CHAPTER 1

Lysophosphatidic Acid (LPA) Receptor Signaling

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1.1. INTRODUCTION

Lysophosphatidic acid (LPA) is a simple phospholipid that has been shown to act as a potent lipid-signaling molecule. LPA acts through defined G protein-coupled receptors (GPCRs) in many developmental and adult processes involving most, if not all, vertebrate organ systems. All LPA molecules contain a phosphate head group attached to a glycerol backbone that is attached to a single aliphatic chain of varied length and saturation, typically ester-linked (with other linkages existing, e.g., alkyl-LPA) (Fig. 1.1). LPA species are present in all eukaryotic tissues at relatively low concentrations that include both structural as well as signaling pools, the latter of which can evoke myriad physiological responses in a wide variety of cell types (1–4).

LPA was long known as a minor component of membrane phospholipid metabolism (5, 6). Hints of LPA’s possible actions as a bioactive lipid were suggested in reports dating from the early 1960s that examined smooth muscle effects including influences on blood pressure (7, 8). The chemically defined LPA species involved emerged years later with LPA’s isolation from soybeans (9). This chemical identity raised mechanistic questions on how it might function, and many theories were proposed that included physical perturbation of the membrane (10), calcium chelation (11), second messenger signaling (12), intracellular receptors (13), and cell surface receptors (14). These competing theories to explain the effects of extracellularly applied LPA as well as other lysophospholipids were clarified upon identification of the first lysophospholipid receptor: a GPCR from the brain initially named “ventricular zone gene-1” because of its expression in the embryonic neuroproliferative layer of the cerebral cortex (15) and which is now known as LPA1 (15, 16). The cloning
and functional identification of this receptor gene led to the deorphansion of other putative receptor genes in the databases based upon their homology to one another (17–19). This collective group of orphan receptors was known by many different receptor names (20), the first of which was “endothelial
differentiation gene” (EDG). This EDG group contained both LPA and sphingosine 1 phosphate (S1P) receptors, which underscored the significant homology among LPA and S1P receptors. At the time of the initial identification, S1P₁ had greatest homology to LPA₁ but was still an orphan receptor, while a homologous known receptor to LPA₁ was the cannabinoid receptor CB₁ (encoded by \( CNR1 \)) that itself interacts with endogenous lipid molecules anandamide and 2-arachidonyl glycerol (21, 22). More recently, three somewhat divergent LPA GPCRs have been identified (LPA₄₋₆) (23–27), which belong to the P2Y purinergic receptor family (Fig. 1.2), providing evidence for the existence of dissimilar clusters of receptors mediating the effects of the same ligand. Other species of bioactive lysophospholipids are also currently being assessed for matching receptors, though none has been identified as of yet (28). An additional dimension to LPA receptor interactions is the likelihood that different chemical forms of LPA may bind preferentially to LPA receptor subtypes (29), although the extreme difficulty of doing classical receptor binding experiments with LPA has prevented direct assessments of this possibility, relying instead on secondary readouts of receptor activity that do support ligand selectivity. All six LPA receptors are type I, rhodopsin-like GPCRs with seven transmembrane domains. Each receptor can couple to one or more of four heterotrimeric \( G \) proteins (\( G_{12/13} \), \( G_{q/11} \), \( G_{i/o} \), and \( G_{s} \)) (Fig. 1.3), resulting in the activation of a wide range of downstream signaling pathways and resulting in diverse physiological and pathophysiological effects documented for LPA signaling.

### 1.2. LPA METABOLISM

LPA is produced both intracellularly and extracellularly from membrane phospholipids (Fig. 1.1). Intracellular LPA is thought to be structural (6) or an intermediate for phospholipid biosynthesis, so it is less likely that it functions as an extracellular pool of signaling molecules (30). Additional LPA-producing pathways also exist (31). The term LPA, at least in an extracellular signaling context, generally refers to 1-acyl-2-hydroxy-sn-glycero-3-phosphate, but distinct chemical forms exist, such as 1-alkyl- or 2-acyl-LPA (32, 33). The acyl chain length and degree of saturation generally depend on the precursor phospholipid, with the most abundant forms of LPA in plasma being 16:0-, 18:2-, and 18:1-LPA (34). The 18:1-LPA form is perhaps the most commonly used LPA species in the laboratory for signaling studies.

The two major pathways involved in LPA production initiate either the sequential activity of phospholipase D (PLD) and phospholipase A₂ (PLA₂) or of PLA₂ and lysophospholipase D (also known as autotaxin, ATX) (Fig. 1.1). The first pathway is mainly involved in cellular LPA production through cell membrane-derived phosphatidic acid hydrolysis, and the second pathway is mainly involved in extracellular LPA production in bodily fluids such as plasma (35). In 1986, it was reported by Tokumura et al. that LPA is produced
**Figure 1.2.** Phylogenetic relationships between known LPA receptors. Non-LPA GPCRs (rhodopsin, S1P1, and the cannabinoid receptors) are included for reference. Amino acid percent identity to LPA1 is shown in parentheses.
in submillimolar concentrations from plasma incubated at 37°C for a long period of time (36). The enzyme responsible for this production of LPA was later identified as the previously known gene Enpp2, which encodes the ATX protein and possesses lysoPLD activity (37, 38). There are at least two additional pathways that can produce intracellular LPA: acylation of glycerol-3-phosphate by glycerophosphate acyltransferase (GPAT) and phosphorylation of monoacylglycerol by monoacylglycerol kinase (MAG-kinase) (39). LPA degradation involves several different enzymes, including LPA-acyltransferase (LPAAT), lipid phosphate phosphatase (LPP), and various lysophospholipases (40). LPA may be converted back to phosphatidic acid by LPAAT, hydrolyzed by LPP-1, -2, and -3, or converted by lysophospholipases into glycerol-3-phosphate (40, 41).

