LPA Receptor Signaling: Pharmacology, Physiology, and Pathophysiology

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Abbreviations and Terminology: GPCR, G protein-coupled receptor; EDG, endothelial differentiation gene; SIP, sphingosine 1-phosphate; 7-TM, 7-transmembrane; cAMP, cyclic adenosine monophosphate; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; LPLs, lysophospholipids; LPC, lysophosphatidylcholine; LPS, lysophosphatidylserine; LPE, lysophosphatidylethanolamine; PLA₁, phospholipase A₁; sPLA₂, secretory phospholipase A₂; ATX, autotaxin; GPAT, glycerophosphate acyltransferase; MAG, monoacylglycerol; CSF, cerebrospinal fluid; PA, phosphatidic acid; LPPs, lipid phosphate phosphatases; LPAAT, lysophosphatidic acid acyltransferase; Enpp2, ectonucleotide pyrophosphatase/phosphodiesterase family member 2; VZ, ventricular zone; NPC, neuroprogenitor cell; OL, oligodendrocyte; SC, Schwann cell; IL, interleukin; DCs, dendritic cells; FH, fetal hydrocephalus; cPLA₂, cytosolic PLA₂; COX-2, cyclooxygenase-2; VEGF, vascular endothelial growth factor; NPSPA, N-palmitoyl serine phosphoric acid; NPTyrPA, N-palmitoyl tyrosine phosphoric acid; NAEPA, N-acyl ethanolamide phosphate; AO-LPA 12b, sn-2-aminooxy analog 12b; α-FMP, α-fluoromethylene phosphonate; α-HMP, α-hydroxymethylene phosphonate; Dialkyl TPA, Dialkyl thiophosphatidic acid; DDP, Dodecyl phosphate; α-MP, α-methylene phosphonate; ODP, Octadecenyl phosphate; TP 18:1, Oleoyl-thiophosphate; DGPP, Diacylglycerol pyrophosphate; BrP-LPA 19b, α-bromomethylene phosphonate analog 19b; TPA 8:0, Thiophosphatidic acid 8:0; TDP, Tetradecyl phosphonate; FDP, Farnesyl diphosphate; Cmpd, Compound
Abstract

Lysophosphatidic acid (LPA) is a small, ubiquitous lipid found in vertebrate and non-vertebrate organisms that mediates diverse biological actions and demonstrates medicinal relevance. LPA’s functional roles are driven by extracellular signaling through at least six, 7-transmembrane G protein-coupled receptors (GPCRs). These receptors are named LPA1-6 and signal through numerous effector pathways activated by heterotrimeric G proteins, including G\textsubscript{i/o}, G\textsubscript{12/13}, G\textsubscript{q}, and G\textsubscript{s}. LPA receptor-mediated effects have been described in numerous cell types and model systems, both \textit{in vitro} and \textit{in vivo}, through gain and loss of function studies. These studies have revealed physiological and pathophysiologica influences on virtually every organ system and developmental stage of an organism. These include the nervous, cardiovascular, reproductive, and pulmonary systems. Disturbances in normal LPA signaling may contribute to a range of diseases, including neurodevelopmental and neuropsychiatric disorders, pain, cardiovascular disease, bone disorders, fibrosis, cancer, infertility, and obesity. These studies underscore the potential of LPA receptor subtypes and related signaling mechanisms to provide novel therapeutic targets.

\textbf{Supplementary key words:} lysophosphatidic acid, lysosphospholipid, autotaxin, brain lipids, cancer, fibrosis, reproduction, obesity, pain, atherosclerosis
Introduction

Lysophosphatidic acid (LPA) is a small glycerophospholipid (molecular weight: 430-480 Da) that acts as a potent extracellular signaling molecule through at least six cognate GPCRs in numerous developmental and adult processes involving virtually all vertebrate systems. All LPA molecules consist of a glycerol backbone connected to a phosphate head group and are commonly ester-linked to an acyl chain of varied length and saturation. These various chemical forms of LPA are derived from multiple sources, such as membrane lipids (1), and exist as bioactive ligands that signal through cognate receptors to produce a wide number of physiological responses (Figure 1).

In the 1960s, studies on smooth muscle and blood pressure hinted at the bioactivity of LPA (2, 3). In later years, various LPA species were isolated and identified from soy beans (4). This raised intriguing questions regarding this lipid’s mechanism of action, which was then thought to include membrane perturbation (5), calcium chelation (6), second messenger signaling (7), intracellular receptors (8), and cell-surface receptors (9). These contending theories were clarified upon the cloning and identification of the first lysophospholipid receptor: LPA₁. This GPCR was previously known as “ventricular zone gene-1” because of its enriched expression in the embryonic neuroproliferative layer of the cerebral cortex (10, 11). The cloning and functional identification of LPA₁ led to the deorphanization of other putative receptor genes based upon sequence homology (12-14), particularly “endothelial differentiation gene” (EDG) members (15) that include LPA and sphingosine 1-phosphate (S1P) receptors. The significant homology between LPA₁ and S1P₁ was underscored by early reports of EDG-1 as a LPA receptor (16). At the time of LPA₁ identification, the only functional homologous receptor was the cannabinoid receptor CB1 (encoded by CNR1) that interacts with the endogenous lipids anandamide and 2-arachidonylglycerol (17, 18). Two other LPA receptors, LPA₂ and LPA₃, were subsequently discovered based on shared homology with LPA₁. In the past ten years, three additional LPA receptors have been discovered (LPA₄-6) (19-23) that are members of the
P2Y purinergic family (Figure 1). These receptors have significantly different encoding sequences than LPA1-3, yet still bind and mediate LPA signaling effects. All six current LPA receptors are class A, rhodopsin-like GPCRs with 7-transmembrane (7-TM) domains. Every receptor couples to at least one or more of the four heterotrimeric Gα proteins (G_{12/13}, G_{q/11}, G_{i/o}, and G_{s}) (Figure 1), resulting in canonical downstream signaling that produce diverse physiological and pathophysiological effects. Other types of lysophospholipids, such as lysophosphatidylserine, lysophosphatidylinositol, and lysophosphatidylethanolamine, have some reported bioactivity and are being evaluated for the involvement of possible cognate receptors (24). An additional important aspect of LPA receptor biology is that various chemical forms of LPA may differentially activate LPA receptor subtypes (25). This finding has been supported by secondary readouts of receptor activity, since direct confirmation through classical receptor binding studies has been difficult.

**LPA Metabolism**

There are two major synthetic pathways for LPA. In the first pathway, the precursor phospholipids (PC, PS, or PE) can be converted to their corresponding lysophospholipids (LPLs) such as lysophosphatidylcholine (LPC), lysophosphatidylserine (LPS), or lysophosphatidylethanolamine (LPE). In platelets, this occurs via phosphatidylserine-specific phospholipase A1 (PS-PLA₁) or secretory phospholipase A2 (sPLA₂) activity. In plasma, LPC is produced by lecithin cholesterol acyltransferase (LCAT) and PLA₁ activity. In either location, LPLs can then be converted to LPA via autotaxin (ATX) activity (Figure 1). In the second major pathway, phosphatidic acid (PA) is first produced from phospholipids through phospholipase D or from diacylglycerol through diacylglycerol kinase. Then, PA is converted directly to LPA by the actions of either PLA₁ or PLA₂ (26) (Figure 1).

Through a separate mechanism, LPA can be generated through the acylation of glycerol-3-phosphate by glycerophosphate acyltransferase (GPAT) and the phosphorylation of
monoacylglycerol (MAG) by monoacylglycerol kinase (MAG-kinase) (27). Additional LPA-producing pathways also exist (26, 28). LPA generation from membrane phospholipids occurs in both intracellular and extracellular fashions (28). Intracellular LPA is an important intermediate for the de novo biosynthesis of complex glycerolipids, including mono-, di-, and triglycerides, as well as phospholipids (28). Extracellular LPA is thought to mediate bioactive effects through LPA receptors (29). Furthermore, a report supporting LPA as a functional ligand for the intracellular transcription factor PPARγ exists, although it remains to be examined (30).

LPA has been found in all eukaryotic tissues examined. The formation of an LPA species depends on its precursor phospholipid, which can vary by acyl chain length and degree of saturation. The term LPA most often refers to 18:1 oleoyl-LPA (1-acyl-2-hydroxy-sn-glycero-3-phosphate), as it is the most commonly used laboratory species. However, there is a growing range of recognized chemical forms of LPA in various biological systems (31, 32) that have been observed in concentrations spanning low nanomolar to micromolar levels. LPA concentrations in blood can range from 0.1 μM in plasma and up to 10 μM in serum, which is well over the apparent nanomolar Kd of LPA1-6 (23, 33-35). The 18:2, 20:4, 16:1, 16:0, and 18:1 LPA forms are particularly abundant in plasma (36-38). Aside from blood, LPA has been quantified in a variety of species, tissues, and fluids, including neural tissue, cerebrospinal fluid (CSF), seminal fluid, urine, saliva, and aqueous humor (7, 36, 39-45) (Table 2). Current methods to detect LPA include indirect enzymatic assays (34), TLC-gas chromatography, liquid chromatography/tandem mass spectrometry (LC-MS), and LC-MS-MS (46-48). These techniques have a growing number of predictive, diagnostic, and therapeutic uses (29, 43, 49, 50).

LPA degradation is mediated by several classes of enzymes, including three types of lipid phosphate phosphatases (LPPs) (51), LPA-acyltransferase (LPAAT), and various phospholipases (e.g., PLA1 or PLA2) (28, 52, 53). LPA may be converted back to phoshatidic
acid by LPAAT, hydrolyzed by LPP(1-3), or converted by phospholipases into glycerol-3-phosphate (28, 54).

**Autotaxin**

Autotaxin (ATX) is one of the best studied enzymes associated with LPA signaling. ATX was first identified and purified as “autocrine motility factor” from human melanoma cells and was perceived to be an ectonucleotide pyrophosphatase/phosphodiesterase (*Enpp2*). Furthermore, since the addition of pertussis toxin reduced cellular motility, ATX’s effects were thought to involve G<sub>i/o</sub> mediated signaling (55). Extracellular LPA was found to be produced in sub-millimolar concentrations by lysophospholipase D activity in plasma (39). The responsible enzyme was subsequently identified as ATX (56, 57). It is clear now that ATX-mediated autocrine signaling induces cell motility through LPA production and G<sub>i/o</sub> mediated LPA receptor signaling (29, 58, 59).

