 1 (Sphk1). (For a review of S1P metabolism see: [48].) This provides a positive signal for neurite outgrowth by activation of $S1P_1$. In addition, $S1P_2$ and $S1P_3$, which inhibit neurite extension, are down-regulated by NGF stimulation. Both $S1P_1$ and Sphk1 are required for NGF-mediated neurite extension. Interestingly, although $S1P_1$ activity accelerates NGF-mediated *extension* of neurites, S1P inhibits NGF-mediated neurite *initiation*.

S1P also appears to regulate synaptic transmission at the neuromuscular junction. In frog motor neurons, addition of S1P increases the frequency of miniature end-plate potentials by a mechanism that is dependent on the mobilization of intracellular Ca^{++} stores [49]. Although the breadth of this phenomenon is unclear, it may more broadly relate to the regulation of neuroexcitability. Indeed, recent studies suggest that S1P increases excitability of cultured rat DRG neurons by both receptor-dependent and receptor-independent mechanisms [50, 51].

Astrocytes (The Most Abundant Neuroglial Cells in the CNS)

LPA

A number of studies have produced a list of responses that are elicited from astrocytes in culture when stimulated by LPA. These include proliferative changes such as DNA synthesis [52, 53] and ERK1/2 phosphorylation [53, 54], Ca^{++} mobilization [54-57], metabolic changes including increased lipid peroxidation and inhibition of glutamate uptake [56], and changes in signaling processes such as the accumulation of inositol phosphates (IP), arachidonic acid (AA) release, and inhibition of cAMP production [53, 54]. LPA also induces Rho-mediated cytoskeletal changes that include the

reversal of stellation [55], stabilization of actin stress fibers, and stimulation of actomyosin contractile forces [58].

These processes are all thought to be mediated through LPA's cognate GPCRs, however, the identities of the involved receptor subtypes remain unclear. Although the presence of LPA_{1-3} has been reported in cultured astrocytes [54, 57, 59], LPA_1 does not appear to be expressed in astrocytes *in vivo* [60]. This may reflect alterations in cell physiology that occur under culture conditions.

S1P

Many of the LPA-mediated responses described above can also be elicited in astrocytes by S1P. These include Ca^{++} mobilization [54, 61, 62], DNA synthesis [62-64], ERK phosphorylation [54, 62, 63], IP production [54, 62], and AA release [54]. In addition, S1P inhibits gap junctions *via* Rho/ROCK activation [65], and stimulates the production of growth factors (GFs) such as fibroblast growth factor-2 [63, 66] and glial-derived neurotrophic factor [64]. The interaction between S1P signaling and additional GFs is likely to explain some of the biological responses elicited by this lipid mediator.

Astrocytes have been shown to express $S1P_1$, $S1P_2$, and $S1P_3$ [54, 62, 67], and can synthesize and release S1P for autocrine/paracrine stimulation [67]. Interestingly, S1P release has been shown to increase in response to fibroblast growth factor-2 exposure [68] further adding to the complexity of the relationship between S1P and peptide growth factors.

Microglia (CNS Resident Macrophages)

LPA

A study by Moller, *et al*. reports some interesting distinctions between LPA signaling in the mouse microglia compared to LPA signaling in rat microglia [69]. Rat cells predominantly express LPA_3, whereas mice primarily express LPA_1. Ca^{++} transients elicited by LPA in rat cells are primarily mediated by influx of extracellular Ca^{++} across the plasma membrane. In contrast, Ca^{++} transients in mouse cells are mediated by thapsigargin-sensitive intracellular stores. Furthermore, increasing the concentration of LPA (3 μM) causes a sustained increase in intracellular Ca^{++} concentration reflecting an inward current that is not apparently observed in rat cells. Another difference between the two species was noted when investigating the effect of LPA stimulation on metabolism. As measured by mitochondrial activity, LPA was able to increase the metabolism/proliferation of microglia in the mouse but not in the rat. Although the cellular consequences of these differences are unclear and may reflect artifacts of manipulating cultured cells (see below), this phenomenon underscores the distinct signaling processes elicited by LPA receptor subtypes.

In contrast to the previous study, Tham, *et al*. report the presence of LPA_1 in both acutely isolated and cultured rat microglia [70]. Interestingly, the presence of LPA_3 was only detectable in cultured cells and only after stimulation with lipopolysaccharide or high concentrations of S1P. Therefore, it is possible that differences in culture conditions (GFs, serum lot, etc.) could account for the differences of receptor expression observed in these studies.

A third study reports that LPA stimulation mediates chemotaxis in an immortalized mouse microglial cell line (BV-2). This response can be disrupted by blocking Ca^{++}-activated K^+ currents [71].

S1P

The only study involving the effect of S1P on microglia reports that while $S1P_{1-3}$ are present in acutely isolated cells, $S1P_3$ expression is lost after ~14 days in culture [70]. In addition to the upregulation of LPA_3 mentioned above, stimulation of these cells with high concentrations of S1P causes the release of tumor necrosis factor-α (TNF-α) which suggests the involvement of S1P in inflammation.