Since LPA is present in low concentrations in all mammalian cells and tissues, it is important to identify biologically relevant concentrations (based upon the half maximal effective concentration [EC50] and/or apparent Kd values of the six LPA receptors). Current LPA detection methods include enzymatic assays, thin-layer chromatography (TLC)–gas chromatography, high-performance liquid chromatography (HPLC)/tandem mass spectrometry, and liquid chromatography–tandem mass spectrometry (LC/MS/MS) (42, 43). LPA concentrations measured in the blood can range from 0.1 μM in plasma to over 10 μM in serum, which is well over the apparent Kd of LPA1–5 (31, 44, 45).
1.3. AUTOTAXIN

ATX is one of the best-studied enzymes associated with LPA signaling. The first reported activity of ATX was as a cell motility-stimulating factor in human melanoma cells (46). The cell motility effect was originally attributed to ATX’s reported function as a nucleotide phosphodiesterase, since ATX shares structural similarities to the nucleotide pyrophosphatase/phosphodiesterase (NPP) family (47). However, the promigratory effects of ATX were blocked by the addition of pertussis toxin, a G_{i/o} inhibitor (46), and G_{i/o} couples to five of the six identified LPA receptors. It is now clear that the cell motility-stimulating activity of ATX is a result of autocrine signaling from the production of LPA and its action on LPA receptors (30, 48, 49). ATX is present in blood and shows fairly broad tissue expression, with relatively high levels of ATX expressed in the brain (especially the choroid plexus), kidney, and lymphoid organs, which leads to high levels of ATX in cerebrospinal fluid and the high endothelial venules (HEVs) in lymphoid organs (50–52).

The physiological importance of ATX was not fully appreciated until the creation of ATX knockout mice (Enpp2−/− mutants). Enpp2−/− mice die around embryonic day 9.5 with prominent vascular and neural tube defects (53, 54). These mutants also have specific deficits in both yolk sac blood vessel formation and large lysosome biogenesis in yolk sac visceral endoderm cells (55). Enpp2+/− heterozygotes survive to adulthood but, importantly, have LPA plasma levels that are half that of wild-type mice. This confirms that ATX activity is the major source of LPA in plasma and is essential for proper embryonic development.

ATX, through its production of LPA, is significantly involved in vascular development. LPA was found to prevent disassembly of blood vessels in cultured allantois explants (54), supporting a role for LPA signaling in maintenance of existing vasculature in addition to assembly and maturation. LPA additionally acts as a vasoregulator in multiple species (9) and has been implicated in the pathology of posthemorrhagic vasoconstriction (56). ATX expression is induced by vascular endothelial growth factor (VEGF), and induces both proliferation and migration of endothelial cells (57–59). LPA-induced endothelial cell migration in a Matrigel migration assay induced expression of matrix metalloproteinase-2 (MMP-2), which is a proteolytic enzyme involved in endothelial cell migration and matrix remodeling during angiogenesis (60). Because angiogenesis and tissue repair require a variety of bioactive mediators, such as growth factors and cytokines that are released from activated platelets, LPA has been implicated in these processes. LPA is known to be released from activated platelets (34), as well as able to induce platelet activation in a positive feedback loop (61, 62), and this LPA production induces mitogenic and migration effects on many of the cell types involved in angiogenesis and tissue repair (4, 58, 63, 64).

Activation of platelets is also heavily associated with cardiovascular disease. LPA is involved in processes relevant to atherosclerosis during both the early
and late stages of plaque formation involving endothelium dysfunction, monocyte attraction and adhesion, LDL uptake, and proinflammatory cytokine release (65–71). LPA both increases the permeability of endothelial cells and rat mesenteric venules (66, 72) and recruits monocytes to the endothelium (67), implicating LPA in the invasion of reactive macrophages in atherosclerosis. LPA was also found to accumulate in the thrombogenic, lipid-rich core of atherosclerotic plaques (61, 73). LPA’s involvement in atherosclerosis is receptor-dependent, involving both LPA1 and LPA2 signaling, and will be discussed in more detail in the succeeding sections.

One of the major causes of damage to cardiac myocytes during myocardial infarction is ischemia and hypoxia. While LPA clearly plays a role in ischemia and hypoxia, the exact nature of its effects require further clarification. LPA levels are elevated under ischemic conditions (66, 74), and while LPA has been shown to protect hypoxia-induced apoptosis in cardiac myocytes and mesenchymal stem cells (75, 76), LPA3 antagonists were reported to protect renal cells from hypoxia-induced apoptosis (77) in vitro. Treatment with an LPA analog, LXR-1035, of a rat model of retinal ischemia/reperfusion injury resulted in decreased neural cell death and improved functional recovery (78). Yet in porcine cerebral microvascular and human umbilical vein endothelial cells, LPA was found to induce specifically oncotic cell death, which was reproduced in both brain explants and retinas in vivo (79). An LPA1 low-affinity antagonist was able to prevent this oncotic cell death. Recently, it was shown, using an ex vivo cortical culturing system and cell culture, that the cellular neurodevelopmental effects of prolonged hypoxia are ameliorated through antagonism or genetic removal of LPA1 (80), mechanisms that were shown to extend at least in part to maternal hypoxic insult in vivo.

ATX influences on LPA signaling are not only involved in platelet activation, but also function in an immunoregulatory capacity. ATX has been identified as a modulator of lymphocyte trafficking into secondary lymphoid organs, where ATX produced by high endothelial cells (HECs) may bind to activated lymphocytes (52). It is proposed that LPA induces the chemokinesis of T cells via the local production of LPA from ATX bound on the lymphocyte cell surface. ATX activity is also induced in T cells treated with lipopolysaccharide (LPS) (81), and LPA can induce Ca2+ signaling in adult B cells (82), which further implicate ATX and LPA in normal immune cell function.

The effects of LPA can also participate in immune misactivation relevant to various autoimmune diseases, where increases in LPA have been identified in systemic sclerosis patients. Notably, fibroblasts from systemic sclerosis patients are hypersensitive to Cl− current activation during LPA exposure (83, 84). LPA is also involved in arthritis, where a functional single-nucleotide polymorphism (SNP) in the promoter region of LPA1 was shown to increase susceptibility to knee osteoarthritis, possibly via upregulation of LPA1 expression (85). Rheumatoid arthritis patients also exhibited increases in ATX in synovial fluid as well as elevated cytokine production in patient fibroblast-like
synoviocytes treated with LPA (86). These results support the proposal that ATX and LPA are involved in facilitating immune system functioning via modulation of lymphocyte trafficking and sensitization of affected cells during autoimmunity.

LPA has also been investigated as a modulator of constructive wound healing. Myriad factors are released from platelets following tissue trauma, including LPA. Treatment of “wounded” endothelial monolayers in vitro with LPA resulted in closure repair (58), and application of LPA to in vivo cutaneous wounds promoted enhanced repair processes (87). Moreover, fibroblast migration into the fibrin wound matrix is an essential step in the process of wound healing, and LPA has been shown to regulate migration of mouse embryonic fibroblasts (MEFs) through LPA₁ signaling (49, 88).