ATX has broad tissue expression, with comparatively high levels in blood, brain (especially the choroid plexus and embryonic floor plate), kidney, and lymphoid organs (60-62). Secretion of ATX leads to high expression and concentration in CSF and in the high endothelial venules of lymphoid organs (63-66). Genetic deletion of ATX in mice (*Enpp2<sup>−/−</sup>*) mutants has contributed significant understanding to this enzyme’s physiological role. These mice die at embryonic day E9.5 with obvious vascular and neural tube defects (67, 68). In addition, they have a poorly formed yolk sac vascular network, as well as enlarged embryonic vessels, malformed allantois, reduced axial turning, kinked neural tube, and an asymmetric neural head fold (68, 69). *Enpp2<sup>+</sup/+</sup>* heterozygotes appear normal, but have a 50% reduction in LPA plasma levels compared to wild-type mice. Thus, ATX activity is significantly responsible for the correct concentration of LPA in plasma, as well as for proper vascular and embryonic development.
ATX and LPA signaling are involved in the vasoregulation and vascular biology of multiple species (4, 70, 71). ATX expression can be induced by vascular endothelial growth factor (VEGF), and the resultant elevation of LPA induces both proliferation and migration of endothelial cells, as well as barrier formation in vitro (72-75). Such endothelial cell behaviors require a variety of additional activated-platelet mediators, such as growth factors and cytokines. LPA is also released from activated platelets (36), which can induce platelet activation through positive feedback (40, 76). In general, LPA production and signaling can induce mitogenic and migratory effects on numerous cell types that are involved in angiogenesis and tissue repair (73, 77-79). Despite these data, some controversy exists as to whether LPA and ATX are truly angiogenic, although both appear to help in vessel maintenance. Interestingly, increased LPA levels can rapidly and reversibly increase blood brain barrier permeability (80), suggesting a concentration-dependent mechanism. Finally, LPA can also regulate vascular tone, such as post-hemorrhagic vasoconstriction in cerebral microvasculature (81) or vasodilation in the thoracic aorta (82). These opposing actions may be context dependent.

LPA Receptors

The diverse and numerous physiological effects of LPA are mediated through six currently recognized LPA receptors, LPA\textsubscript{1-6}: protein names LPA\textsubscript{1–6} and gene names \textit{LPAR1-LPAR6} (human) and \textit{Lpar1-Lpar6} (non-human) (11, 83). These 7-TM GPCRs couple to at least one or more of the four G\textsubscript{a} proteins (G\textsubscript{12/13}, G\textsubscript{q/11}, G\textsubscript{i/o}, and G\textsubscript{s}) that initiate a variety of signaling cascades. The spatiotemporal distribution of LPA and LPA receptors - typically differential receptor subtypes in specific tissues - drives the abundance of the physiological and pathophysiologial processes noted here.

LPA\textsubscript{1}
LPA₁ was the first lysophospholipid receptor identified (10) and is the best studied of the six LPA receptors. The *LPAR1* gene (human chromosome locus 9q31.3) encodes a 41-kDa protein containing 364 amino acids with 7-TM domains. In mice, the *Lpar1* gene (mouse chromosome locus 4 32.2 cM) encodes five exons with a conserved intron (shared amongst *Lpar1-3*) interrupting TM domain 6. There is a variant of *Lpar1* (mrec1.3) that results in an 18-amino acid deletion of the N terminus (84), but its biological significance is unknown. LPA₁ shares ~50-60% amino acid sequence identity with LPA₂ and LPA₃. While no complete crystal structures have been reported for any LPA receptor to date, crystallization of other GPCRs have facilitated constraint modeling for LPA₁, particularly in the second extracellular loop (85). Additionally, computer-modelled mutagenesis studies have identified three key residues in LPA₁-₃ signaling. R3.28A and K7.36A are both important for the efficacy and potency of LPA, while Q3.29A decreased ligand interaction and activation (86), based primarily on secondary read-outs. Future structural analyses of LPA₁ will help clarify key residues.

LPA₁ couples with three types of Gα proteins: G₁₁₀, G₁₁₁, and G₁₂/₁₃ (Figure 1), which initiate downstream signaling cascades though phospholipase C, mitogen-activated protein kinase (MAPK), Akt, and Rho. LPA₁ activation alters a range of cellular responses, including cell proliferation and survival, cell-cell contact through serum-response element activation, cell migration and cytoskeletal changes, Ca²⁺ mobilization, and adenylyl cyclase inhibition (15, 77, 87).

There is wide distribution of Lpar1/LPAR1 expression in both adult mice and humans, including in the brain, uterus, testis, lung, small intestine, heart, stomach, kidney, spleen, thymus, placenta, and skeletal muscle (13, 87, 88). Lpar1 expression is more restricted during embryonic development, including portions of the brain, dorsal olfactory bulb, limb buds, craniofacial region, somites, and genital tubercle (89, 90). Lpar1 expression is regulated both spatially and temporally. For example, in the developing nervous system, (77, 87), Lpar1 is found in several areas, including the germinal neuroepithelium or ventricular zone (VZ), portions
of the midbrain, and superficially in the marginal zone and meninges (10, 43, 89, 91). Though the VZ disappears at birth, \textit{Lpar1} expression continues in the hindbrain and the white matter tracks during oligodendrocyte myelination of the postnatal brain (92). Visualization of LPA\textsubscript{1} expression (as well as other LPA receptors) has been hampered by lack of validated antibodies.

LPA signaling has numerous effects on key neurodevelopmental processes, including cerebral cortex formation and function (93-95), neural cell proliferation, survival, growth cone retraction, migration, and adhesion (90, 95-102). Interestingly, studies have also found evidence of concentration-dependent LPA-mediated necrosis as well as apoptosis, possibly through oxidative stress (103-105). \textit{In vitro} experiments have demonstrated the effects of LPA\textsubscript{1}-mediated stimulation that increase the proliferation and differentiation on primary neuroprogenitor cell (NPC) cultures, neuronal cell lines, and primary neurons (90, 106, 107). Additional LPA\textsubscript{1}-mediated neural effects have also been observed in non-mammalian organisms, in which analogs of LPA\textsubscript{1} and LPA\textsubscript{2} could regulate cortical actin assembly in \textit{Xenopus} embryos (108).

LPA can generate diverse cellular responses, and the interacting proteins that mediate downstream signaling pathways are being uncovered. Towards elucidating these interactions, one study demonstrated that LPA\textsubscript{1} (but not other tested LPA receptors) is trafficked to early endosomes through a PDZ-mediated motif that facilitates its binding to GIPC, which attenuates LPA-mediated Akt signaling from APPL signaling endosomes (109). Other studies identified LPA as a critical regulator of the Hippo-YAP pathway, which controls LPA-induced gene expression, cell migration, and cell proliferation. This pathway was predominantly activated through G\textsubscript{13} stimulation, presumably via the RhoA-ROCK pathway, while G\textsubscript{s} activity inhibited YAP function (110, 111). While the critical LPA receptors in this pathway are still being investigated, there is evidence for varying LPA\textsubscript{1}, LPA\textsubscript{2}, and LPA\textsubscript{3} involvement within different cell types.
Removal of Lpar1 by constitutive deletion has provided insight into the in vivo functions of this receptor. Lpar1−/− mouse litters have 50% perinatal lethality related to impaired suckling behavior (90), which was attributed to defective olfaction. Surviving Lpar1−/− mice have a significantly reduced body size, craniofacial dysmorphism with blunted snouts, and increased apoptosis in sciatic nerve Schwann cells (90). When the original Lpar1−/− line was expanded, a spontaneous variant arose that was named “Málaga LPA1” (maLPA1) (93). The maLPA1 variant has negligible perinatal lethality, but displays more severe brain defects than the Lpar1−/− line. Neurodevelopment defects in maLPA1 include reduced NPC proliferation, increased cerebral cortical apoptosis, decreased cortical size, and premature expression of neuronal markers (93). Defects in the proliferation, differentiation, and survival of new neurons was also impacted during adult hippocampal neurogenesis (112). Additionally, maLPA1 mice showed several behavioral deficits, including inhibition of fear extinction (113) and aggravation of chronic stress-induced impairment to hippocampal neurogenesis (114). Recently, an Lpar1−/− rat hypomorph generated by ENU mutation also showed some physical defects reminiscent of the mouse knockout (115). In addition to NPC and neurons, LPA1 signaling is also prominently involved in glial biology, which includes macroglia such as astrocytes, oligodendrocytes and Schwann cells, as well as microglia. Astrocytes are the most abundant type of glia and play a significant role in developmental, functional, and pathological processes (116). While basal in vivo astrocytes have undetectable levels of LPA1 (91, 92, 117), cultured astrocytes express LPA1−5 (118) and are responsive to LPA. Such effects include calcium influx, phosphorylation of ERK, morphological changes, and stabilization of stress fibers (119-121). LPA1 is also implicated in astrocyte proliferation and astrogliosis, although the relevant receptors have not been conclusively identified (122). Lipopolysaccharide or interleukin-1β-primed astrocyte migration appears to involve a G12/13 phosphatidylinositol 3-kinase (PI3K) /Rac1 response to LPA, in contrast to their normal proliferative response via G12/13 (123). However, some controversy regarding this proliferation pathway exists, possibly due to different culture systems (124).
addition, both LPA₁ and LPA₂ signaling can increase neuronal differentiation by LPA-primed astrocytes through unidentified astrocyte-derived soluble factors (125), possibly epidermal growth factor (EGF) (126) or nerve growth factor (127), hinting at a rich LPA-mediated biology between astrocytes and neurons.