Oligodendrocytes (Myelinating Cells of the CNS)

LPA

Initial studies identified expression of LPA receptor genes in oligodendrocyte lineages [60, 72] indicating that LPA has a direct effect on oligodendrocytes by eliciting PTX-sensitive Ca^{++} transients in mature cells, but not their precursors [73]. This effect is likely to be mediated by LPA_1 which is absent in the neonatal murine brain but becomes widely expressed within the first postnatal week in the mature, myelinating oligodendrocytes [60]. In contrast, Stankoff, *et al*. reported that LPA stimulated ERK1/2 phosphorylation in mature rat oligodendrocytes, but did not evoke Ca^{++} transients [74]. This may reflect differences between rat and mouse cells or differences in culture conditions. (The latter study used primary cultures of mature oligodendrocytes while the former study used oligodendrocytes differentiated from precursor cells *in vitro*.) Another interpretation of these data involves event-dependent changes in the sensitivity of oligodendrocytes to LPA stimulation. LPA may only evoke Ca^{++} transients in mature oligodendrocytes before myelination, a response which is subsequently decoupled from LPA signaling. Such a mechanism could distinguish "occupied" oligodendrocytes from a "ready" myelinating pool.

Another interesting distinction in stage-specific, LPA-mediated effects on oligodendrocytes was that of process retraction. This Rho-mediated response was reported to occur in oligodendrocyte precursors, but not in differentiated cells [75]. Taken together, these studies suggest that LPA is an important mediator of differentiation and myelination of oligodendrocytes.

S1P

As with many other cell types, oligodendrocytes also express S1P receptor genes [70, 76, 77]. They exhibit proliferative responses to S1P exemplified by an increase in ERK phosphorylation, an event which is MAPK-, PLC-, and PKC-dependent, but PTX-insensitive [76]. In addition, the cell survival effect of neurotrophin-3 on oligodendrocytes appears to be mediated, at least in part, by the formation of S1P by Sphk1 [68]. In contrast to LPA, S1P does not appear to produce Ca^{++} transients. Like LPA, however, S1P stimulates Rho-dependent process retraction in immature cells only, but can also act as a survival factor for mature oligodendrocytes in a PTX-sensitive manner [77]. The responses of oligodendrocytes to S1P are primarily mediated by $S1P_5$ (see below). These studies revealing stage-specific effects of S1P again underscore the significance of LP receptor-mediated signaling in developmental processes.

Schwann Cells (Myelinating Cells of the Peripheral Nervous System)

LPA

LPA has been shown to affect a number of processes in Schwann cells (SCs). Both LPA_1 and LPA_2 are expressed in SCs [78, 79] and there is a notable upregulation of LPA_1 following sciatic nerve resection injury. Although it does not generate a proliferative stimulus, LPA is a potent survival factor, as demonstrated by the inhibition of apoptotic cell death in cultured cells [80]. This response is dependent on Akt, G_i-coupled GPCRs, and PI-3-kinase. LPA stimulation also mediates cytoskeletal changes which are seen with the formation of circumferential f-actin "wreath" structures and focal adhesions [78]. Furthermore, LPA promotes cell clustering by the formation of N-cadherin cell-cell junctions. These processes are Rho-dependent and PTX-insensitive and are also associated with increases in cell migration [81]. Similarly, another study demonstrated that LPA stimulates the formation of actin stress fibers, promotes survival, and increases expression of myelin protein P0, which is indicative of Schwann cell maturation [79]. All of these studies demonstrate that LPA regulates many processes that are important for migration, differentiation, and myelination of

Schwann cells during development and remodeling following injury.

S1P

In contrast to LPA, S1P is not survival factor for SCs despite expression of at least one S1P receptor [80]. S1P does, however, activate RhoA and Rac1, induce the formation of lamellipodia and actin wreaths, and increase cell migration similar to that seen with LPA stimulation. However, only Rac1 (not RhoA) is required for this actin rearrangement [81]. SCs can also synthesize S1P by the activity of Sphk for autocrine/paracrine stimulation.

Knockout Mice

An important way to understand the functions of an individual receptor is to examine the consequences of its specific loss in an intact model organism. In the context of nervous system function, the LPA_1 knockouts have been instructive. These mice are characterized by smaller size, shorter snouts, cranial hematomas, and a 50% perinatal mortality rate [82], with grossly normal cerebral cortical development in the survivors [83]. The mortality is due to poor suckling behavior which is likely to be caused by a defect in olfaction (central and/or peripheral). Additionally, consistent with studies of isolated Schwann cells, $LPA_1^{-/-}$ mice show increased apoptotic cell death in the sciatic nerve. Furthermore, Schwann cells derived from these knockout animals have a marked reduction in LPA-mediated actin rearrangement [78]. The additional loss of LPA_2 in these animals manifests only a slight exacerbation of $LPA_1^{-/-}$ phenotypes, characterized by an increase in the occurrence of hematomas. However, fibroblasts obtained from these animals have notably decreased responses to LPA.

Functional deletion of LPA_3 causes no obvious neurological defects, but is characterized by reproductive phenotypes resulting in reduced embryonic viability [84]. Implantation of embryos into the uterine wall is delayed in knockout females by about 24 hours. Once implanted, these embryos are inappropriately spaced, clustering near the cervix rather than having the normal even distribution along the uterine horns. The implantation delay is associated with a reduction of COX2 expression and prostaglandin levels and can be rescued by exogenous administration of prostaglandins. This demonstrates that LPA_3 is an upstream regulator of prostaglandin-mediated on-time implantation. These defects are attributed to loss of maternal rather then embryonic function since $LPA_3^{-/-}$ embryos implant normally when transferred to pseudopregnant wild-type females.