There is currently a wealth of data explicitly implicating ATX and LPA signaling in cancer progression. LPA signaling has been associated with many of the dysregulated processes involved in cancer development, including proliferation, survival, metastasis, and promotion of angiogenesis (reviewed in References 3 and 89–91). De Alvarez and Goodnell first suggested the involvement of LPA in cancer in 1964 when lysolecithin (known also as lysophosphatidylcholine, LPC), LPA’s precursor, was found to be significantly increased in the serum of patients with ovarian cancer (5). Later, ATX was specifically identified as a motility-stimulating factor for cancer cells (46), although ATX had yet to be identified as having lysoPLD activity. Other early clues to LPA’s involvement in cancer included the observation that LPA enhanced invasiveness of lung cancer cells in vitro (92). Myriad other cancer cell lines have shown responsiveness to LPA in regards to enhanced proliferation, migration, and survival. These cell lines include ovarian, gastrointestinal, breast, prostate, mesothelioma, pancreatic, liver, and glioma (93–102). LPA levels are increased in the ascites and plasma of ovarian cancer patients (93, 103), and a variety of cancer cell lines (99, 104, 105) and primary tumor tissues have increased ATX expression (106–110). In breast cancer in particular, antagonists against ATX and LPA receptors prevent breast cancer cell (BCC) migration and promote tumor regression in vivo (106, 111, 112). Increased ATX expression in breast cancer and melanoma cells has also been implicated in Taxol resistance (Bristol-Meyers Squibb, New York, New York) (113), and forced expression of ATX promotes bone metastasis through activation of osteoclasts (114), which highlights the importance of developing a better understanding of ATX and LPA signaling in cancer. Indeed, LPA receptor mutations and aberrant expression of receptors have been found in osteosarcoma, colon, lung, and liver cancer cells (115–118), further suggesting roles in aspects of cancer. In addition, many tumors require significantly increased blood flow, and ATX/LPA signaling promotes angiogenesis through VEGF and MMPs (119–121). There is interest and effort in developing ATX inhibitors as anticancer chemotherapeutics (122), and a further understanding of how ATX and LPA affect processes like angiogenesis, metastasis, and cancer proliferation could
aid therapeutic modulation of ATX and LPA in understanding and treating cancer.

1.4. LPA RECEPTORS

The numerous reported physiological effects of LPA are primarily mediated through the six currently recognized LPA receptors, \( \text{LPA}_{1-6} \). These GPCRs couple to all four \( G_\alpha \) proteins (\( G_{12/13}, G_{q/11}, G_{i/o}, \) and \( G_s \)), which initiate a variety of signaling cascades. The interplay among different LPA receptors, primarily modulated by differential receptor subtypes in specific tissues, drives the many biological and pathological processes noted here as well as in subsequent chapters.

1.4.1. \( \text{LPA}_1 \)

\( \text{LPA}_1 \) was the first receptor identified for any lysophospholipid (15) and is the best studied of the six recognized LPA receptors. \( \text{LPARI} \) (human chromosomal locus 9q31.3) encodes a 41-kDa protein containing 364 amino acids with seven putative transmembrane domains. In mice, the \( Lpar1 \) gene encodes five exons with a conserved intron (shared among \( Lpar1-3 \)) interrupting transmembrane domain 6. There has been one reported variant of \( Lpar1 \) (mrec1.3) that results in an 18 amino acid deletion of the N terminus (123), but the biological significance of this variant has not been elucidated. \( \text{LPA}_1 \) is highly homologous to \( \text{LPA}_{2-3} \), sharing a ~50–60% amino acid sequence identity. While there are currently no crystal structures available for any of the LPA receptors, mutagenesis studies have identified several residues in \( \text{LPA}_{1-3} \) signaling. R3.28 and K7.36A are both important for the efficacy and potency of \( \text{LPA}_1 \), while Q3.29 decreased ligand interaction and activation (124), based primarily on secondary readouts.

\( \text{LPA}_1 \) couples with three types of \( G_\alpha \) proteins: \( G_{i/o} \), \( G_{q/11} \), and \( G_{12/13} \) (Fig. 1.3). These form heterotrimeric G proteins that initiate signaling cascades through downstream molecules such as mitogen-activated protein kinase (MAPK), phospholipase C (PLC), Akt, and Rho. \( \text{LPA}_1 \) activation induces a variety of cellular responses, including altered cell–cell contact through serum response element activation, cell proliferation and survival, cell migration and cytoskeletal changes, \( \text{Ca}^{2+} \) mobilization, and adenylyl cyclase inhibition (reviewed in References 4, 20, and 125).

Expression of \( Lpar1/LPARI \) is widely distributed in both adult mice and humans, including in the brain, uterus, testis, lung, small intestine, heart, stomach, kidney, spleen, thymus, placenta, and skeletal muscle (17, 125, 126). Expression of \( Lpar1 \) is more spatially restricted during embryonic development, but is enriched in parts of the brain, limb buds, craniofacial region,
somites, and genital tubercle (127). In the developing nervous system in particular, Lpar1 expression is regulated both spatially and temporally (reviewed in References 4 and 125). During embryogenesis, central nervous system (CNS) expression is enriched in the neocortical neurogenic region called the ventricular zone (VZ) and superficially in a layer that includes the marginal zone and meninges (15). The VZ disappears just prior to birth, at the end of cortical neogenesis, but Lpar1 expression continues in oligodendrocytes, particularly within the white matter tracks of the postnatal brain and this expression coincides with myelination.

Much of what is known regarding LPA signaling during neurodevelopment has been gleaned from the use of Lpar1−/− mice. Of the four LPA receptor-null mouse lines that have been reported (Lpar1−4−/−), Lpar1−/− mice are the only ones to demonstrate obvious neurodevelopmental defects. These mice show 50% perinatal lethality because of a defect in suckling behavior (128), which could be attributable to olfactory deficits. Surviving Lpar1−/− mice have a significantly reduced body size, craniofacial dysmorphism with blunted snouts, and increased apoptosis in sciatic nerve Schwann cells (SCs) (129). During colony expansion of the original Lpar1−/− line, a variant arose spontaneously that was dubbed “Málaga” (maLPA1) for its geographic location in Spain (130). The maLPA1 variant exhibits more severe developmental brain defects than the Lpar1−/− line, yet has negligible perinatal lethality. Defects in maLPA1 neurodevelopment include reduced proliferative populations, increased cortical apoptosis, and premature expression of neuronal markers (130), as well as similar effects on adult hippocampal neurogenesis (131).