Another glial cell type, the oligodendrocyte (OL), also expresses LPA₁ (91, 92, 128). These cells produce myelin, ensheathing neurons in the CNS. Basally, Lpar1 co-localizes with proteolipid protein and myelin basic protein, but not glial fibrillary acidic protein (91, 92). During development, Lpar1 expression in OLs emerges just prior to maturation/myelination, suggesting an important role in controlling these processes, although no effect of LPA on OL survival, maturation, myelination, or cytoskeleton organization was reported in vitro (129). However, both the rat OL precursor cell line CG-4 and freshly isolated precursors respond via Rho-ROCK (Rho-associated protein kinase) cell rounding upon LPA exposure, in contrast to differentiated OLs (130). Similarly, LPA increases the network area of dendritic processes and MBP expression in differentiating OLs (131). Therefore, LPA may regulate OL function in a maturation stage-specific manner. Additional studies will better define LPA signaling activity in this cell type.

Schwann cells (SCs), which are counterparts to OL in the peripheral nervous system, have also been implicated in LPA signaling. Two main types of SCs exist: myelinating SCs express Lpar1 and possibly Lpar2 (100, 132), while terminal SCs express both Lpar1 and Lpar3 (100, 132). SC survival is increased by LPA through LPA₁ and G/PI3K/Akt in vitro (133), which is supported in vivo by observations that Lpar1⁻/⁻ mice have increased apoptosis of SCs in sciatic nerves (90). In addition to SC survival, LPA also induces morphological and cell adhesion changes. In vitro, LPA induces SC wreath formation and enhances focal adhesions, as well as promoting N-cadherin dependent cell aggregation (100), responses that are dramatically reduced in Lpar1⁻/⁻ SCs (100). LPA can also increase the expression of P0 protein in SCs through LPA₂ signaling, possibly contributing to SC differentiation (134). Recently, LPA₁
was shown to influence embryonic SC migration, adult myelination, and cell-to-axon segregation, processes that appear to be defective in Lpar1<sup>-/-</sup> mice (135).

**LPA<sub>2</sub>**

LPA<sub>2</sub> was first identified through a gene sequence search for orphan GPCRs. It shows 55% amino acid similarity to LPA<sub>1</sub>. LPAR2 (human chromosome 19p12) encodes a 351 amino acid protein with a calculated molecular mass of 39.1 kDa (136), while mouse Lpar2 (mouse chromosome 8 33.91 cM) encodes for a 348 amino acid protein with molecular weight of 38.9 kDa. Mutagenesis studies of LPA<sub>2</sub> have found two key residues that, when altered, can decrease LPA<sub>2</sub> activation (Q3.29E and R5.38A) (86). During development, expression of Lpar2 is more diffuse than Lpar1, although it is clearly present in the embryonic brain, limb buds, craniofacial regions, and Rathke’s pouch (89, 137-139).

Compared with Lpar1/LPAR1, Lpar2/LPAR2 expression is more restricted in adult mice and humans, with high LPAR2 in leukocytes and the testis, and Lpar2 in the kidney, uterus, and testis (13, 87). More moderate levels of LPAR2 are found in the prostate, spleen, thymus, and pancreas, and lower levels of Lpar2 expression are found in the lung, stomach, spleen, thymus, postnatal brain, and heart (140).

LPA<sub>2</sub> couples to the G<sub>i/o</sub>, G<sub>q/11</sub>, and G<sub>12/13</sub> family of heterotrimeric G proteins, similar to LPA<sub>1</sub> (Figure 1). These G proteins initiate downstream signaling pathways through molecules such as Ras, Rac, PI3K, MAPK, PLC, diacylglycerol, and Rho (90). LPA<sub>2</sub> activation is associated with cell survival and cell migration. LPA<sub>2</sub>-initiated migration appears to be promoted through interactions with the focal adhesion molecule TRIP6 (141, 142) and several PDZ-domain and zinc finger proteins, through interactions with the carboxy-terminal tail of LPA<sub>2</sub> (143). Notably, the PDZ-binding domain of LPA<sub>2</sub> regulates NHERF2 behavior, activating PLC-β3 and AKT/ERK signaling while inhibiting CTFR. These pathways stimulate cell migration, enhance survival, and alter gene expression, accounting for many of the functions attributed to LPA<sub>2</sub>. This intracellular
coupling is a unique feature of LPA₂. Also, LPA₂ activation can inhibit EGF-induced migration and invasion of pancreatic cancer cells through the G₁₂/₁₃/Rho pathway (144). These data lend support to the pattern of transactivation and cross-regulation between GPCRs and receptor tyrosine kinases in a variety of settings (145) and expand the signaling effects of LPA beyond single subtype receptor mechanisms.

Phenotypically, Lpar2⁻/⁻ mice appear mostly normal, with expected Mendelian birth ratios and normal prenatal and postnatal viability (146). Like LPA₁, LPA₂ is involved in several aspects of nervous system function and development. Activation of LPA₂ increases P0 protein in cultured SCs, implicating this receptor in myelination. In fact, LPA₂ is upregulated, along with LPA₁, after neural injuries such as nerve transection and neuropathic pain (100, 147). The interaction of LPA₂ with plasticity related gene-1 (PRG-1) signaling has also been reported to modulate excitatory transmission in the hippocampus (148).

Mice that are Lpar₁⁻/⁻/Lpar₂⁻/⁻ null have also been generated. Such mutants mostly phenocopy Lpar₁⁻/⁻ mice and have similar body size reduction, craniofacial dysmorphism with blunted snouts, and 50% perinatal lethality, but also exhibit an increased incidence of frontal hematomas. Studies in these mice have also shed light on LPA₁ and LPA₂ signaling redundancy in distinct neural and vascular phenotypes. Lpar₁⁻/⁻/Lpar₂⁻/⁻ cells display altered responsiveness to LPA in vitro, ranging from significant reduction to complete abrogation (95, 149). Exogenous LPA exposure in ex vivo cerebral cortical cultures decreased cell death, increased NPC cell cycle exit, and produced early terminal mitosis. This resulted in precocious neuronal production, cortical thickening, and folding which resembles gyri in humans (95). These effects were abolished in Lpar₁⁻/⁻/Lpar₂⁻/⁻ embryonic cerebral cortices.

Lpar₁⁻/⁻/Lpar₂⁻/⁻ mice have also been helpful in understanding the effects of LPA on the vascular system. Specifically, LPA₁ and LPA₂ were found to exhibit opposite effects on primary vascular smooth muscle cells (VSMCs) and injury-induced neointimal hyperplasia (149). VSMC migration was increased in Lpar₁⁻/⁻ mice but was partially attenuated in Lpar₁⁻/⁻/Lpar₂⁻/⁻ mice,
demonstrating LPA$_1$ and LPA$_2$’s opposition as negative and positive chemotactic mediators, respectively. Additionally, exogenous LPA exposure can increase vascular permeability, which was blocked using the dual LPA$_{1/3}$ receptor antagonist Ki16425 (150). Ki16425 also inhibited neointima formation and SMC recruitment to the injury site after wire-induced carotid injury (151). Neither LPA$_1$ nor LPA$_2$ was required for dedifferentiation of SMCs following vascular injury in vivo or LPA exposure ex vivo, nor were they required for LPA-induced blood pressure increases (149). These observations indicate the likely involvement of other LPA receptor subtypes in these processes.

A similar dynamic between LPA$_1$ and LPA$_2$ signaling also exists in immune system function. LPA receptors are found on virtually all immune cell types and organs, including lymphocytes (144), dendritic cells (DCs) (152, 153), thymus, and spleen (20, 77, 154). In T cells, LPA can stimulate or attenuate cellular activity, depending on the cell’s activation state and receptor expression. In unstimulated T cells, LPA augments chemotaxis and blocks interleukin-2 (IL-2) production through LPA$_2$ (155, 156). In activated T cells, where LPA$_2$ is downregulated and LPA$_1$ is upregulated, the reverse is true; LPA inhibits chemotaxis, activates IL-2 and IL-13 production, and promotes cell proliferation (156, 157). In addition, LPA affects immature and mature DCs differently. LPA$_{1-3}$ are expressed in both immature and mature DCs, and LPA appears to affect immature DCs by enhancing maturation and cytokine production (152, 153). Furthermore, LPA$_3$-specific activation induces chemotaxis of immature, but not mature, DCs (158). DCs from Lpar2$^{-/-}$ mice are hyperactive compared with wild-type counterparts and less susceptible to inhibition by different LPA species, suggesting that LPA via LPA$_2$ may inhibit DC activation (159). Thus, LPA may differentially affect DC maturity and stage-specific functions through multiple LPA receptors.

LPA$_3$
*Lpar3* was discovered and cloned in a similar fashion to *Lpar2*, using homology searches for orphan GPCRs and a degenerate, PCR-based cloning method (14, 160).

**LPAR3/Lpar3** (human chromosomal locus 1p22.3, mouse chromosome locus 3 71.03 cM) encodes a 353 amino acid sequence GPCR with a molecular mass of ~40 kDa that, in humans, is ~54% identical to LPA₁ and ~49% identical to LPA₂ (14). Mutagenesis studies on LPA₃ have identified two specific residues involved in LPA₃ activation (W4.64A and R5.38N), as well as a residue that increased the EC₅₀ of LPA₃ by a factor of ten (K7.35A) (86). During development, *Lpar3* expression is observed in the heart, mesonephros, and in three spots in the otic vesicle (89). In the adult, **LPAR3/Lpar3** expression is most prominent in the human heart, testis, prostate, and pancreas, (14, 160) as well as mouse lung, kidney, uterus, and testis. **LPAR3/Lpar3** is less highly expressed in the human lung, ovary, and brain, as well as mouse small intestine, brain, heart, stomach, placenta, spleen, and thymus (87, 140). Expression differences of LPA₃ between human and mouse tissue warrant further study.

LPA₃ couples with Gα₁/q and Gα₁/q₁₁ to mediate LPA-induced Ca²⁺ mobilization, adenylyl cyclase inhibition and activation, PLC activation, and MAPK activation ([Figure 1](#)) (161). LPA₃ has been reported to prefer 2-acyl-LPA containing unsaturated fatty acids (14, 162). In addition, *Lpar3*−/− mice are viable and appear normal, with no reported neural deficits even though LPA₃ is found in the frontal cortex, hippocampus, and amygdala (14, 160). However, female null mutants have a striking reproductive phenotype, with delayed embryo implantation, embryo crowding, and reduced litter size (163). In the uterus, *Lpar3* is solely found in the endometrial epithelium of the lumen during the transient window of embryo implantation (163, 164) and is regulated by progesterone and estrogen (165). LPA is found in follicular fluid from healthy women, which underscores the LPA-LPA₃ signaling axis in the uterus (166). During pregnancy, ATX concentration is upregulated in the serum and placenta of healthy women in the third trimester. Interestingly, patients with pregnancy-induced hypertension (preeclampsia) harbored decreased ATX levels in the third trimester compared with healthy women (56, 167, 168). LPA
receptors are also expressed in the testis (19, 77, 88), suggesting a role for LPA signaling in male sperm behavior and fertility. These data support numerous LPA-mediated roles in reproduction.