Interestingly, mice lacking both $S1P_2$ and $S1P_3$ receptors were also remarkable for their reduced litter sizes, indicating a defect somewhere in the reproductive system. These mutants do not display obvious defects in the development or function of the CNS, despite characteristic reductions of S1P signaling [85]. However, other studies of $S1P_2$ knockout mice revealed behavioral and electrophysiological defects resembling seizure activity [86] (see below). The absence of seizures in independently derived $S1P_2$ knockout animals is likely the result of the sensitivity of this phenotype to genetic background.

Similar studies of the roles of $S1P_1$ are complicated by the embryonic lethality of these knockout animals [87]. These mice die *in utero* due to hemorrhaging caused by a defect in vascular maturation. Similarly, while loss of either sphingosine kinase 1 or 2 individually caused no phenotypic abnormalities, simultaneous loss of both isoforms resulted in embryonic lethality [88, 89]. The developing brains of the double-null embryos are characterized by decreased proliferation and increased apoptosis.

It is likely that the essential functions performed by individual LP receptors in CNS development are protected by redundant and overlapping functions of other receptor subtypes. Additional genetic manipulations will help elucidate these mechanisms.

CHEMICAL MODULATORS OF LYSOPHOSPHOLIPID RECEPTOR FUNCTION

A number of compounds have been developed that agonize or antagonize lysophospholipid receptors (Fig. (**2**), Tables **1** & **2**). These compounds have potential to be useful in pharmacologically elucidating both the signaling mechanisms of LPs and the identity of the receptors that mediate such reactions[1]. The *in vivo* specificity of all of these compounds remains to be determined. Nevertheless, their use as chemical tools provides important approaches to assessing the functional consequences of LP receptor agonism and antagonism, particularly when combined with molecular genetic approaches [90, 91]. This section summarizes the characteristics of these compounds.

LPA Receptor Modulators

LPA analogs

Extensive work has been performed in an effort to generate LPA derivatives that can act as receptor-specific agonists or antagonists. Initial work involving the replacement of the glycerol backbone of LPA with an aromatic moiety generated a number of weak agonists with EC_{50} values in the high nanomolar to low micromolar range [92]. Later, additional compounds were synthesized to contain non-hydrolyzable phosphonate groups for the purpose of serving as lipid phosphate phosphohydrolase (LPP) inhibitors [93]. Two of these compounds (wls31 and wls60) also exhibited low affinity agonism for LPA_1 and LPA_2 but not for LPA_3. However, the potency of wls31 and wls60 should, theoretically, be higher since their resistance to LPP-mediated degradation would facilitate a longer biological half-life.

Perhaps the most interesting compounds generated by this group were made by modifying an LPA derivative that carries an ethanolamine backbone. This lead compound itself, *N*-acyl ethanolamide phosphate (NAEPA), is an LPA receptor agonist in that it can elicit Ca^{++} transients and inhibit adenylyl cyclase activity at concentrations similar to that of LPA [94]. These activities can be desensitized by LPA but not by S1P. In an effort to exploit this LPA mimetic, MacDonald and Lynch subsequently described a series of derivatives with differential activities as agonists or antagonist that have varying specificities for LPA receptors. Addition of a methyl (VPC12086), methylene hydroxyl (VPC31143), or methyl amine (VPC12178) increased the potency of NAEPA against LPA_1 with a lesser effect toward LPA_2 and LPA_3 [95]. Other modifications generated an LPA_1/LPA_2-specific agonist (NAEPA-17), an LPA_1-specific agonist (NAEPA-19), and a non-hydrolyzable LPA_1-specific agonist (NAEPA-11) [96]. In contrast, addition of a bulky hydrophobic group (benzyl-4-oxybenzyl) resulted in an LPA_1/LPA_3-selective competitive antagonist. This compound (VPC12249) was validated *in vivo* by its ability to protect against LPA_3-mediated renal ischemia-reperfusion injury [97] and by its inhibition of LPA-stimulated motility of pancreatic cancer cells [98].

VPC12249 was further modified in an effort to improve receptor specificity [99]. This process generated antagonists of moderate affinity that are specific for either LPA_1 or LPA_3, but in addition, generated a high-affinity LPA_1/LPA_3 antagonist (10t), which was subsequently used as a lead compound to generate a non-hydrolyzable, phosphatase-resistant, LPA_3 selective antagonist (13d) [100].

A report from another group describes a different approach in designing LPA analogs which involves replacing the glycerol backbone with a carbohydrate scaffold [101]. This scheme generated 4 compounds of interest: two LPA_3-specific agonists (#2 and #13), an LPA_1-specific agonist (#15), and an LPA_1-specific antagonist (#14).

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1. Note that few studies have involved LPA_4, and LPA_5. This reflects their recent identification and divergence from the other LPA receptors [11, 16].

