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Critical Review

Lysophosphatidic Acid in Vascular Development and Disease

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Summary

Lysophosphatidic acid (LPA) is a small signaling lipid that is capable of stimulating a plethora of different cellular responses through the activation of its family of cognate G protein-coupled receptors. LPA mediates a wide range of biological effects in many tissue types that have been recently reviewed; however, its effects on vasculature development and function have received comparatively less examination. In this review, literature on the actions of LPA in three main aspects of vascular development (vasculogenesis, angiogenesis, and vascular maturation) is discussed. In addition, evidence for the roles of LPA signaling in the formation of secondary vascular structures, such as the blood brain barrier, is considered, consistent with significant roles for LPA signaling in vascular development, function, and disease.
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Keywords lysophosphatidic acid; G protein-coupled receptors; vasculature development; angiogenesis.

INTRODUCTION

LPA Receptor-Mediated Signaling

Lysophosphatidic acid (LPA) is a bioactive lipid component of serum that is also produced by many cell types such as activated platelets (*1*) and postmitotic neurons in culture (*2*). LPA stimulates many different cellular activities by activating a family of cognate G protein-coupled receptors. Five mammalian cell-surface LPA receptors have been identified to date and they are designated LPA_1-LPA_5. LPA_1 is the most widely expressed receptor, with high mRNA levels in the brain, colon, small intestine, placenta, and heart, and more modest expression in the pancreas, ovary, and prostate, as well as other loci (*3*). LPA_2 and LPA_3 have more restricted gene expression patterns compared with LPA_1. In mice, LPA_2 gene expression is found to be most abundant in the embryonic brain, kidney, and testis, with low levels found in numerous other organs, while LPA_3 is present in the lung, kidney, and testis (*4, 5*). In contrast, LPA_4 shows adult gene expression at very low levels in most human tissues, but is expressed significantly in the ovary (*6*). LPA_5 has broad, low levels of expression in many tissues including the embryonic brain, and is enriched in the small intestine and moderate levels in skin, spleen, stomach, thymus, lung, and liver (*7*). During development, LPA_1 and LPA_2 are expressed in the embryonic brain during neurogenesis, with high levels of LPA_1 present in the cerebral cortical ventricular zone. LPA_3 is expressed during the early postnatal period in the brain (*4, 5*).

LPA induces a variety of cellular responses in many cell types, including intracellular calcium mobilization, stress fiber formation, cell rounding, neurite retraction, proliferation, and survival (*8*) (Fig. 1). The great variety of cellular and biological actions of LPA is explained by the fact that LPA receptors can couple to at least four distinct G protein families defined by their alpha subunits ($G\alpha_i$, $G\alpha_{12/13}$ $G\alpha_{q/11}$, and $G\alpha_s$), which, in turn, feed into multiple effector systems (*9*). LPA activates $G\alpha_{q/11}$ and thereby stimulates phospholipase C (PLC), with subsequent phosphatidylinositol-bisphosphate hydrolysis and generation of multiple second messengers leading to protein kinase C activation and changes in cytosolic calcium. LPA also activates $G\alpha_i$ (*10*), which leads to at least three distinct signaling routes: inhibition of adenylyl cyclase with inhibition of cyclic AMP accumulation; stimulation of the mitogenic RAS–MAPK (mitogen-activated protein kinase) cascade (*11*); and activation of phosphatidylinositol 3-kinase (PI3K), leading to activation of the downstream RAC GTPase, a key regulator of the cell morphology and motility (*12*), as well as to activation of the AKT/PKB antiapoptotic pathway (*13*). LPA receptors activate $G\alpha_{12/13}$, leading to activation of the small GTPase RhoA, which drives cytoskeletal contraction and cell rounding (*14*). In addition, $G\alpha_s$ activation can increase cAMP levels through LPA_4.