Most LPA receptors are expressed in the nervous system, and LPA is abundantly present in the brain. LPA signaling can influence many neurodevelopmental processes, including cortical development and function (130, 132), growth and folding of the cerebral cortex (133), growth cone process retraction (134–136), cell survival (133), migration (137), adhesion (129), and proliferation (128, 133). LPA1 signaling was first reported to influence proliferation and differentiation of primary neuroprogenitor cells (NPCs) and neurosphere cultures (128, 138, 139). Nonmammalian models have also demonstrated LPA1 effects in the CNS, where analogs of both LPA1 and LPA2 were reported to regulate normal cortical actin assembly in Xenopus embryos (140). A number of in vitro experiments have demonstrated the effect of LPA stimulation on NPC cultures, as well as a variety of neuronal cell lines and primary neurons. These studies reported LPA-induced neurite retraction, growth cone collapse, and migration (136, 137, 141–144).

In addition to NPC and neuronal cell types, LPA1 signaling is involved in the biology of glial cell types. Astrocytes are the most abundant type of glia and play a significant role in developmental, functional, and pathological processes. Astrocytes express LPA1–5 (145) and, upon treatment with LPA, initiate a wide range of effects in vitro, including morphological changes and stabilization of stress fiber (146, 147). These responses are potentially relevant to neurodegeneration, where astrogliosis can be prominent. Injections of LPA
into the striatum can induce astrogliosis (148), although the receptors through which these processes are mediated are unidentified as of yet. LPA₁ has been implicated in astrocyte proliferation, with the caveat that some controversy does surround this claim, possibly a result of disparate cell culture systems (reviewed in Reference 149). LPS- or interleukin (IL)-1B-primed astrocytes were reported to have a specific G_{ai} migration response to LPA compared with their normal proliferative response via G_{a12/13} (150). In addition, both LPA₁ and LPA₂ signaling has been reported to increase neuronal differentiation through astrocytes primed with LPA (151). The effect of LPA₁ signaling in astrocytes awaits further study.

LPA₁ is also expressed in oligodendrocytes, the myelin-forming glial cells in CNS (152–154). Lpar1 was shown to colocalize with myelin basic protein (MBP) and proteolipid protein (PLP), but not with glial fibrillary acidic protein (GFAP) (152, 153). During development, Lpar1 expression in oligodendrocytes appears shortly before maturation/myelination, suggesting an important role in controlling this process (155), although no effect of LPA on oligodendrocyte survival, maturation, myelination, and cytoskeleton organization was reported in vitro (155). However, using the oligodendrocyte precursor cell line CG-4, it was reported that oligodendrocytes respond differently to LPA during various developmental stages (156). A Rho–Rho-associated protein kinase (ROCK) pathway-dependent cell process retraction is only seen in oligodendrocyte precursors, not in differentiated oligodendrocytes (156). Similarly, LPA increases dendritic process network area and MBP expression in differentiating oligodendrocytes (157). Therefore, it seems plausible that LPA regulates oligodendrocyte functions in a temporally specific manner, and further study will better define the activities of LPA signaling in this CNS cell type.

SCs have also been implicated in LPA signaling in the nervous system. SCs are myelin-forming cells in the peripheral nervous system (PNS) that express LPA₁ and possibly LPA₂ (129, 158). LPA increases SCs’ survival in culture by activating LPA₁ and the downstream Gₐ–phosphatidylinositol 3-kinase (PI3K)–Akt pathway (159). In vivo experiments also support this finding, showing that Lpar₁⁻/⁻ mice have increased apoptosis of SCs in the sciatic nerves (128). In addition to SC survival, LPA also induces morphological changes and adhesion. In vitro, LPA induces wreath formation in SCs and appears to enhance focal adhesions, as well as promoting cell aggregation via N-cadherin-based cell–cell adhesion (129). These effects of LPA are greatly reduced in the Lpar₁⁻/⁻ SCs, implicating LPA₁ signaling in these responses to LPA (129). LPA has also been reported to increase the expression of P₀ protein in SCs through LPA₂ signaling, possibly contributing to SC differentiation (160).

SCs have been implicated in neuropathic pain, or peripheral neuropathy, which is associated with a primary trauma or inflammation of the nervous system. Direct injections of LPA elicit a pain response, similar to that seen in neuropathic pain, through the overactivation of LPA₁. This activation of LPA₁ initiates the release of the pronociceptive factor substance P (161, 162), and direct intrathecal injection of LPA produced allodynia and hyperalgesia in
Lysophosphatidic acid (LPA) receptor signaling is modulated by LPA1 activation in wild-type mice (163) that is prevented in \(Lpar1^{-/-}\) mice. Furthermore, partial sciatic nerve ligation (PSNL) nociception was completely blocked in \(Lpar1^{-/-}\) mice, and the demyelination common to neuropathic pain pathology was abolished in C3-treated mice, indicating Rho pathway involvement (163). In a following \textit{ex vivo} study, LPA also induced demyelination in isolated dorsal root fiber and decreased MBP expression (164). In addition, ATX was shown to induce neuropathic pain through the conversion of LPC to LPA (165–167). \(Enpp2^{+/+}\) mice, which have a 50% decrease in ATX activity and LPA concentrations, also have a 50% recovery from neuropathic pain induced by PSNL (165). LPA therefore appears to modulate important SC function through LPA1 activation, and could serve as an important therapeutic target for myelinating diseases, especially neuropathic pain.

A large body of accumulating evidence suggests that many psychological diseases have a neurodevelopmental origin. This evidence, in part, comes from a variety of studies linking prenatal risk factors, such as hypoxia, prenatal hemorrhaging, and immune activation, to the development of such neuropsychiatric disorders as autism and schizophrenia (168–171). As previously noted, LPA, particularly through LPA1, is involved in both immune system function and hypoxia. The mechanism for the effects of hypoxia appear to involve LPA1 potentiation via the actions of the receptor kinase GRK2, linking LPA receptor signaling during prenatal hypoxia to clinically relevant neurodevelopmental diseases, such as autism, schizophrenia, and epilepsy.