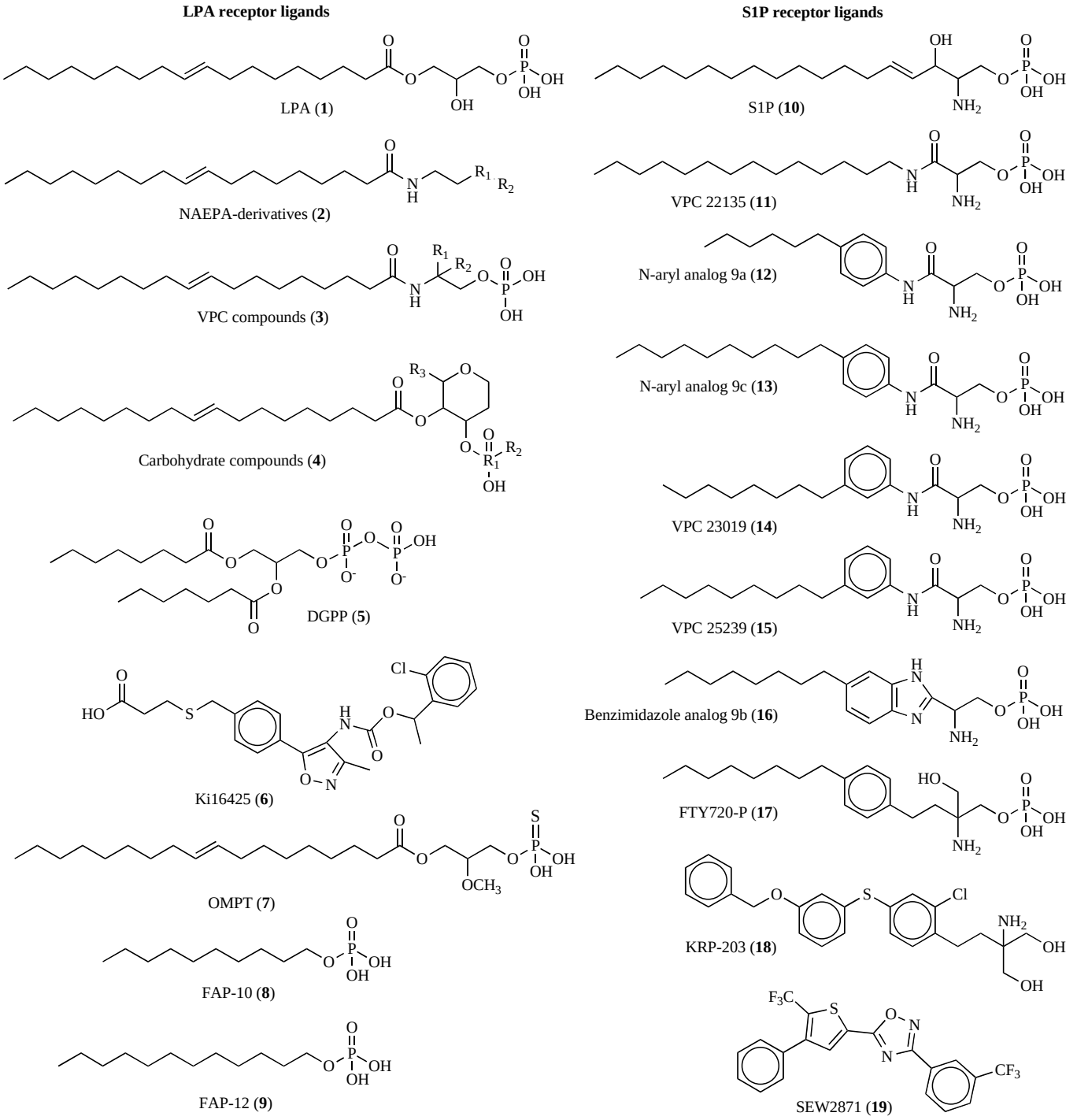

Fig. (2). Structures of lysophospholipid receptor modulators.

Finally, Gueguen, *et al*. [102] described a number of candidate LPA agonists based on their ability to induce a platelet aggregation response that could be desensitized by LPA. These LPA analogs (in descending order of potency), 2-N-Palmitoyl-norleucinol-1-phosphate (PNPA), 2-N-Palmitoyl-serine-3-phosphate (NAPS), and, dioxazaphosphocane bearing a P-OH bond (DOXP-OH) are not stereoselective in their activities. In contrast, one of these compounds (NAPS) was previously reported to be stereoselective in activating LPA receptors in human breast cancer cells with 2 orders of magnitude greater potency than that which is required to elicit platelet aggregation [103]. These observations are in agreement with reports that the platelet response may signal through unidentified, low affinity receptors [93, 104, 105]. Therefore, the specificity of the compounds identified here should be regarded with caution, since the high concentrations of lipid mediators used to elicit a platelet response may complicate analyses by involving promiscuous interactions with other phospholipid receptors.