In addition, LPA<sub>3</sub> appears to be involved in determining vertebrate left-right patterning during embryogenesis, a crucial process for proper organ formation and function. Downregulation of <i>Lpar3</i> or inhibition of LPA<sub>3</sub> signaling with Ki16425 disrupted asymmetric gene expression and organ asymmetry in zebrafish, a phenotype which was rescued by <i>Lpar3</i> overexpression (169). Downregulation of ATX by oligonucleotide knockdown or inhibition of ATX by HA130 similarly disrupted left-right patterning and developmental asymmetry.

**LPA<sub>4</sub>**

LPA<sub>4</sub> was the first identified lysophospholipid receptor to show a dissimilar amino acid sequence from the other lysophospholipid receptors, LPA<sub>1-3</sub>, sharing only ~20% amino acid identity with LPA<sub>1</sub> (140) and slightly greater homology to other LPA receptors (19). It was previously known as the orphan GPCR P2Y9, based on its similarity to P2Y purinergic receptors. However, it was reclassified as LPA<sub>4</sub> after demonstrating responsiveness to LPA but not to any nucleotides or nucleosides during ligand screening using a calcium mobilization assay (11, 19).<i>LPAR4</i>/<i>Lpar4</i> (human chromosome Xq21.1, mouse chromosome X region D) encodes a 370 amino acid protein with predicted molecular weight of 41.9 kDa.

During development, <i>Lpar4</i> is found in the mouse embryonic brain, maxillary processes, branchial arches, limb buds, liver, and somites (89). In adults, <i>LPAR4</i> is prominently found in the human ovary, while less prominent <i>LPAR4</i> can be found in the thymus, pancreas, brain, heart, small intestine, testis, prostate, colon, and spleen. <i>Lpar4</i> is present in mouse heart, ovary, skin, thymus, and bone marrow (89, 140).

LPA<sub>4</sub> is a 7-TM GPCR that couples with G<sub>α</sub>-proteins, including G<sub>12/13</sub>, G<sub>q/11</sub>, G<sub>ι/0</sub>, and G<sub>s</sub> (170). This receptor induces neurite retraction and stress fiber formation through G<sub>12/13</sub> and
subsequent Rho/ROCK pathway activation, as seen with activation of other LPA receptors (170, 171). In addition, LPA₄ facilitates both ROCK dependent cell aggregation and N-cadherin dependent cell adhesion, using a cell line that heterologously expresses this receptor (171). Moreover, LPA₄ induces intracellular cyclic adenosine monophosphate (cAMP) accumulation through Gₛ activation; currently this is the only known LPA receptor with this activity (170). LPA₄ can also influence the differentiation of immortalized hippocampal progenitor cells (172). LPA-induced cell migration (e.g., via LPA₁) is inhibited by activation of LPA₄, and LPA₄-deficient cells have increased sensitivity to LPA exposure, with increased lamellipodia formation and transwell movement (173). This noteworthy ability of LPA₄ to negatively regulate cell motility opposes LPA’s traditional status as a chemoattractant and indicates that differential effects may be achieved through combinatorial LPA receptor expression.

Adult Lpar₄⁻/⁻ mice appear grossly normal (173), yet exhibit increased trabecular bone volume, number, and thickness (174, 175). This is in contrast to Lpar¹⁻/⁻ mice, which display decreased bone mass (176). This suggests that LPA₄ negatively regulates osteogenesis and may counteract LPA₁-initiated osteogenesis. Consistent with these observations, knockdown of LPAR4 in a human mesenchymal stem cell line resulted in the inhibition of its differentiation into osteoblasts (177). During embryo development, there is decreased prenatal survival for Lpar₄⁻/⁻ mutants. Observed phenotypes in these null mice include pericardial effusions, severe edema, and hemorrhage, abnormally dilated blood and lymphatic vessels and lymph sacs, and impaired pericyte recruitment (178). This indicates important pleiotropic roles for LPA₄ in circulatory and lymphatic system development. Vasculogenesis in extraembryonic membranes (such as the chorioallantoic membrane) has also been recently linked to an ATX-mediated product ion of LPA. The responsible receptor may be LPA₄, although the concomitant presence of Lpar₁, Lpar₂, and Lpar₆ make this subtype identification unclear (179).
In 2006, LPA$_5$ was identified through unbiased screening approaches that led to the deorphanization of GPR92 (20, 21). LPAR5 shares 35% homology with LPAR4, but only ~22% homology to LPAR1-3 (21). LPAR5/Lpar5 (human chromosome 12p13.31, mouse chromosome 6 59.21 cM) encodes a 372 amino acid protein. LPAR5 is highly expressed in spleen, and to a lesser degree in heart, small intestine, placenta, colon, and liver. Lpar5 in murine tissues is highly expressed in small intestine, and more moderately in lung, heart, stomach, colon, spleen, thymus, skin, liver, platelets, mast cells, gastrointestinal lymphocytes, and dorsal root ganglia (20, 21, 180, 181). Additionally, Lpar5 was found in the early embryonic mouse forebrain, rostral midbrain, and hindbrain. This expression pattern becomes more ubiquitous from E9.5-E12.5, showing signals throughout the embryo, as well as diffuse patterns in the developing brain and choroid plexus that suggest a possible role for LPA$_5$ in brain development (89).

Mutagenesis studies have found that several residues are likely involved in the binding of LPA to LPA$_5$, including one mutant that abolished receptor activation (R2.60N) and three separate mutants that greatly reduced receptor activation (H4.64E, R6.62A, R7.32A) (182).

LPA$_5$ couples to G$_{12/13}$ and G$_{q/11}$ (21). LPA$_5$-expressing cell lines demonstrate LPA-induced neurite retraction, stress fiber formation, and receptor internalization in vitro through the G$_{12/13}$ pathway (21). LPA$_5$ can also activate G$_q$ to increase intracellular calcium levels and induce cAMP accumulation (21). However, this cAMP accumulation is not changed by G$_s$ minigene administration, suggesting the involvement of an alternative G protein (20, 21). In melanoma cells, LPA inhibits migration through LPA$_5$ and the cAMP-PKA pathway, acting as a chemorepellent that is similar to its LPA$_4$-mediated effect. Moreover, LPA$_5$ demonstrated a 10-fold preference for alkyl, rather than acyl, 18:1 LPA (183). LPA$_5$ signaling may also affect the colon, where it may be involved in water absorption (184). In intestinal epithelial cells, LPA promoted Na$^+$-dependent water absorption through Na$^+$/H$^+$ exchanger 3 (NHE3) via an interaction between LPA$_5$ and Na$^+$/H$^+$ exchanger regulatory factor 2 (NHERF2), which then recruits NHE3 to the microvilli (184). In mast cells, LPA$_5$ is the main LPA receptor responsible
for LPA-induced release of MIP-1β (also known as chemokine ligand 4 or CCL-4) (181). The diverse expression patterns of LPA₆ suggest that additional physiological effects will be uncovered in the future.

**LPA₆**

The most recent addition to the LPA receptor family is LPA₆. In 1993, receptor 6H1 was isolated from a chicken T cell library (185) and subsequently renamed to p2y₅ because of sequence homology with P2Y receptors (186). Genetic studies indicated roles for LPA₆ in hypotrichosis simplex, a complex of diseases involving familial hair loss (22). Most recently, studies using a chimeric G₁₃ protein indicated that LPA induced multiple effects, including p2y₅-mediated cAMP accumulation, Rho-dependent changes in cell morphology, [³H]LPA binding, and [³⁵S]guanosine 5’-3-O-(thio)triphosphate binding (23). p2y₅ has now been reclassified as an LPA receptor and renamed LPA₆ (22, 83). 2-acyl-LPA appears to have a higher activity to LPA₆ than 1-acyl-LPA. Interestingly, many of the performed assays require extraordinarily high concentrations of LPA (up to 10 μM) to show an effect, compared to the nanomolar concentrations needed for activating LPA₁₋₅. When LPA₆ was co-expressed with a promiscuous Gᵦ protein, LPA₆ was activated by LPA and resulted in increased intracellular Ca²⁺, reduced forskolin-stimulated [cAMP]ᵢ, and ERK1/2 activation (187).

While several studies have now identified LPA₆ mutations in hypotrichosis patients (22, 188, 189), there have also been reports of lipase member H (LIPH) (also known as PA-PLA₁α) mutations in hypotrichosis that are associated with both a decrease in LPA production as well as reduced or abrogated LPA₆ activation in cells expressing the receptor (190, 191). An additional study has demonstrated that LIPH regulates hair follicle development by modulating epidermal growth factor receptor (EGFR) signaling through a tumor necrosis factor α converting
enzyme and the transforming growth factor α pathway (192). These findings suggest LPA₆, and possibly EGFR, as candidates for therapeutic intervention in forms of human hair loss.

**LPA Signaling Agonists and Antagonists**

There is a rapidly growing number of reported LPA signaling agonists and antagonists, all with varying selectivity and potency (Table 1). Most of these receptor modulators are directed at LPA receptors, particularly against LPA₁-₃, although a few compounds have demonstrated some LPA₄ and LPA₅ activity with limited selectivity (182, 193, 194). Additionally, several compounds target enzymes that regulate the production of LPA, notably ATX and LPPs (195, 196). The vast majority of these studies have relied heavily upon in vitro assays for validation thus far, but a growing number of compounds have some demonstrated in vivo efficacy (Table 1). An LPA₁ antagonist named AM966 has shown efficacy in inhibiting lung fibrosis in a bleomycin injury model (197). The dual LPA₁/₃ antagonist Ki16425 has shown preventative efficacy in a mouse model of hydrocephalus (43), as well as therapeutic reduction of bone metastases from breast cancer in a xenograft tumor model (198). In murine renal ischemia-reperfusion injury, opposing effects can be seen in which the LPA₃-selective agonist OMPT enhanced kidney damage, whereas the LPA₁/₃ dual antagonist VPC12249 reduced the injury via LPA₃ inhibition (199). These compounds require further validation in vivo. LPA₁ antagonists are also being tested clinically for disease amelioration: SAR-100842 for systemic sclerosis and AM-152 for idiopathic pulmonary fibrosis (200). Exogenous LPA exposure can also increase blood brain barrier permeability, which may be relevant to diseases and drug delivery into the brain (80). In the next section, LPA mediated pathophysologies across organ systems will be discussed, with specific mention of LPA signaling modulators in therapeutic contexts (Table 1).