Most recently, a striking effect of LPA signaling on the developing brain was shown by Yung et al. in relation to fetal (congenital, or prenatal forms of post-hemorrhagic) hydrocephalus (FH) (172). FH is a neurodevelopmental disorder characterized by accumulation of cerebrospinal fluid (CSF), an enlarged head, and neurological dysfunction. Prenatal injections of LPA induced many of the classical symptoms of FH in an LPA1 receptor-dependent manner. In addition, one of the major risk factors for FH is prenatal intracranial hemorrhaging. In a mouse model of intracranial hemorrhage, which induces FH with about 50% penetrance, \(Lpar1^{-/-}/Lpar2^{-/-}\) mice were protected from developing FH (172).

It is therefore plausible that excessive LPA exposure occurring in development—through hemorrhage or infection—may induce some of the developmental disturbances seen in neuropsychiatric diseases. Indeed, the removal of LPA1 signaling during development can significantly impact the neuropsychiatric profile of mice. \(Lpar1^{-/-}\) mice exhibit prepulse inhibition deficits, alterations in serotonin (5-HT) neurotransmitter levels, and abnormalities in glutamatergic synapses (132, 173, 174), as well as a reduction in entorhinal cortex gamma oscillations and parvalbumin-positive neurons (175). \(maLPA1^{-/-}\) mice display defects in olfaction, pain sensing, exploration, anxiety, and memory retention, as well as many cortical developmental defects. All of these neural dysfunctions are reminiscent of the pathological and behavioral symptoms of those suffering from schizophrenia and schizophrenia animal models (130, 132, 176, 177). In addition, expression of the LPA-synthesizing enzyme cytosolic PLA2 is increased in schizophrenic patients and inhibition of cytosolic PLA2 in control populations induces deficits in prepulse inhibition (reviewed in...
Reference 178). There appears to be a balance of LPA availability and LPA receptor signaling that is relevant to aspects of schizophrenia. The removal of LPA$_1$ signaling is important to this balance, while perhaps the signaling of other LPA receptors may also contribute. maLPA$^{−/−}$ mice also display craniofacial dysmorphism and defects in adult hippocampal neurogenesis, both of which are associated with autism (128, 131). LPA infusion has also been shown to enhance long-term spatial memory in mice (179). This wealth of data implicates LPA and LPA$_1$ receptor signaling in schizophrenia and possibly other neuropsychiatric diseases.

Obesity in both adults and children is a growing problem in the developed world. One of the most important regulators of fat deposition and accumulation is the ratio of adipocyte precursor cells to differentiated adipocytes. Numerous factors modulate the proliferation and differentiation of preadipocytes, including LPA. LPA is released by adipocytes, but not preadipocytes, both in vivo and in vitro (180, 181). This release was linked to the secretion of ATX during adipocyte differentiation, leading to the proliferation and motility of preadipocytes (182). Genetically obese–diabetic db/db mice (type II diabetes), as well as glucose-intolerant obese human subjects, display preadipocyte proliferation in response to ATX release and LPA production (182, 183). LPA-induced glucose lowering was reported in normal mice as well as in streptozotocin-induced type I diabetic mice (184). However, LPA production was not altered in the type I diabetic mouse, unlike the type II diabetic mouse (182). In culture, preadipocyte proliferation in response to LPA was specifically dependent on LPA$_1$ signaling (185), possibly through extracellular signal-regulated kinase 1/2 (Erk1/2) activation (186), while LPA$_1$ activation of PPAR$\gamma$2 seems to inhibit the differentiation of preadipocytes (187), leading to a specific antiadipogenic response. Indeed, despite a lower body weight, Lpar1$^{−/−}$ mice have higher adiposity than their wild-type littermates (188) even when controlled for excessive food consumption (189). Lpar1$^{−/−}$ adipose tissues contain more preadipocytes than could be differentiated in culture (187). Overall, these observations implicate ATX and LPA functions in adipose tissues, with possible therapeutic relevance.

LPA$_1$ signaling has also been linked to fibrosis (88, 190). Fibrosis, the formation of excess fibrous connective tissues, is associated with a number of pathological conditions including pulmonary and tubulointerstitial fibrosis (TIF). Pulmonary fibrosis studies identified increased LPA levels in the bronchoalveolar lavage fluid after bleomycin-induced lung injury, which were associated with pulmonary fibrosis, vascular leakage, and mortality. These pathologies were significantly reduced in Lpar1$^{−/−}$ mice (88). Specifically, in the absence of LPA$_1$, fibroblast recruitment and vascular leakage was decreased. LPA levels were also increased in the bronchoalveolar lavage fluids in patients following segmental allergen challenge (191). Similar effects are also seen in renal fibrosis, albeit through a slightly different mechanism. LPA effects were examined in a TIF kidney fibrosis model using unilateral urethral obstruction (UUO). UUO fibrosis initiated increases in LPA$_1$ expression and decreases in LPA$_3$ expression, and LPA levels in conditioned media from kidney explants were also increased (192). LPA also induced connective tissue growth factor
(CTGF) expression in renal fibroblast cell lines. Renal fibrosis was markedly reduced in both $Lpar1^{-/-}$ mice and following treatment with Ki16425, an LPA$_{1/3}$ antagonist, in this model (190). Furthermore, LPA and ATX levels are also increased following hepatitis C-induced liver fibrosis, presumably through stellate cell and hepatocyte proliferation (45), which are the main contributors to extracellular matrix accumulation in the liver (193, 194).

### 1.4.2. LPA$_2$

LPA$_2$ was first identified from a GenBank search for orphan GPCR genes because of its ~60% amino acid similarity to LPA$_1$. $LPAR2$ (located on chromosome 19p12) encodes a 348 amino acid protein with a calculated molecular mass of ~39 kDa (195). Mutagenesis studies of LPA$_2$ have identified two specific residues that decrease LPA$_2$ activation (Q3.29E and R5.38A) (124). $Lpar2$/$LPAR2$ expression is relatively restricted in adult mice and humans, compared with $Lpar1$/$LPAR1$. $LPAR2$ is highly expressed in the testis and leukocytes, and $Lpar2$ is highly expressed in the kidney, uterus, and testis (17, 125). 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, brain (fetal and postnatal), and heart. Expression of $Lpar2$ is much more diffuse than that of $Lpar1$ during development, yet $Lpar2$ is clearly present in the limb buds, craniofacial regions, Rathke’s pouch, and the embryonic brain (127).