DGPP

The naturally occurring lipid diacylglycerol pyrophosphate (DGPP) was shown recently to act as an LPA_1/LPA_3 antagonist [106, 107]. The authors theorize that similarities between DGPP and the LPA pharmacophore allow recognition of the LPA receptor binding pocket that is stabilized by the increased hydrophobicity

Table 1. Specificities of LPA Receptor Agonists and Antagonists

		Relative Affinities					Reference
		LPA_1	**LPA_2**	**LPA_3**	**LPA_4**	**LPA_5**	
Agonists	wls31 & wls60	+	+	-	N/D	N/D	[93]
	VPC12086, 12178, 31143	+++	++	++	N/D	N/D	[95]
	NAEPA-11	++	+	-	N/D	N/D	[96]
	NAEPA-17	+++	++	-	N/D	N/D	
	NAEPA-19	++	-	-	N/D	N/D	
	Carbohydrate compound 2	-	N/D	+++	N/D	N/D	[101]
	Carbohydrate compound 13	-	-	+++	N/D	N/D	
	Carbohydrate compound 15	+++	-	-	N/D	N/D	
	OMPT	-	-	+++	N/D	N/D	[117]
	FAP-10	-	+	++ (antagonist)	N/D	N/D	[161]
	FAP-12	+ (antagonist)	++	++ (antagonist)	N/D	N/D	
Antagonists	VPC12249	++	+	++	N/D	N/D	[95]
	VPC12249-10t	+++	-	+++	N/D	N/D	[99]
	VPC12249-10t-13d	-	-	+++	N/D	N/D	[100]
	Carbohydrate compound 14	-	-	+++	N/D	N/D	[101]
	DGPP	++	-	+++	N/D	N/D	[106]
	Ki16425	+++	+	+++	N/D	N/D	[113]

+++: Strong agonist/antagonist, affinity approaches or exceeds that of LPA. ++: Moderate agonist/antagonist, EC_{50}/IC_{50} within 2 orders of magnitude relative to LPA. +: Low affinity agonist/antagonist. -: No activity. N/D = not determined. Other compounds with unknown selectivity: Agonists; LPA analogs with aromatic substitutions [92], NAEPA [94], PNPA [102], NAPS [102], cAGP [102], DOXP-OH [102]. Antagonists; DOXP-H [102].

Table 2. Specificities of S1P Receptor Agonists and Antagonists

		Relative affinities					Reference
		$S1P_1$	**$S1P_2$**	**$S1P_3$**	**$S1P_4$**	**$S1P_5$**	
Agonists	FTY720-P	+++	-	+++	+++	+++	[126, 128]
	SEW2871	++	-	-	-	-	[91]
	VPC22135	++	+	++	N/D	-	[119]
	N-aryl analog 9a	++	-	-	++	++	[120]
	N-aryl analog 9c	+++	-	++	++	++	
	Benzimidazole analog 9b	+++ (5%)	-	-	+++ (85%)	+++ (50%)	[122]
	VPC23019	+++ (antagonist)	+++	++ (antagonist)	+++	+++	[121]
	VPC25239	+++ (antagonist)	+++	+++ (antagonist)	+++	+++	
	KRP-203	+++	N/D	-	N/D	N/D	[140]
Antagonists	JTE-013	-	+++	-	N/D	N/D	[145, 148]

+++: Strong agonist/antagonist, affinity approaches or exceeds that of S1P. ++: Moderate agonist/antagonist, EC_{50}/IC_{50} within 2 orders of magnitude relative to S1P. +: Low affinity agonist/antagonist. -: No activity. N/D = not determined. Benzimidazole analog 9b exhibits high affinity agonism but with varying efficacy. Numbers in parenthesis indicate percent maximal response relative to that obtained by S1P.

from the second short-chain fatty acid moiety. These receptors then remain inactive since their activation is dependent upon binding a ligand with a long-chain fatty acid [108, 109]. DGPP does not antagonize LPA_2. A number of studies have validated the use of DGPP as an antagonist: 10μM DGPP inhibits LPA-evoked Cl^- currents in corneal keratinocytes by ~60% [110], LPA-induced platelet aggregation and shape change were reduced by ~50% with 1 μM DGPP and completely abrogated with 10 μM DGPP [111], preincubation of eosinophils with 10μM DGPP inhibited LPA-induced Ca^{++} transients, actin rearrangement, and production of reactive oxygen species [112], and DGPP inhibited the LPA-mediated clonal expansion of neural stem cells [24].

Ki16425

Ki16425 was identified from a library of 150,000 compounds by the Kirin Brewery Company (Japan) as the best candidate for an LPA receptor antagonist [113]. Since Ki16425 was not designed *a priori* to compete with LPA for receptor binding, its structure bears little resemblance to the native ligand. Despite this structural dissimilarity, Ki16425 was shown to act as an effective competitive inhibitor of LPA_1 and LPA_3 with little effect on LPA_2 or the structurally related S1P receptors. In contrast to DGPP which is more effective against LPA_3, Ki16425 is slightly more effective against LPA_1. LPA-induced responses that can be inhibited by Ki16425 include: Ca^{++} transients, inositol phosphate production, and proliferation of fibroblasts [113]; migration of multiple carcinoma cells expressing LPA_1 [3, 98, 114]; and neurite retraction in PC12 cells [115]. Furthermore, this compound has been used to inhibit proliferation and bone metastasis of breast cancer cells *in vitro* and *in vivo* [116].

OMPT

A thiophosphate derivative of LPA (1-oleoyl-2-O-methyl-rac-glycerophosphothionate, OMPT) proved to be a potent agonist of LPA_3 with little detectable activity toward LPA_1 or LPA_2 [117]. Notably, it was a ~100-fold more potent mitogen than LPA in a murine mesenchymal cell line. Furthermore, OMPT was able to exacerbate LPA_3-mediated renal ischemia/reperfusion injury *in vivo* [97].