**Pathophysiology**
Neurological disorders

Many neurodevelopmental disorders have unclear etiologies, yet are associated with prenatal and perinatal risk factors such as hemorrhage, hypoxia, infection, and immune activation. These include CNS malformations such as heterotopias, layer malformations, and hydrocephalus, as well as neurological disorders such as cerebral palsy, schizophrenia, and autism (201-203). LPA signaling can be significantly altered during all of these insults, and could induce or contribute to developmental disturbances.

Recently, a prominent effect of LPA signaling on the developing brain was shown in relation to post-hemorrhagic fetal hydrocephalus (FH) (43). FH is a neurodevelopmental disorder characterized by the excessive accumulation of cerebrospinal fluid, macrocephaly, enlarged ventricles, and neurological dysfunction. A main risk factor for FH is bleeding within the germinal matrix of the cerebral cortex. Prenatal injections of LPA directly into fetal brains induced most of the symptoms of FH in an LPA₁ dependent manner. In a mouse model of intracranial hemorrhage (with lower levels of LPA), blood components induced FH with 25-50% penetrance, while Lpar₁⁻/⁻/Lpar₂⁻/⁻ mice were protected from developing FH (43). Additionally, the effects of hypoxia involve LPA₁ potentiation via the receptor kinase GRK2, linking LPA receptor signaling to another common FH risk factor (204). Postnatal brain exposure to blood appears to induce ventriculomegaly without hydrocephalus, possibly due to spatiotemporal LPA receptor and ligand distribution (205, 206).

Neuropsychiatric disorders are also thought have a neurodevelopmental origin. Many share the same risk factors cited above, based on studies linking hypoxia, prenatal hemorrhage, and immune activation to diseases such as schizophrenia and autism (207-209). LPA, particularly through LPA₁, is involved in both immune system function and hypoxia. Indeed, the removal of LPA₁ signaling during development significantly alters the neuropsychiatric profile of mice. Lpar₁⁻/⁻ mice exhibit deficits in pre-pulse inhibition of the startle reflex, altered levels of
serotonin, abnormalities in glutamatergic synapses (94, 210, 211), and a reduction in entorhinal cortex gamma oscillations and parvalbumin-positive neurons (212). Furthermore, maLPA1−/− mice display defects in olfaction, pain sensing, exploration, anxiety, and memory retention in addition to cortical developmental defects. These syndromic dysfunctions are reminiscent of the pathological and behavioral symptoms exhibited by schizophrenic patients and animal models (93, 94, 213, 214). Expression of the LPA-synthesizing enzyme cytosolic PLA2 (cPLA2) is increased in schizophrenic patients, while cPLA2 inhibition in control groups reduces deficits in pre-pulse inhibition (215). These observations suggest that altered LPA1 signaling may be relevant to schizophrenia and related psychiatric symptoms. Lpar1−/− and maLPA1−/− mice also display craniofacial dysmorphism and defects in adult hippocampal neurogenesis, traits that are associated with autism (112). These data strongly implicate LPA and LPA1 receptor signaling in neurodevelopmental and neuropsychiatric disorders.

LPA and LPA1 signaling are also involved in nerve injury responses. Neuropathic pain, with symptomatic allodynia and hyperalgesia, is often associated with peripheral neuropathy and Schwann cell demyelination, commonly caused by trauma or inflammation of the nervous system. Intrathecal injections of LPA over-activate LPA1 and elicit both allodynia and hyperalgesia in wild-type mice, which were prevented in Lpar1−/− mice (147). LPA1 activation also induces a nociceptive response that appears to mediate the release of substance P, a neuropeptide implicated in inflammation and pain (216). Furthermore, partial sciatic nerve ligation (PSNL) nociception was completely blocked in Lpar1−/− mice. Allodynia and demyelination were also reduced using Rho pathway inhibitors, implicating LPA1-mediated Rho activation in these neuropathic pain-associated pathologies (147). Finally, PSNL-induced neuropathic pain was inhibited through a distinct pathway involving LPA5 and cAMP in the dorsal horn neurons despite continued upregulation of several pain-related markers (217).

In earlier ex vivo studies, LPA induced demyelination in isolated dorsal root fiber and decreased myelin basic protein expression (218). ATX has also been shown to induce
neuropathic pain through the conversion of LPC to LPA (219). This is supported by the fact that Enpp2−/− mice, which have a 50% decrease in LPA plasma concentration, also have a 50% recovery from PSNL-induced neuropathic pain (220). Interestingly, intrathecal LPC-mediated pain involved the feed forward production of LPA through ATX and LPA₃ (221). Cyclic phosphatidic acid has been reported to attenuate PSNL-induced allodynia and hyperalgesia (222). LPA-mediated nociception may be dependent on specific LPA forms, as pain responses were strongly elicited by 18:1 LPA but not by 16:0 LPA or 18:0 LPA (223). Interestingly, intraperitoneal administration of Ki16425 (LPA₁/₃ antagonist) within a short window straddling the induction of pain appears to completely block LPA-induced nociception (223). These proof-of-concept studies indicate that LPA and LPA₁ signaling can serve as important therapeutic targets for myelinating diseases, including neuropathic pain.

Atherosclerosis and Cardiovascular Disease

As LPA receptors are integral to vascular integrity and the immune response, it comes as no surprise that dysregulated LPA signaling may be implicated in atherosclerosis. In particular, LPA is involved in inflammatory cytokine release, monocyte attraction and adhesion, abnormal endothelial cell behavior, endothelial permeability, and LDL uptake for plaque formation (224-228). These factors can lead to the excessive macrophage invasion seen in atherosclerosis. Additionally, LPA accumulation has been observed within the thrombogenic core of atherosclerotic plaques (76, 229), potentially implicating the presence of LPA in disease progression.

LPA signaling may also be involved in cardiovascular disease and myocardial infarction, as excessive platelet activation is associated with cardiovascular disease (230). Activated platelets release LPA which can initiate a positive feedback loop where LPA induces platelet activation and aggregation (36, 40, 76). During myocardial infarction, cardiac myocytes can sustain major damage due to ischemia and hypoxia. LPA signaling has also been implicated in
both of these conditions. For example, elevated LPA levels have been detected in ischemia and have been shown to increase cardiomyocyte and mesenchymal stem cell survival (231-234). During injury recovery, LPA stimulates the immune cell reaction, endothelial cell migration, cell survival, and proliferation processes involved in repair and angiogenesis (73, 77, 140). While prolonged LPA expression may exacerbate ischemia, the involvement of LPA in injury response may be important during myocardial infarction recovery. In addition to the potential cardioprotective effects of LPA, LPA signaling has been shown to be neuroprotective during ischemic injury. Treatment of a rat model of retinal ischemia/reperfusion injury with an LPA analog protected neural cells from apoptosis and improved recovery outcomes (235). However, these data are inconclusive, as LPA has also been shown to induce cell death in cerebral vascular cells, umbilical endothelial cells, brain explants, and retinas (236). This apoptotic response was reduced with an LPA$_1$ antagonist.

**Inflammation and Autoimmunity**

LPA is a potent mediator of the immune response and can contribute to improper immune activation, upregulating the production of inflammatory cytokines such as IL-2 and IL-6 (140, 237). In addition, LPA$_1$ and LPA$_2$ signaling can activate microglial cells in the brain, triggering immune invasion. Importantly, LPA has been linked to several inflammatory and autoimmune disorders, including systemic sclerosis, asthma, and arthritis.

Abnormally high levels of LPA have been identified in fibroblasts from systemic sclerosis patients. These primary cells are hypersensitive to Cl$^-$-current activation during LPA exposure (238, 239). The bronchoalveolar fluids of allergen-provoked asthmatic patients showed concomitant increases in ATX levels and LPA concentration with increased 22:5 and 22:6 polyunsaturated fatty acids (240). In a mouse model of allergic asthma, the blockade of ATX activity and LPA$_2$ knockdown attenuated the Th2 cytokines and allergic lung inflammation (240).
LPA is also involved in several aspects and forms of arthritis. Rheumatoid arthritis (RA) patients display increased ATX concentrations in synovial fluid, and elevated cytokine production was found in patient fibroblast-like synoviocytes treated with LPA (241). Recent studies have also demonstrated that LPA/LPA₁ contributes to the development of RA via cellular infiltration, Th17 differentiation, and osteoclastogenesis (242), underlining LPA's effects on inflammation and immune regulation. Consistent with LPA receptor activation in this context, a functional single-nucleotide polymorphism discovered in the promoter region of LPA₁ increased susceptibility to knee osteoarthritis, possibly through LPA₁ upregulation (243). These results support the proposal that ATX and LPA are involved in facilitating immune system functions via modulation of lymphocyte trafficking and sensitization of affected cells during autoimmunity.

Understanding these pathways could lead to the use of LPA signaling modulators in the treatment of human autoimmune disorders. Use of Ki16425 or BrP-LPA (a pan-LPA antagonist) attenuated arthritis in a mouse model of inflammatory joint disease (244). Specifically, treatment with Ki16425 reduced synovial inflammation, cell infiltration, and bone destruction, while decreasing the presence of inflammatory mediators in the joint tissue (242). These studies suggest that LPA receptor inhibition may be a future treatment option for arthritic patients.