LPA$_2$ couples with the same three types of $G_\alpha$ proteins as does LPA$_1$: $G_{i/o}$, $G_{q/11}$, and $G_{12/13}$ (Fig. 1.3). These associated heterotrimeric G proteins initiate signaling cascades through downstream molecules such as Ras, MAPK, PI3K, Rac, PLC, diacylglycerol, and Rho (Fig. 1.2) (128). LPA$_2$ activation is generally associated with cell survival and cell migration (185, 188, 196–198). It is interesting to note that several reports have provided evidence that LPA$_2$ signals through other pathways than the three reported $G_\alpha$ pathways. LPA$_2$-initiated migration has been reported to be promoted through interactions with the focal adhesion molecule TRIP6 (199, 200), and several PDZ domain proteins and zinc finger proteins have also been reported to interact with the carboxyl-terminal tail of LPA$_2$ (201). In addition, LPA$_2$-mediated signaling can inhibit epidermal growth factor-induced migration and invasion of pancreatic cancer cells through the $G_{12/13}$/Rho pathway (202). These studies provide evidence that there is cross-regulation between classical G protein signaling cascades and other signaling pathways in LPA$_2$ signaling, increasing the range of signaling effects mediated by LPA and a single receptor subtype.

$Lpar2^{-/-}$ mice are, for the most part, phenotypically normal, with normal prenatal and postnatal viability and expected Mendelian birth ratios. However, $Lpar1^{-/-}$/Lpar2$^{-/-}$ double mutants have an exacerbation of the low-frequency frontal hematomas present in $Lpar1^{-/-}$ mice, and primary fibroblasts and embryonic cortical cells display significantly reduced responses to exogenous LPA in vitro (133, 188). This functional redundancy between LPA$_1$ and LPA$_2$
signaling is further illustrated through use of the double mutants in elucidating the receptor specificity of neural and vascular phenotypes seen upon LPA exposure.

Like LPA₁, LPA₂ is also involved in some aspects of nervous system development and function. Activation of LPA₂ upregulates myelin P0 protein in cultured SCs, implicating LPA₂ signaling in SC function. In fact, LPA₂ is upregulated, along with LPA₁, after injuries such as nerve transection and neuropathic pain (129, 163). LPA₁’s interaction with proteoglycan 1 (PRG-1) signaling has also been reported to modulate excitatory transmission in the hippocampus (203). Exogenous LPA exposure in an ex vivo cerebral cortical culture system increased terminal mitosis of NPCs, which resulted in cortical thickening and folding that resembles gyri in humans, presumably through a decrease in cell death and early cell cycle exit (133). These effects are completely absent in embryonic cerebral cortices from Lpar₁⁻/−/Lpar₂⁻/− mice.

Lpar₁⁻/−/Lpar₂⁻/− mice have been especially illuminating in regards to LPA’s effects on the vascular system and on vascular smooth muscle cells (VSMCs). Specifically, LPA₁ and LPA₂ were found to exhibit opposite effects on primary VSMCs derived from knockout mice (188). Migration of VSMCs was increased in Lpar₁⁻/− mice, but was attenuated in Lpar₁⁻/−/Lpar₂⁻/− mice, thus depicting LPA₁ and LPA₂ as acting in opposition to each other as negative and positive chemotactic mediators, respectively. LPA has been shown to increase vascular permeability, and treatment with the dual LPA₁/3 receptor antagonist Ki16425 blocked the permeability increases (204). Ki16425 also inhibited neointima formation and SMC recruitment to the injury after wire-induced carotid injury induction (205). However, neither LPA₁ nor LPA₂ was required for dedifferentiation of SMCs following vascular injury in vivo or LPA exposure ex vivo (39), which may indicate the involvement of additional LPA receptor subtypes in this process.

Out of all the LPA receptors currently known to be involved in cancer, LPA₂ is associated with protumorogenic activities, along with LPA₃. LPA₂ overexpression is found on many types of cancer cells studied in vivo and in vitro (97, 206, 207), and it has been associated with invasion and metastasis of ovarian, endometrial, mesothelioma, and colon cancer cells (208–210). This is most likely through induction of several of the following signaling molecules, all of which have been implicated in LPA₂-mediated cancer cell motility: VEGF, epithelial growth factor receptor transactivation, metalloproteinase, urokinase-type plasminogen activator, cyclooxygenase-2 (COX-2), and Akt/ERK1/2 (95, 208, 211–214). Importantly, knockdown or removal of LPA₂ reduced tumorgenesis in many of the studies previously mentioned. LPA₃ also seems to play a complimentary role to LPA₂, initiating invasion and metastasis in the same cancer cell types (212, 215–217).

LPA₁ is also found in many cancer cell lines and primary tumors, but it may play both an opposing, and sometimes complimentary, role to LPA₂-3 signaling, depending on the cancer cell type. LPA₁ has been shown to reduce the proliferation and metastasis of ovarian cancer cells (OCCs) and BCC in vitro and
in vivo (170), but forced LPA₁ expression in BCCs induced metastasis (216, 218) and initiated motility in human pancreatic cancer cells (101). In gastrointestinal cells, LPA₁ initiated colony scattering in some cell lines, but inhibited it in other cell lines (219). Mutations in LPA₁ were also found in an osteosarcoma cell line (118), as well as in lung and liver tumors in rats (117). It was even reported that LPA₂ initiated inhibition of pancreatic cancer cell migration, whereas LPA₁ induced a migratory response to LPA (202). LPA receptor signaling promotes invasion and metastasis of many different types of cancers, but the roles each receptor plays may be different in each cancer type. These pro- or antitumorigenic roles of LPA₁–₃ may in part reflect the expression of a variety of growth factor peptides and receptors.

LPA₁ and LPA₂ signaling appear to have a similarly interesting dynamic in immune cell function. LPA receptors are expressed on most immune cells and immune organs, including lymphocytes (202) and dendritic cells (DCs) (220, 221), as well as in the spleen and thymus (4, 24, 222). In T cells, LPA can either stimulate or attenuate cellular activity, depending on the cell activation state. LPA₂ is predominantly expressed in unstimulated T cells, whereas LPA₁ is predominantly expressed in stimulated T cells. While cell survival in T cells is influenced by both LPA₁ and LPA₂ signaling (197), the effects of LPA differ depending on the expressed receptor. In unstimulated T cells, LPA enhances chemotaxis and inhibits IL-2 production (185, 198, 223). In activated T cells, where LPA₂ is downregulated and LPA₁ is upregulated, LPA inhibits chemotaxis, activates IL-2 and IL-13 production, and promotes cell proliferation (198, 224). In addition, LPA has differing effects on immature and mature DCs. LPA₁–₃ are expressed in both immature and mature DCs, and LPA appears to affect immature DCs by enhancing maturation and cytokine production (220, 221). Furthermore, LPA₃-specific activation induces chemotaxis of immature, but not mature, DCs (225). Thus, the effect of LPA on DCs appears to be stage specific, though the nature and receptor specificity of this regulation remains largely uncharacterized.