Fatty Alcohol Phosphates

In another approach to generate subtype-specific LPA receptor agonists, Gabor Tigyi's group reasoned that a phosphorylated fatty acid would provide a minimal structure that could satisfy the LPA pharmacophore. This method generated two compounds, decyl phosphate (FAP-10) and dodecyl phosphate (FAP-12), that specifically activate LPA_2. In RH7777 cells stably transfected with LPA_2, the potency of FAP-12 to induce Ca^{++} transients approached that of LPA but with a half maximal response, whereas FAP-10 was able to elicit a 100% maximal response but at a 10-fold lower potency. Interestingly, neither compound was able to elicit a response from either LPA_1 or LPA_3. In fact, both compounds were *antagonistic* to LPA_3. Chain-length was a decisive factor for these structures, since fatty acid phosphates less then 10 or greater than 14 carbons were devoid of activity. Moreover, FAP-12 inhibits LPP activity which may further enhance LPA signaling by increasing the availability of endogenous substrate. Surprisingly, FAP-12 was unable to act as either an agonist or antagonist to 4 of the 5 known S1P receptors ($S1P_4$ not tested) despite the simplicity of the compound and its similarity to S1P. Efficacy of FAP-12 was demonstrated *in vitro* by its ability to induce release of proinflammatory cytokines from human mast cells at high concentrations similar to that of LPA [118].

S1P Receptor Modulators

S1P Analogs

The earliest work toward synthesizing receptor-specific S1P analogs generated a number of low-affinity, broad-specificity analogs [119]. However, one compound (VPC22135) exhibited preference toward $S1P_1$ and $S1P_3$, with no activity toward $S1P_5$. Further improvements in specificity were reported with the synthesis of N-aryl amide compounds [120]. Of note, derivative 9a has moderate affinity for three S1P receptors with no activity toward $S1P_2$ and $S1P_3$, and derivative 9c has high affinity for $S1P_1$ with significantly lower activity toward $S1P_{2\text{-}5}$. The signaling properties of these compounds were subsequently modified by altering the aryl linkage to the fatty acid [121]. The lead compound for this study, VPC23019, is a high affinity agonist for $S1P_{2\&4}$, a partial agonist for $S1P_5$, and a potent antagonist for $S1P_1$ and (to a lesser extent) $S1P_3$. From this, a number of interesting derivatives were generated including VPC25239 which resembles the activity of the lead compound but with more potent antagonism for $S1P_3$.

Even greater specificity was achieved with the use of benzimidazole-based S1P analogs [122]. This scheme produced a number of agonists with a strong preference for $S1P_4$. Notably, while compound 9b was unable to activate $S1P_{2\&3}$, it exhibited high affinity for $S1P_{1\&5}$ but only elicited 5% and 50% maximal response (respectively) relative to S1P. In contrast, $S1P_4$ was activated at high affinity and efficacy by 9b.

FTY720 (FTY720-P)

The compound FTY720 deserves special consideration in any discussion of S1P receptor modulators as it is the first such compound to be used clinically in humans. Its potential therapeutic value as an immunosuppressive agent was first identified in 1996 with the report that administration of FTY720 could significantly increase the survival of canine kidney allograft recipients by causing peripheral lymphopenia [123]. Although the mechanism of action was unknown at the time, its similarity to sphingosine led researchers to believe that it in some way disrupted sphingolipid metabolism and lead to apoptosis of lymphocytes. It was later discovered, however, that FTY720 actually effects immune cell trafficking and results in the sequestration of lymphocytes to the lymph nodes [124]. Furthermore, it was recently revealed that FTY720 does not disrupt the formation S1P, and may in fact prevent its catabolism by inhibiting the only enzyme known to catalyze its irreversible degradation, S1P lyase [125].

The primary mechanism of FTY720's action is mediated by its phosphorylation by sphingosine kinase [126, 127]. This phosphorylated species (FTY720-P) quickly reaches equilibrium as the dominant metabolite and is a potent agonist of four of the five known S1P receptors (not $S1P_2$) [128]. Reports also suggest that FTY720-P may serve as a functional antagonist *in vivo* by causing the irreversible internalization and inactivation of S1P receptors upon binding [129, 130]. A number of analogs have since been generated in an effort to improve potency and specificity [126, 131-133]. There have been many demonstrations of the efficacy of FTY720 as an immunosuppressive agent. (For comprehensive reviews see [134-136].) Novartis Pharmaceuticals is currently conducting Phase III clinical trials on FTY720 for suppression of renal transplant rejection as well as Phase II clinical trials for the treatment of Multiple Sclerosis.

SEW2871

Despite the efficacy of FTY720, its low receptor subtype selectivity may be associated with undesirable physiologic effects such as bradycardia [137]. Therefore, to minimize side effects of S1P-agonist drugs and to provide additional tools with which to study the biological roles of individual S1P receptors, the laboratory of Dr. Hugh Rosen has identified a novel $S1P_1$-specific agonist (SEW2871) [91, 130]. This compound is able to regulate lymphocyte trafficking and induce peripheral lymphopenia, like FTY720, but is not associated with the bradycardia that results from stimulation of $S1P_3$. Additionally, in an *in vitro* monocyte adhesion assay, treatment with SEW2871 inhibited monocyte/endothelial interactions similar to S1P, thus implicating $S1P_1$ as a key mediator of this

effect [138]. Furthermore, administration of SEW2871 *in vivo* was able to ameliorate renal ischemia/reperfusion injury in a mouse model, thus implicating its usefulness as a therapeutic agent for the treatment of acute renal failure [139].