Fibrosis

LPA signaling has been linked to injury response and fibrosis, which can involve the formation of excess fibrous connective tissues in a wide range of organ systems, resulting in complications such as pulmonary, renal, and tubulointerstitial fibrosis. Increased LPA has been found in bronchoalveolar fluid in pulmonary fibrosis after bleomycin-induced lung injury, which was associated with increased vascular leakage, fibroblast recruitment, and mortality. These pathologies were significantly reduced in Lpar1⁻/⁻ mice, linking LPA₁ signaling to this disorder (245). Renal fibrosis is also linked to LPA₁ signaling, albeit through a slightly different
mechanism that also involves LPA₃ (246). In particular, LPA₁ activation in mesothelial cells can lead to stimulated migration, cell proliferation, and upregulated expression of a profibrotic factor called connective tissue growth factor in epithelial cells and fibroblasts (247). The subsequent cell growth can result in the pathogenic fibroblast proliferation seen in fibrosis. Lpar2 may also be involved in pulmonary fibrosis, as knockout mice demonstrated protection against bleomycin-induced lung injury, fibrosis, and death (248). In addition, LPA and ATX levels are increased following hepatitis C-induced liver fibrosis, presumably through induction of stellate cell and hepatocyte proliferation (35, 249), which are the main contributors to extracellular matrix accumulation in the liver (250).

Furthermore, LPA₂ signaling can activate αᵥβ₆ integrin, causing transforming growth factor-β1 activation in epithelial cells and stimulating growth (200). Both LPA₂ and αᵥβ₆ integrin have been shown to be upregulated in animal models and patient samples of lung fibrosis. Not only does LPA mediate the progression of fibrosis via cell growth, but LPA₁ has also been implicated in the initiation of disease through excessive fibroblast recruitment and persistent maintenance of leaky vasculature (251). While fibroblast presence and vascular permeability are important factors in the early stages of injury repair, high fibroblast levels and prolonged vascular leakage have both been implicated in disease.

Inhibition of LPA signaling has been demonstrated to attenuate many of the negative responses seen in fibrosis. For example, administration of Ki16425 in an animal model of lung fibrosis inhibited fibroblast chemotaxis (200). Usage of LPA₁/LPA₃ dual antagonist VPC12249 reduced the incidence of lung fibrosis from radiation therapy, counteracting the increased LPA₁/LPA₃ and CTGF expression seen in these mice (252). Administration of this drug pre- and post-irradiation resulted in a dose-dependent response including prolonged survival, inhibited fibroblast growth, reduced collagen deposits, and recovered lung structure. Another study utilized ischemia-reperfusion injury to the kidney as a model of fibrosis (253). This study implicated LPA₂-stimulated Smad2 phosphorylation and TGF-β transactivation in proximal
tubule cells as mediators of kidney fibrosis. In the presence of the pan-LPA receptor antagonist HLZ-56, but not the LPA₁/LPA₃-specific antagonist Ki16425, Smad2 activation was attenuated. Reduced Smad2 prevented the activation of pro-fibrotic factors TGF-β, CTGF, and PDGF-β. Another model simulated dermal fibrosis in the mouse by measuring changes in collagen content and dermal thickness (254). This study showed that the fibrosis was prevented in *Lpar1*⁻/⁻ mice and its severity reduced by administration of the LPA₁ antagonist AM095. In addition, myofibroblast differentiation into smooth muscle was diminished in *Lpar1*⁻/⁻ mice and nuclear Smad2 reduced in null and drug-treated mice. The disease course was unaffected in *Lpar2*⁻/⁻ mice. These data show that LPA₁ or LPA₂ inhibition may be therapeutically beneficial in patients with varying types of fibrosis. In particular, phase I clinical trials are already being carried out with LPA₁ antagonists; Sanofi is testing SAR-100842 for systemic sclerosis and Bristol-Myers Squibb is assessing AM152 for idiopathic pulmonary fibrosis (200).

**Bone Development and Disease**

LPA signaling has also been implicated in aspects of bone development and pathology (255). The two major cell classes in bone are osteoblasts and osteoclasts which build and remove bone, respectively. LPA can induce differentiation of human mesenchymal stem cells into osteoblasts through LPA₁ and LPA₄ (177), although these receptors appear to mediate opposing actions. *Lpar4*⁻/⁻ mice have increased trabecular bone volume, number, and thickness (177). An interplay between osteoblasts and osteoclasts has also been hypothesized (256), based on observations that osteoblasts can produce LPA, while exposure to LPA causes osteoclast lamellipodia retraction, disrupted peripheral actin belts, increased survival, and resorptive activity (257). This process was driven mainly by LPA₁ G₁/o coupling. However, G₁₂/₁₃ coupling with an as yet unidentified LPA receptor (LPA₂, LPA₄, LPA₅, or LPA₆) may also underlie osteoclast function (257).
Reproductive System and Infertility

LPA$_3$ signaling has an established role in regulating aspects of male and female reproduction and its dysregulation is implicated in mammalian infertility and reproductive problems (258). In fact, Lpar3$^{-/-}$ null mice have delayed embryo implantation, embryo crowding, and reduced litter size. These defects were traced to maternal influences, as wild-type embryos transferred into Lpar3$^{-/-}$ dams failed to implant normally (163). Mice lacking cyclooxygenase-2 (COX-2), an enzyme that produces prostaglandins downstream of LPA$_3$, also have similar defective phenotypes. Mechanistically, exogenous prostaglandin administration to Lpar3$^{-/-}$ dams rescues delayed implantation and reduced litter size, demonstrating that LPA$_3$-mediated signaling is upstream of prostaglandin synthesis (163). However, this treatment failed to rescue embryo crowding, indicating that LPA$_3$ signaling mediates aspects of implantation in both prostaglandin-dependent and -independent fashions (259). The mechanism underlying spacing defects in Lpar3$^{-/-}$ mice remains to be fully understood, but may involve cytosolic phospholipase A2α (cPLA$_{2\alpha}$) or Wnt/β-catenin signaling, as cPLA$_{2\alpha}$ removal or Wnt/β-catenin signaling inhibition result in similar embryo crowding phenotypes (260, 261). Injection of Ki16425 into bovine blood decreased progesterone and PGE$_2$ secretion, suggesting yet another role for LPA signaling in the reproductive system and pregnancy (262). This change in progesterone and PGE$_2$ secretion is LPA$_1$-specific, since use of an LPA$_3$-selective inhibitor, DGPP, did not alter production (263).

In addition to being integral to female reproduction, LPA$_3$ appears to play an important role in male fertility (88). One study demonstrated that lipid phosphate phosphatase-1 (LPP-1) overexpression resulted in impaired spermatogenesis (264), suggesting that the presence of LPA may be important in sperm development. There is also evidence for LPA involvement in sperm motility (265), although triple genetic deletion of LPA$_{1-3}$ showed no detectable deficits in sperm motility. However, deletion of LPA$_{1-3}$ did result in decreased germ cell survival and increased prevalence ofazoospermia in aging mice (264). In addition to the above reproductive
phenotypes, additional studies implicate LPA signaling in male sexual function, ovarian function, fertilization, decidualization, pregnancy maintenance, and parturition (88). This indicates that LPA$_{1-3}$ signaling is important in both male and female reproduction.

**Obesity**

Obesity is quickly becoming one of the greatest problems in the developed world. Not only is obesity the most common childhood disorder, but it raises the risk of other potentially fatal co-morbidities (266). The long-term effects of being overweight correlate with premature death, cardiovascular disease, metabolic morbidities, and asthma, among other problems (267). Many factors modulate the propensity to accumulate fat cells, including an increased ratio of adipocyte precursor cells to differentiated adipocytes (268). In this vein, LPA signaling may play a role in fat storage, as LPA influences adipocyte precursor differentiation. Additionally, ATX is secreted during adipocyte differentiation and LPA is produced by mature adipocytes (269-271). When obesity and type II diabetes is genetically induced in db/db mice, LPA production increased (241). Moreover, LPA and ATX production have been linked to preadipocyte proliferation (270, 272), a process which may be LPA$_1$ dependent (195). LPA$_1$-mediated activation of the mitogenic Erk1/2 pathway (273) and the differentiation-inhibiting PPAR$_\gamma$2 receptor (274) has been implicated in this response. These data are supported by the fact that Lpar1$^{-/-}$ mice have higher adiposity compared to wild-type littermates, despite lowered body weight (149, 275).

In addition to affecting adipocyte proliferation, LPA$_1$ signaling may be more directly involved in mediating glucose and insulin in diabetic patients. LPA has been shown to lower glucose levels in normal and Type I diabetic mice, though LPA production was unaltered (276). Furthermore, administration of LPA to obese, prediabetic, glucose intolerant mice inhibited insulin secretion and lowered glucose tolerance (277, 278). This effect was prevented by the antagonist Ki16425. These studies also showed that circulating LPA levels were elevated in the
high-fat diet mice compared to normal diet controls and that long-term administration of Ki16425 improved insulin tolerance, glycogen storage, and glucose use in these mice, possibly by increasing the population of pancreatic islets. Therefore, ATX, LPA production, and LPA₁ signaling have been implicated in the propagation of adipose tissue and the instigation of abnormal glucose and insulin levels in obese patients and mice. These data suggest that reducing LPA levels or inhibiting LPA₁ signaling in obese or diabetic patients may be a possible treatment option for these disorders.

Cancer

LPA and ATX signaling are implicated in the cancer field as important factors in the migration, invasion, metastasis, proliferation, survival, and angiogenesis processes of tumorigenic cells (140, 279-281). Increased LPA levels have been measured in ovarian cancer patients and several cell lines (282-286). In particular, LPA₂ overexpression has been observed in several cancers in vivo and in vitro (287, 288). LPA₂ signaling has been associated with invasion and metastasis of ovarian, endometrial, mesothelioma, and colon cancer cells (289, 290), likely through the activation and induction of VEGF, EGFR, metalloproteinases, urokinase-type plasminogen activator, COX-2, or Akt/Erk1/2 (289, 291-294). LPA₃ may play a complementary role, initiating invasion and metastasis of the same cancer cell types (295-297).