1.4.3. LPA₃

Lpar3 was discovered in a similar way to Lpar2, using homology searches for orphan GPCRs and a degenerate, polymerase chain reaction (PCR)-based cloning method (18, 226). LPAR3 (human chromosomal locus 1p22.3-p31.1) encodes a ∼40 kDa GPCR that, in mice, is ∼50% identical in amino acid sequence to LPA₁ and LPA₂. 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 LPA₃’s EC₅₀ by a factor of 10 (K7.35A) (124). LPAR3 expression is found in the human heart, testis, prostate, pancreas, lung, ovary, and brain (18, 226) and is most prominent in the mouse testis, kidney, lung, small intestine, heart, stomach, spleen, brain, and thymus (125). During development, Lpar3 expression was reported in the heart, mesonephros, in a linear profile between the lateral nasal process and the maxillary process, and...
LPA receptors

in three spots in the otic vesicle (127). Like both LPA₁ and LPA₂, LPA₃ can couple with G_{α_{i/o}} and G_{α_{q/11}} to mediate LPA-induced PLC activation, Ca^{2+} mobilization, adenyl cyclase inhibition and activation, and MAPK activation (Fig. 1.3) (227). LPA₃ has been reported to prefer 2-acyl-LPA containing unsaturated fatty acids (18, 228).

Lpar₃−/− mice are viable and grossly normal, and even though LPA₃ is expressed in the frontal cortex, hippocampus, and amygdala (18, 226), there were no reported phenotypes regarding LPA₃ and the nervous system. However, female nulls show a striking phenotype in relation to the reproduction system (229). LPA is present in the follicular fluid of healthy individuals (230), and ATX activity is enhanced in the serum and placenta of normal pregnant women in the third trimester of pregnancy, which is further increased in patients at risk for preterm delivery (38, 231, 232), supporting important potential roles in aspects of reproduction. Lpar₃ is exclusively expressed in the luminal endometrial epithelium during the short window of implantation (229), and its expression is regulated by progesterone and estrogen (233). This tight regulation of Lpar₃ expression suggested specific functionality during embryo implantation. Analyses of Lpar₃−/− mutant mice identified delayed embryo implantation, embryo crowding, and reduced litter size that were traced to maternal effects, based on transfer of wild-type embryos into Lpar₃−/− dams that failed to implant normally (229). These defects are remarkably similar to the phenotypes of mice lacking COX-2, an enzyme that produces prostaglandins. LPA₃-mediated signaling appears to be upstream of prostaglandin synthesis in this system because exogenous administration of prostaglandins to Lpar₃−/− dams rescues the delayed implantation and reduced litter sizes (229). However, this treatment failed to rescue the embryo crowding, indicating that LPA₃ signaling mediates implantation in both a prostaglandin-dependent and prostaglandin-independent manner (234). The mechanism underlying the spacing defect in Lpar₃−/− mice remains obscure, but may involve either cytosolic phospholipase A₂α (cPLA₂α) or Wnt/β-catenin signaling, since both cPLA₂α removal and Wnt/β-catenin signaling inhibition show similar embryo-crowding phenotypes as that observed in Lpar₃−/− mice (235, 236).

The expression of LPA receptors in the testis (4, 23, 126) also suggested a role for LPA signaling in male reproduction. Overexpression of LPP-1, an LPA-degrading enzyme, resulted in impaired spermatogenesis (237), indicating the importance of lipid phosphatases, and potentially LPA, in this process. There is also evidence for LPA functioning in sperm motility (238), although triple genetic deletion of LPA₁-3 showed no detectable deficits in sperm motility. However, genetic deletion of LPA₁-3 did result in pronounced defects in germ cell survival and an increased prevalence of azoospermia in aging mice (237), indicating that the combined signaling of LPA₁-3, as well as potential involvement of other receptor subtypes, are important for both male reproductive processes as well as female reproductive processes. These data add to other studies indicating that LPA signaling is involved with many reproductive
processes, including spermatogenesis, male sexual function, ovarian function, embryo implantation, fertilization, decidualization, pregnancy maintenance, and parturition (reviewed in Reference 126).

1.4.4. **LPA₄**

LPA₄ was the first identified lysophospholipid receptor to show a dissimilar predicted amino acid sequence from the other lysophospholipid receptor genes for LPA₁-₃ and S1P₁-₅. LPA₄ was identified through ligand screening using a calcium mobilization assay (23). It had been previously known as an orphan GPCR name P2Y9 for its similarity to P2Y purinergic receptors, sharing only 20–24% sequence homology to LPA₁-₃ (23). However, it responds to LPA but not to any nucleotides or nucleosides (23). Located on chromosome Xq21.1, **LPAR4** encodes a 370 amino acid protein, while the mouse homolog **Lpar4** is also located on chromosome X (region D). **Lpar4** is present in multiple murine tissues including heart, skin, thymus, bone marrow, and embryonic brain (239). Additional in situ data also confirm the developmental expression of **Lpar4** in the mouse brain, maxillary processes, branchial arches, limb buds, liver, and somites. This expression pattern of **Lpar4** in the developing brain suggests a possible role in brain development (127).

As with the other LPA receptors, LPA₄ is a GPCR with seven transmembrane domains and couples to several different Gα-proteins, including G₆, G₁, G₄, and G₁₂/₁₃ (239). Through G₁₂/₁₃ and subsequent Rho/ROCK pathway activation, LPA₄ induces neurite retraction and stress fiber formation seen with activation of other LPA receptors (239, 240). LPA₄ mediates ROCK-dependent cell aggregation and N-cadherin-dependent cell adhesion in the B103 rat neuroblastoma cell line heterologously expressing LPA₄ (240). LPA₄ induces intracellular cyclic adenosine monophosphate (cAMP) accumulation through the activation of Gαₛ and was also the first LPA receptor identified that could evoke Gαₛ activity (239). When coexpressed with c-Myc and Tbx2, LPA₄, along with LPA₁ and LPA₂, can transform MEF in vitro and is dependent on Gαₛ-induced ERK and PI3K signaling (241). In addition, LPA₄ has been reported to control the differentiation of immortalized hippocampal progenitor cells (242). Notably, LPA₄ negatively modulates cell motility, whereas LPA is traditionally seen as a chemoattractant, indicating that differential effects can be activated through specific LPA receptors for cell functions. LPA₄ specifically inhibits LPA-induced cell migration and LPA₄-deficient cells are hypersensitive to LPA exposure, with more lamellipodia formation and increased transwell movement (243).