KRP-203

A series of recent reports describe the potential effectiveness of another novel S1P receptor agonist (KRP-203) in preventing allograft rejection. Structurally similar to FTY720, this compound acts as an $S1P_1$ agonist but, like SEW2871, does not affect $S1P_3$ [140]. Administration of KRP-203 is associated with improved outcome in rodent models for skin allograft, heart transplant, renal transplant, aortic transplant, and autoimmune hepatitis [140-144]. Studies involving its co-administration with commonly used immunosuppressive agents suggest that KPR-203 may be used as an adjuvant to improve the efficacy of mycophenolic acid or reduce the toxicity associated with cyclosporine by allowing for decreased dosage.

JTE-013

A compound developed by the Japan Tobacco Incorporation was recently described by researchers as an $S1P_2$-specific antagonist [145]. This compound (JTE-013) has been used to identify the involvement of $S1P_2$ in Rho-mediated coronary artery smooth muscle contraction [146] and Rho-mediated antiproliferation of hepatocytes [147]. The use of JTE-013 helped clarify the opposing effects that different S1P receptor subtypes have on cell migration [145, 148]. For example, the predominant S1P receptors expressed by endothelial cells and melanoma cells are $S1P_1$, $S1P_2$ and $S1P_3$. Stimulating these cells with S1P normally has a net inhibitory effect on migration. However, if $S1P_2$ is inhibited by pre-treatment with JTE-013, S1P *stimulates* migration through $S1P_1$- and $S1P_3$-mediated activation of Rac.

POSSIBLE INDICATIONS FOR DRUGS TARGETING LYSOPHOSPHOLIPID RECEPTORS

As previously mentioned, lysophospholipid receptors are widely expressed and are responsible for the regulation of many cellular processes. Therefore, it is not surprising that LPA and S1P signaling have been implicated in a number of disease pathologies. There is extensive evidence for their involvement in tumorigenesis and cardiovascular disease and, as such, these topics have been comprehensively reviewed elsewhere [48, 149-153]. The following section describes studies that identify diseases involving the CNS and immune system that may be treated with drugs that target lysophospholipid receptors.

Brain Injury

Both LPA and S1P are present in serum at high concentrations (micromolar) and their release from activated platelets is thought to be a major source of these signaling lipids [105, 154]. Therefore, it is likely that LP receptors in the brain are activated during the sudden influx of ligand when the blood-brain barrier is disrupted by traumatic injury. Considering the effects of LPA and S1P on cytoskeletal reorganization, migration and proliferation in cells of the CNS it is likely that these lipid signaling molecules mediate some aspects of inflammation and remodeling.

Astrogliosis is a hallmark response to many types of brain injury characterized by a dramatic increase in glial fibrillary acidic protein (GFAP)-positive astrocytes. Although the functional consequences of this process are incompletely resolved, it appears that glial scarring is inhibitory to axonal regeneration. In addition to the proliferative responses of astrocytes to LPA and S1P *in vitro* (described above) it has been demonstrated that intercranial injection of either lipid is sufficient to induce astrogliosis *in vivo* [59]. Furthermore, GF stimulation of astrocytes can modulate LP signaling. For example, one study shows that long-term stimulation of astrocytes with a GF cocktail significantly reduces LPA- and S1P-mediated IP accumulation [155]. The authors speculate that GF signaling following injury buffers a dramatic, acute response of astrocytes to LP stimulation. However, further studies are needed to fully characterize the events that are regulated by LPs during acute responses and remodeling following brain injury.

Seizure Disorder

Evidence for the role of S1P in CNS neuroexcitability was unexpectedly derived from the study of $S1P_2$ knockout mice. These animals initially appeared phenotypically normal in their development, anatomy, and physiology including such indexes of nervous system integrity as peripheral innervation, neuroanatomy, and cell death in the CNS [85, 86]. However, it was noticed that homozygous knockout mice occasionally exhibited seizure activity characterized behaviorally by a 2-10 second wild running episode followed by a 15-60 second period of freezing [86]. These events occurred only in 3-7 week old mice and were accompanied by changes in electrical activity of the brain. Electroencephalogram recordings revealed the presence of high amplitude polyspike discharges characteristic of epileptic seizures. Patch-clamp studies of neocortical pyramidal neurons revealed no defect in membrane potential, but there were notable increases in both the frequency and amplitude of spontaneous excitatory postsynaptic currents. The authors conclude that $S1P_2$ plays a role in the developmental, rather than physiological, stabilization of neuronal excitability. That is, the loss of $S1P_2$ may cause the association of inappropriate axonal/dendritic synapses that lead to promiscuous depolarization. This study provides insight into the pathophysiology of seizure disorders and identifies potential therapeutic strategies. Since this phenotype was not observed for independently derived nulls (discussed above), the generality of this mechanism remains for future work.