LPA₁ is also upregulated in many cancer cell lines and primary tumors and may play an important role in regulating cancer malignancy. Studies showed that induction of LPA₁ expression induced metastasis in breast cancer cells (296, 298) and initiated motility in human pancreatic cancer cells (299). Additionally, genetic knockdown or LPA₁ inhibition reduced cytokine production, tumor proliferation, and bone lesions or metastases with breast and ovarian cancer cells (198). LPAR1 mutations were also found in an osteosarcoma cell line (300) and in lung and liver tumors in rats (301). Moreover, LPA₁-initiated colony scattering (a prerequisite for metastasis) occurred in some, but not all, gastrointestinal epithelial cancer lines (302),
suggesting that this phenomenon may require cross-talk with other signaling molecules. It was
even reported that LPA₂ inhibited pancreatic cancer cell migration while LPA₁ induced migration
in response to LPA (144). LPA receptor signaling promotes invasion and metastasis of several
cancer types, but the role of each receptor may be different in each case. These pro- and anti-
tumorigenic effects of LPA₁₋₃ may reflect the differential expression of various growth factors
and receptors.

Finally, ATX itself has been implicated in stimulating cancer cell motility (58), and may
promote bone metastasis (303). Additionally, ATX was shown to be overexpressed in several
primary tumor types (175, 304-307). ATX expression may also confer resistance to cancer
treatments such as Taxol (308). Overexpression of LPA₁₋₄ and autotaxin has been reported to
enhance tumor aggression for numerous cancer types, whereas knockdown studies mitigate
tumorigenic behaviors (309, 310). These responses may be mediated, at least in part, by Gα-
induced RhoA and Rac signaling leading to cytoskeletal remodeling and migration (311). In
addition, LPA receptor signaling stimulates cell proliferation through Ras signaling and
suppresses apoptosis through PI3K and Akt activation (279).

LPA is also a known vasculature-altering factor, participating in the formation and
stabilization of new blood vessels (70). These properties may cause LPA signaling to assist
invasion and metastasis more indirectly by stimulating the rapidly growing vasculature of the
cancer microenvironment to form leaky blood vessels, feeding the cancer cells and allowing
easier extravasation of malignant cells. To this end, LPA has been reported to stimulate VEGF
production, a prominent angiogenic factor and target for cancer therapy research (312-314). In
addition to enhancing blood supply to tumors, VEGF has been shown to increase cancer cell
migration, proliferation, invasion, and survival. In a study involving epithelial ovarian cancer,
LPA-induced VEGF expression was discovered to be mediated by NF-κB through Gα₁ signaling
(315).
Several LPA receptor antagonists have been utilized in the study of cancer development. Notably, these drugs seem to attenuate metastatic and migratory behaviors in cancer cells. In one study, the pan-LPA antagonist BrP-LPA was synthesized and applied to MDA-MB-231 breast cancer cells \textit{in vitro} and in a mouse xenograft model (195). The treated cancer cells demonstrated reduced migration, inhibition of cancer cell invasion, tumor regression, and decreased blood vessel density surrounding the tumor. In addition, use of LPA\textsubscript{1}/LPA\textsubscript{3} specific inhibitors reduced pancreatic and breast cancer invasion and metastasis, but not tumor size (316, 317), while administration of the LPA\textsubscript{2}-selective antagonist Compound 35 to HCT-116 colorectal cancer cells inhibited mitogenic Erk phosphorylation and reduced cell proliferation (318). Finally, BrP-LPA sensitized the radiation-resistant vascular endothelial cells surrounding mouse gliomas to radiation therapy (196). This evidence points to active LPA signaling as an important factor in the cancer field and a possible target for cancer therapies.

\section*{Conclusion}

The past 20 years has seen the cloning and identification of a growing family of LPA receptors and signaling pathways, coupled with a rapidly burgeoning field aimed at understanding the physiological and pathophysiological relevance of these lipids. It is now an exception, rather than the rule, to find an organ system that is not impacted by LPA and LPA signaling. Moreover, there is ever-growing understanding and appreciation that numerous human disease mechanisms are explicitly linked to altered LPA signaling. Most exciting is the development of novel pharmacological modulators that serve as both research tools and potential medicinal therapies aimed at reducing human suffering. The prospects are bright for expanding insights and contributions in LPA biology.
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References


Prevalent LIPH founder mutations lead to loss of P2Y5 activation ability of PA-PLA1alpha in autosomal recessive hypotrichosis. *Human Mutation* **31**: 602-610.


214. Estela Castilla-Ortega, Jorge Sánchez-López, Carolina Hoyo-Becerra, Elisa Matas-Rico, Emma Zambrana-Infantes, Jerold Chun, Fernando Rodríguez De Fonseca, Carmen Pedraza,


are differentially regulated by LPA3-mediated lysophosphatidic acid signaling in mice. *Biology of Reproduction* **77**: 954-959.


acid-induced urokinase plasminogen activator activation, cell invasion, and migration in ovarian cancer SKOV-3 cells. *Croatian Medical Journal* **49**: 175-181.


Figure Legends

Figure 1. Summary of the major routes of LPA synthesis and the activated signaling pathways via the six cognate LPA receptors. Enzymes are indicated in red. Abbreviations: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylserine (LPS), phosphatic acid (PA), phospholipase D (PLD), autotaxin (ATX), phospholipase A1 (PLA₁), phospholipase A2 (PLA₂), lecithin-cholesterol acyltransferase (LCAT).