Adult **Lpar4**⁻/⁻ mice do not display obvious abnormalities (243), however there is a decrease in the prenatal survival of **Lpar4**⁻/⁻ mice likely caused by hemorrhage associated with abnormal and dilated blood vessels (244). Similarly, the lymphatic system of **Lpar4**⁻/⁻ mice is also affected, showing enlarged lymphatic vessels and dilated lymph sacs, which indicates an important role for LPA₄ in circulatory system development (244). Additionally, LPA₄ was
shown to be involved in osteogenesis regulation via the inhibition of osteoblastic differentiation of stem cells, using a human mesenchymal stem cell line that revealed increased alkaline phosphatase activity and mineralization in LPAR4 knockdown cells (245). Lpar4−/− mice also exhibit increased trabecular bone volume, number, and thickness, suggesting that LPA4 negatively regulates osteogenesis and may counteract LPA1-initiated osteogenesis (106, 246).

1.4.5. LPA5

LPA5 was first identified in 2006 (24, 25). LPAR5 shares 35% homology with LPAR4, but is more dissimilar compared to LPAR1-3 (25). LPAR5 (chromosomal locus 12p13.31) encodes a 372 amino acid protein, while the homolog in mice (Lpar5) is located on chromosome 6F2. Lpar5 is expressed in many murine tissues, with high expression in spleen, heart, platelets, gastrointestinal lymphocytes, and dorsal root ganglia (DRG) (24, 25, 247). Expression of Lpar5 was also identified specifically in the early embryonic mouse brain, with ubiquitous expression later in development, suggesting a potential role for LPA5 in brain development (127). Recent mutagenesis studies have implicated several residues involved in LPA5 ligand recognition, including one mutant that abolished receptor activation (R2.60N) and three separate mutants that greatly reduced receptor activation (H4.64E, R6.62A, and R7.32A) (248).

Like other GPCRs, LPA5 possesses seven transmembrane domains and couples to Gα12/13 and Gαq (25). In vitro experiments have shown that LPA5-expressing cell lines produce neurite retraction and stress fiber formation, as well as receptor internalization, through the Gα12/13 pathway (25). LPA5 also activates Gαq to increase intracellular calcium levels (25), as well as induce cAMP accumulation in LPA5-expressing cells. However, this cAMP accumulation is unaltered by Gαs minigene administration, suggesting alternative G protein involvement (24, 25). LPA5 may also affect intestinal water absorption (249). In intestinal epithelial cells, LPA induced Na+/H+ exchanger 3 (NHE3). This LPA effect is mediated through the interaction between LPA5 and Na+/H+ exchanger regulatory factor 2 (NHERF2), which then recruit NHE3 to the microvilli (249). These data suggest a clinical possibility of using LPA or LPA receptor agonists against diarrheal syndromes.

1.4.6. LPA6

The most recent addition to the LPA receptor family was LPA6. Previously known as an orphan GPCR called P2Y5, LPA6 is also a member of the P2Y group of receptors, along with LPA4 (27). A chimeric Gα13 protein indicated that LPA induced LPA6-mediated cAMP accumulation, along with Rho-dependent cell morphology alterations, [3H]LPA binding, and LPA-induced [35S]guanosine 5’-3-O-(thio)triphosphate binding (26). 2-acyl-LPA does appear to have a higher activity to LPA6 than 1-acyl-LPA, and many of the tests
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**Antagonists**

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Note: EC50, IC50, and/or Ki values are represented by: ++++, <1 nM; ++++, 1–10 nM; ++, 10–100 nM; +, 100–1000 nM; +, 100–5000 nM; +/-, >5000 nM; −, no activity; NA, not applicable.
performed required unusually high concentrations of LPA (up to 10 µM) to show an effect, compared to the nanomolar concentrations needed for activating LPA₁₋₅. Activation of LPA₆ with LPA resulted in increased intracellular Ca²⁺ when LPA₆ was coexpressed with a promiscuous G₃₉ protein, reduction in forskolin-stimulated [cAMP]ᵢ, and ERK1/2 activation (250).

When LPA₆ was first characterized, it was reported as a genetic risk factor for an autosomal recessive form of hypotrichosis simplex, a complex of diseases involving rare, familial forms of hair loss in humans. Several studies have now identified mutations in LPA₆ in hypotrichosis patients (27,251,252). There have also been reports of lipase member H (LIPH) mutations in hypotrichosis that are associated with both a decrease in LPA production when expressed in cell culture as well as reduced or completely abrogated LPA₆ activation in cells expressing the receptor (253,254). These findings suggest LPA₆ as a candidate for therapeutic intervention in forms of human hair loss.

1.5. LPA RECEPTOR AGONISTS AND ANTAGONISTS

There are many different reported LPA receptor agonists and antagonists, all with a variety of selectivities and potency (Table 1.1). Most of these pharmaceutical modulators focus on LPA₁₋₃, although a few recent studies have focused on LPA₄ with limited selectivity (255,256). The vast majority of these studies have relied heavily upon in vitro assays for validation, but a few have been reported as functional in vivo. For example, the LPA₃-selective agonist OMPT enhanced murine renal ischemia–reperfusion injury, whereas the LPA₁/₃ dual antagonist VPC12249 reduced the injury via LPA₃ inhibition (77). Currently, an antagonist to LPA₃ has shown efficacy in inhibiting lung fibrosis in a bleomycin injury model (257), and the dual LPA₁/₃ antagonist Ki16425 has demonstrated reduction of the metastatic potential of breast cancer in a xenograft tumor model (258). A dual activity pan-LPA receptor antagonists/ATX inhibitors, named BrP-LPA, has also been used to initiate breast, lung, and colon cancer tumor regression (112,259,260). All of these compounds require further validation, particularly within specific assays, especially if they involve delivery in vivo, where pharmacodynamic and pharmacokinetic issues are critical. Nevertheless, they indicate the feasibility of developing pharmaceutical agents that can therapeutically target LPA receptors, as proven for the lysophospholipid S1P receptor modulator FTY720 (fingolimod, Gilenya) that has become a medicine for the treatment of multiple sclerosis (91).

REFERENCES