Pain

LPA was shown to induce a nociceptive response in peripheral sensory neurons in a mouse model [44]. In this study, LPA introduced by intraplantar injections stimulated a 3-fold increase in flexor response relative to saline controls. This response was PTX-sensitive (G_i-mediated) and prevented by administration of a substance P receptor antagonist. The nociceptive response was shown to be LPA_1-mediated by use of antisense oligonucleotide knockdown experiments [45] and LPA_1 knockout animals [46]. S1P did not elicit a similar response. Furthermore, intrathecal injection of LPA induced transient demyelination of the dorsal root, similar to that induced by ligation injury to the sciatic nerve. The demyelination from both stimuli was fully abrogated in the LPA_1 knockout animal. This supports the model that LPA release from activated platelets is required for the initiation of neuropathic pain following nerve injury. Therefore, antagonists of LPA_1 might be therapeutically useful for administration to early stages of nerve injury.

Transplant Rejection

Most cells of the immune system, including lymphocytes, mast cells, dendritic cells, and macrophages, either produce LPs and/or respond to them *via* stimulation of their cognate GPCRs. (For comprehensive reviews see: [135, 156, 157].) However, interest in understanding the roles of LPs in immune system function was compounded by the somewhat surprising discovery that the immunosuppressant FTY720-P is an S1P receptor agonist (see above). Conventional immunosuppressants (like cyclosporine, a calcineurin inhibitor) prevent transplant rejections by inhibiting T-cell activation. These drugs have dramatically improved the success of kidney and liver transplants since their introduction more than 20 years ago, but have a narrow therapeutic range. At doses high enough to prevent acute allograft rejection, calcineurin inhibitors can be associated with such side effects as infection and toxicity. Since the molecular target and mode of action of FTY720 does not overlap that of cyclosporine, it is thought that the use of FTY720 as adjunc-

tive therapy would facilitate the use of conventional therapies at lower doses, thus decreasing the risk of toxicity and improving efficacy. Indeed, early clinical trials of FTY720 combined with cyclosporine therapy shows similar efficacy for prevention of allograft rejection compared to conventional combination therapy [158]. Importantly, there is no apparent toxicity attributable to FTY720 [159]. Furthermore, since FTY720 merely sequesters lymphocytes away from the periphery (rather than inactivating or depleting them), it is predicted that this treatment is less likely to inhibit appropriate immune responses to infection compared to currently used therapies. Continued clinical studies are needed to assess consequences, particularly the safety, of longer-term exposure to FTY720.

Multiple Sclerosis

The efficacy of FTY720 in preventing transplant rejection suggests its potential use in the treatment of autoimmune disorders. Therefore, it is not surprising that researchers have used this compound in a convincing demonstration of its ability to clinically improve the symptoms of an animal model for multiple sclerosis (MS). MS is a progressive autoimmune disorder which causes demyelination of the CNS. Its pathology is replicated in mice with a manipulation known as experimental autoimmune encephalomyelitis (EAE). EAE is induced by immunization of a myelin protein peptide fragment. Animals treated in this way display symptoms characteristic of multiple sclerosis accompanied by plaques of demyelination as is also seen in MS. Interestingly, EAE animals show a striking improvement of clinical symptomology following treatment with FTY720, and this effect is observed whether the drug is administered before or after the peak onset of the disease [160]. This effect is reversible (drug-withdrawal caused an exacerbation of the symptoms) and parallels the dose-response of FTY720-mediated lymphopenia. The correlation between lymphopenia and clinical score is imperfect, however, which suggests other mechanisms by which FTY720 acts as an immune modulator.

CONCLUSION

LPs mediate a diverse range of biological processes that are mediated by cognate G protein-coupled receptors. The complexity of LP signaling systems is compounded by the overlapping expression of LP receptors. Advances in understanding LP signaling through genetic approaches have identified new physiological as well as pathophysiological roles for individual as well as combined receptor function. The growing availability of compounds that can be used to modulate LP signal transduction will provide important chemical tools, of particular utility *in vivo* when combined with genetic models. There is hope that these studies will ultimately lead to the development of novel therapies for the growing range of involved diseases.

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ABBREVIATIONS

AA	=	Arachidonic acid
CNS	=	Central nervous system
DOXP	=	Dioxazaphosphocane
EGF	=	Epidermal growth factor
GF	=	Growth factor
GFAP	=	Glial fibrillary acidic protein
GPCR	=	G protein-coupled receptor
IP	=	Inositol phosphate
LP	=	Lysophospholipid
LPA	=	Lysophosphatidic acid
LPA_{1-5}	=	High-affinity LPA receptors
LPP	=	Lipid phosphate phosphohydrolase
NAPS	=	2-N-Palmitoyl-serine-3-phosphate
NAEPA	=	*N*-acyl ethanolamide phosphate
NGF	=	Nerve growth factor
RT-PCR	=	Reverse transcriptase polymerase chain reaction
S1P	=	Sphingosine 1-phosphate
$S1P_{1-5}$	=	High-affinity S1P receptors
SC	=	Schwann cell
SVZ	=	Subventricular zone
PNPA	=	2-N-Palmitoyl-norleucinol-1-phosphate
PTX	=	Pertussis toxin
PLC	=	Phospholipase C
TTX	=	Tetrodotoxin
VZ	=	Ventricular zone

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