Figure 2. Effects of LPA receptor agonists and antagonists. LPA receptor modulating compounds are classified according to their potency against each receptor, LPA₁-LPA₆. Ranges of compound EC₅₀, IC₅₀, or Ki values are given on the x-axis, progressing from strong antagonism on the left to strong agonism on the right. A subset of these compounds is shown in more detail in Table 1. This graph was prepared using GraphPad Prism software. Abbreviations: NPSPA, N-palmitoyl serine phosphoric acid; NPTyrPA, N-palmitoyl tyrosine phosphoric acid; NAEPa, N-acyl ethanolamide phosphate; AO-LPA 12b, sn-2-aminooxy analog 12b, α-FMP, α-fluoromethylene phosphonate; a-HMP, α-hydroxymethylene phosphonate; Dialkyl TPA, Dialkyl thiophosphatic acid; DDP, Dodecyl phosphate; a-MP, α-methylene phosphonate; ODP, Octadecenyl phosphate; TP 18:1, Oleoyl-thiophosphate; DGPP, Diacylglycerol pyrophosphate; BrP-LPA 19b, α-bromomethylene phosphonate analog 19b; TPA 8:0, Thiophosphatic acid 8:0; TDP, Tetradecyl phosphonate; FDP, Farnesyl diphosphate; Cmpd, Compound.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Target</th>
<th>Characteristics</th>
<th>Physiology or Disease Relevance</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPA (18:1)</td>
<td>LPA₁₋₅ and weak</td>
<td>Lyosphospholipid present in blood and tissues</td>
<td>Appropriate levels of signaling are essential for development, neurogenesis, cell migration, cell survival, vasculogenesis, and reproduction, among other processes</td>
<td>(25, 140, 319)</td>
</tr>
<tr>
<td>N-palmitoyl serine phosphoric acid (NPSPA)</td>
<td>Weak LPA₁₋₃ agonist</td>
<td>Lipid analogue</td>
<td>Agonizes intracellular Ca²⁺ mobilization in mammalian cells (agonizes in Xenopus)</td>
<td>(320-322)</td>
</tr>
<tr>
<td>N-palmitoyl tyrosine phosphoric acid (NPTyrPA)</td>
<td>weak LPA₁/₃ antagonist, weak LPA₂/₆ agonist</td>
<td>Lipid analogue</td>
<td>Agonizes intracellular Ca²⁺ mobilization in mammalian cells (agonizes in Xenopus)</td>
<td>(320-322)</td>
</tr>
<tr>
<td>N-acyl ethanolamide phosphate (NAEPA)</td>
<td>LPA₁/₂ and weak LPA₃ agonist</td>
<td>Lipid analogue</td>
<td>Agonizes platelet aggregation</td>
<td>(25, 323)</td>
</tr>
<tr>
<td>Oleoyl-thiophosphate</td>
<td>LPA₁/₃ agonist</td>
<td>Thiophosphate lipid analogue</td>
<td>Elevates transient intracellular Ca²⁺ mobilization</td>
<td>(322, 324)</td>
</tr>
<tr>
<td>oleoyl-sn-glycero-3-phosphate (L-α-LPA)</td>
<td>LPA₁/₂ agonist</td>
<td>LPA analogue</td>
<td>Increases uterine smooth muscle contraction tension, amplitude, and frequency</td>
<td>(325)</td>
</tr>
<tr>
<td>sn-2-Aminooxy analog, 12b (AO-LPA 12b)</td>
<td>LPA₂ and weak LPA₁/₄ agonist, LPA₃ antagonist</td>
<td>Aminooxy lipid analogue</td>
<td>Enhances intestinal endothelial cell migration, activates Rho/Rac1 signaling, potential use in repair of gastrointestinal epithelium</td>
<td>(194)</td>
</tr>
<tr>
<td>Dialkyl thiophosphatidic acid 8:0</td>
<td>LPA₁/₃ and weak LPA₂ agonist</td>
<td>Thiophosphate lipid analogue</td>
<td>Elevates transient intracellular Ca²⁺ mobilization</td>
<td>(322)</td>
</tr>
<tr>
<td>T13</td>
<td>LPA₁ and weak LPA₃ agonist</td>
<td>Thiophosphate lipid analogue</td>
<td>Increases intracellular Ca²⁺ mobilization</td>
<td>(322, 326)</td>
</tr>
<tr>
<td></td>
<td>LPA&lt;sub&gt;1/3&lt;/sub&gt; agonist</td>
<td>Thiophosphate lipid analogue</td>
<td>Activates cell migration</td>
<td>(322, 326)</td>
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</tr>
<tr>
<td>Dodecyl phosphate</td>
<td>LPA&lt;sub&gt;2/3&lt;/sub&gt; agonist</td>
<td>Lipid analogue</td>
<td>Elevates transient intracellular Ca&lt;sup&gt;2+&lt;/sup&gt; mobilization</td>
<td>(322, 327)</td>
</tr>
<tr>
<td>α-methylene phosphonate</td>
<td>LPA&lt;sub&gt;2&lt;/sub&gt; and weak LPA&lt;sub&gt;4&lt;/sub&gt; agonist</td>
<td>Lipid analogue</td>
<td>Elevates transient intracellular Ca&lt;sup&gt;2+&lt;/sup&gt; mobilization</td>
<td>(193, 322)</td>
</tr>
<tr>
<td>1-oleoyl-2-O-methyl-rac-glycerophosphothionate (OMPT)</td>
<td>LPA&lt;sub&gt;3&lt;/sub&gt; and weak LPA&lt;sub&gt;2&lt;/sub&gt; agonist</td>
<td>Lipid analogue, S-enantiomer is more potent and LPA&lt;sub&gt;3&lt;/sub&gt;-selective</td>
<td>Enhances kidney damage after renal ischemia-reperfusion injury, increases uterine smooth muscle contraction tension and amplitude but decreases frequency</td>
<td>(25, 199, 325)</td>
</tr>
<tr>
<td>Alkyl OMPT</td>
<td>LPA&lt;sub&gt;1/3/6&lt;/sub&gt; agonist</td>
<td>Lipid analogue</td>
<td>Induces cancer cell migration and fibroblast proliferation, activates Ca&lt;sup&gt;2+&lt;/sup&gt; mobilization</td>
<td>(322, 328)</td>
</tr>
<tr>
<td>α-fluoromethylene phosphonate</td>
<td>LPA&lt;sub&gt;3&lt;/sub&gt; and weak LPA&lt;sub&gt;1/2&lt;/sub&gt; agonist</td>
<td>Lipid analogue</td>
<td>Not available</td>
<td>(329)</td>
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<tr>
<td>α-hydroxymethylene phosphonate</td>
<td>LPA&lt;sub&gt;3&lt;/sub&gt; agonist</td>
<td>Lipid analogue</td>
<td>Not available</td>
<td>(329)</td>
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<td>Octadecenyl phosphate</td>
<td>LPA&lt;sub&gt;4&lt;/sub&gt; agonist</td>
<td>Lipid analogue</td>
<td>Induces platelet shape change</td>
<td>(182, 322)</td>
</tr>
<tr>
<td>α-bromomethylene phosphonates (BrP-LPA)</td>
<td>Antagonist of ATX and all LPA receptors</td>
<td>Lipid analogue, diasteriomer</td>
<td>Attenuates arthritis, reduces breast cancer migration and invasion, causes tumor regression, radiosensitizes tumor vasculature</td>
<td>(195, 196, 330)</td>
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<tr>
<td>HLZ-56</td>
<td>Antagonizes all LPA receptors</td>
<td>Lipid analogue</td>
<td>Prevents SMAD2/TGF-B activation in kidney fibrosis</td>
<td>(193, 253)</td>
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<td>Tetradecyl-</td>
<td>Weak LPA&lt;sub&gt;1-3&lt;/sub&gt;</td>
<td>Lipid analogue</td>
<td>Inhibits transient intracellular Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>(322, 330)</td>
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<td>Phosphonate Antagonist</td>
<td>Mobilization</td>
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<tr>
<td>Ki16425</td>
<td>LPA&lt;sub&gt;1/3&lt;/sub&gt; and weak LPA&lt;sub&gt;2&lt;/sub&gt; antagonist</td>
<td>Inhibits cell migration, attenuates autoimmune arthritis, inhibits cancer metastasis, inhibits progesterone and PGE&lt;sub&gt;2&lt;/sub&gt; secretion from bovine reproductive tract</td>
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<tr>
<td>Ki16198</td>
<td>LPA&lt;sub&gt;1&lt;/sub&gt; and weak LPA&lt;sub&gt;2/3&lt;/sub&gt; antagonist</td>
<td>Attenuates pancreatic cancer invasion and migration</td>
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<td>Debio 0719</td>
<td>LPA&lt;sub&gt;1/3&lt;/sub&gt; antagonist</td>
<td>R-stereoisomer of Ki16425, higher potency</td>
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<td>LPA&lt;sub&gt;1/3&lt;/sub&gt; antagonist</td>
<td>Lipid analogue of NAEPA/NASPA</td>
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<td>VPC32183</td>
<td>LPA&lt;sub&gt;1/3&lt;/sub&gt; antagonist</td>
<td>Lipid analogue of NAEPA/NASPA</td>
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<td>Thiophosphatidic acid 8:0</td>
<td>LPA&lt;sub&gt;1/3&lt;/sub&gt; antagonist</td>
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<td>Compound 12</td>
<td>LPA&lt;sub&gt;1/3&lt;/sub&gt; antagonist</td>
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<td>H2L-5765834</td>
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<td>H2L-5987411</td>
<td>LPA&lt;sub&gt;4&lt;/sub&gt; and weak LPA&lt;sub&gt;5&lt;/sub&gt; antagonist</td>
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<tr>
<td>AM095</td>
<td>LPA&lt;sub&gt;1&lt;/sub&gt; antagonist</td>
<td>Small molecule,</td>
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<tr>
<th>Compound</th>
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<td>Ki16425</td>
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<td>Thiophosphatidic acid 8:0</td>
<td>182, 324</td>
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<td>Compound 12</td>
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<td>AM966</td>
<td>LPA₁ antagonist</td>
<td>Small molecule, developed by Amira</td>
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<td>AM152</td>
<td>LPA₁ and weak LPA₂₅ antagonist</td>
<td>Small molecule, developed by Amira</td>
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<td>Compound 35</td>
<td>LPA₂ and weak LPA₁/₃ antagonist</td>
<td>Small molecule</td>
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<tr>
<td>Diacylglycerol pyrophosphate 8:0 (DGPP)</td>
<td>LPA₃ and weak LPA₁ antagonist</td>
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<td>NSC161613</td>
<td>LPA₆ antagonist</td>
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<td>Farnesyl diphosphate</td>
<td>LPA₅ agonist, LPA₃ and weak LPA₂₄ antagonist</td>
<td>Lipid analogue</td>
</tr>
<tr>
<td>SAR-100842</td>
<td>LPA₁ antagonist</td>
<td>Small molecule, developed by Sanofi</td>
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**Table 1. Summary of LPA receptor modulators, receptor subtype target and activity, and disease relevance.** Compounds have agonist (blue shading), antagonist (red shading), or dual agonist/antagonist (purple shading) activities. Activity, compound characteristics, and effects in various disease models are emphasized. If information in a disease context was not available,
effects on cellular physiology were included instead. “Weak” effects are classified as having EC$_{50}$, IC$_{50}$, or Ki values greater than 1000 nM. Note that not all compounds were tested against all LPA receptors (see Figure 2 for known interactions). References are indicated in parentheses.
<table>
<thead>
<tr>
<th>Tissues/Fluids</th>
<th>[LPA]</th>
<th>[LPC]</th>
<th>Method of measurement</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic brain</td>
<td>0.32-0.35 pmol/mg</td>
<td>Not available</td>
<td>LC-MS</td>
<td>(43)</td>
</tr>
<tr>
<td>Adult brain</td>
<td>3.7-35 pmol/mg</td>
<td>Not available</td>
<td>GC-MS, LC-MS-MS</td>
<td>(32, 47, 339, 340)</td>
</tr>
<tr>
<td>Nerve (spinal cord)</td>
<td>0.79 pmol/mg</td>
<td>Not available</td>
<td>B103 bioassay</td>
<td>(42)</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.7 μM / 0.17-0.63 μM</td>
<td>100-140 μM / ~440 μM</td>
<td>LC-MS, RH7777 bioassay</td>
<td>(38, 239, 299, 341)</td>
</tr>
<tr>
<td>Serum</td>
<td>4-15.5 μM</td>
<td>234 μM</td>
<td>TLC-G, enzymatic assay, LC-MS-MS</td>
<td>(166, 342, 343)</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>0.025-0.2 pM</td>
<td>Not detected</td>
<td>RH7777 bioassay</td>
<td>(344)</td>
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<td>Seminal fluid</td>
<td>Negligible</td>
<td>8-19 μM</td>
<td>RH7777 bioassay, enzymatic cycling</td>
<td>(41)</td>
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<tr>
<td>Saliva</td>
<td>0.785 nM</td>
<td>Not available</td>
<td>FAB-MS</td>
<td>(345)</td>
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<tr>
<td>Lacrimal gland fluid</td>
<td>1.3 μM</td>
<td>Not available</td>
<td>MS</td>
<td>(45)</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>0.2 μM</td>
<td>Not available</td>
<td>MS</td>
<td>(45)</td>
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<tr>
<td>Follicular fluid</td>
<td>25.3 μM</td>
<td>157 μM</td>
<td>TLC-GC</td>
<td>(166)</td>
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<tr>
<td>Acites</td>
<td>0.62 μM</td>
<td>Not available</td>
<td>RH7777 bioassay</td>
<td>(299)</td>
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<tr>
<td>Fertilized hen white</td>
<td>8.0-9.6 μM</td>
<td>Not available</td>
<td>LC-MS</td>
<td>(179)</td>
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<tr>
<td>Pathological</td>
<td>Nerve (injury)</td>
<td>74.8 pmol/mg</td>
<td>B103 bioassay</td>
<td>(42)</td>
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<tr>
<td>Serum</td>
<td>5.5 μM</td>
<td>130 μM</td>
<td>LC-MS-MS</td>
<td>(239)</td>
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<tr>
<td></td>
<td>Concentration</td>
<td>Method</td>
<td>Reference</td>
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<tr>
<td>Serum (sepsis)</td>
<td>Not available</td>
<td>43.5 μM</td>
<td>Enzymatic assay (343)</td>
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<td>Acites (pancreatic cancer patients)</td>
<td>2.7 μM</td>
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<tr>
<td>Plasma (chronic liver injury)</td>
<td>0.3-0.4 μM</td>
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<td>Enzymatic assay (346)</td>
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<tr>
<td>Plasma (obesity)</td>
<td>Not available</td>
<td>83.5-84.4 μM / ~420-460 μM</td>
<td>LC-MS (341)</td>
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</table>

Table 2. Summary of LPA concentrations in various tissues and biological fluids.

Measurements were performed using a variety of techniques under numerous conditions, resulting in a range of values (associated errors were omitted for simplicity). References are indicated in parentheses. Values from non-human organisms and humans are indicated in red and blue, respectively. Abbreviations: Fast atom bombardment mass spectrometry (FAB-MS); liquid chromatography-mass spectrometry (LC-MS); thin layer chromatography-gas chromatography (TLC-GC).
Figure 1
Figure 2