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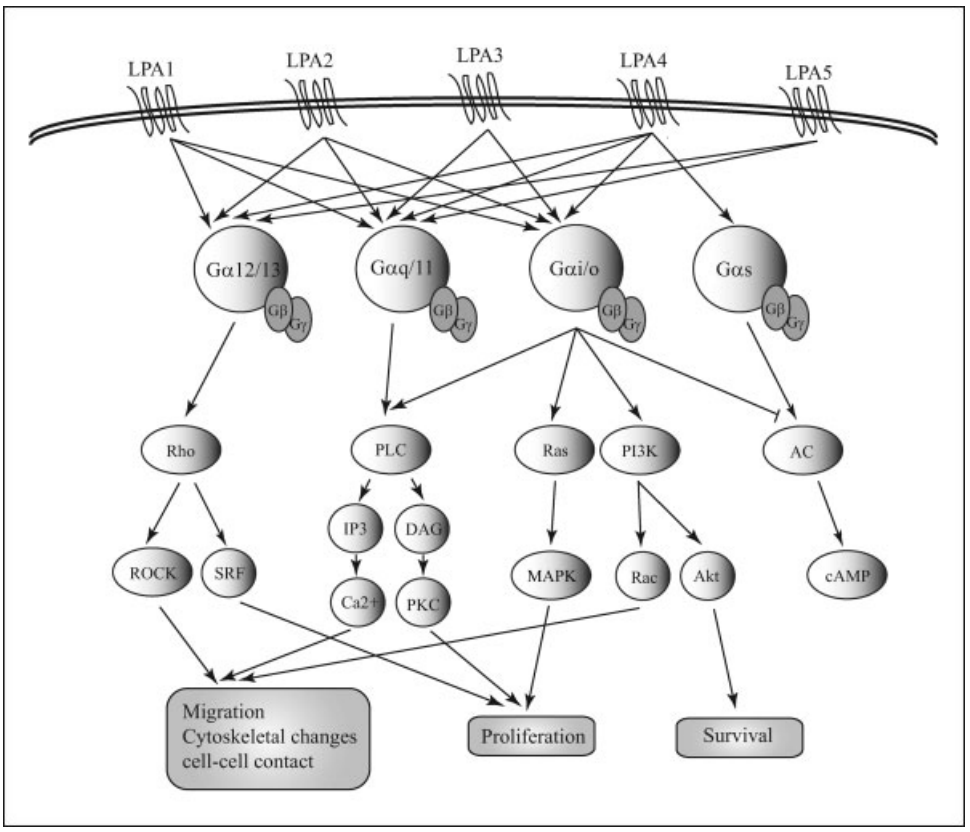

Figure 1. LPA signaling pathways mediated by LPA receptors.

There are at least two different metabolic pathways postulated for the production of LPA. The first pathway is predominant in serum and plasma, and involves the conversion of phospholipids (PLs) to lysophospholipids (LPLs) by either phospholipase A1 (PLA_1) and A2 (PLA_2), and subsequent conversion to LPA by lysophospholipase D (lysoPLD). Recent studies have identified autotaxin (ATX) as the predominant lysoPLD (*15*) responsible for most of the LPA present in normal serum. In platelets and certain cancer cells, the second pathway occurs with the conversion of PLs and diacylglycerol (DAG) by phospholipase D (PLD) and diacylglycerol kinase (DGK), respectively, to phosphatidic acids (PAs). The PAs are subsequently deaceylated to LPA by either PLA_1 or PLA_2 (*16–18*). This combined regulation of ligand and receptor distribution, in addition to the diversity of second messenger activation, provides an expansive range of biological roles for LPA signaling in different tissues and developmental stages.

LPA Receptor Knockout Mice

To date, four of the five receptors ($LPA_{1\text{-}4}$) have been characterized by gene knockout studies in mice. Because the expression pattern of these LPA receptors is heterogeneous, the phenotype of these knockout mice varies considerably. LPA_1 knockout mice suffer from suckling defects, resulting in a 50% neonatal lethality rate. The impaired suckling defect was attributed to faulty olfaction, possibly because of a defect in olfactory bulb or cortex development. The surviving knockouts exhibit a reduced body size, craniofacial dysmorphism, embryonic frontal cranial hematomas, and increased apoptosis in sciatic nerve Schwann cells, which correlates with the expression pattern of LPA_1 (*19*). The deletion of LPA_2 did not cause any obvious phenotypic defects, with mice born at the expected frequency. Furthermore, $LPA_{1,2}$ double knockout mice did not result in any additional phenotypic abnormalities relative to those observed in LPA_1 single knockout mice, except for an increased frequency of frontal hematomas in the head, which provides evidence for the involvement of these receptors in vascular development. However, $LPA_{1,2}$ double knockout embryonic fibroblasts showed a severe reduction or absence of LPA-induced responses, including phospholipase C activation, calcium mobilization, proliferation, JNK activation, Akt activation, and stress fiber formation. This reduction of LPA responsiveness was less pronounced in LPA_1 or LPA_2 single knockout fibroblasts, indicating redundancy of the LPA_2 receptor with respect to LPA_1 (*20*).

The targeted deletion of LPA_3 resulted in a considerably different phenotype characterized by pronounced reproductive

defects. Litters born to $LPA_3^{-/-}$ breeder female mice were less than half those of controls, with no dependence on stud male genotype, indicating defects in female reproduction. LPA_3 knockout mice exhibit delayed embryo implantation and altered embryo spacing, resulting in delayed embryonic development and hypertrophic placentas and embryonic death. This was attributed to a down regulation of cyclooxygenase 2 (COX2) which led to reduced levels of prostaglandins E_2 and I_2, which are essential players in implantation (*21*).

LPA_4-deficient mice did not exhibit any apparent abnormalities, and were born at the statistically expected Mendelian rate. However, LPA_4-deficient mouse embryonic fibroblasts (MEFs) were found to be hypersensitive to LPA-induced cell migration. LPA_4 deletion caused a potentiation of Akt and Rac activation, implying that LPA_4 negatively regulates the phosphatidylinositol-3-kinase pathway, which is in contrast to activation of this pathway by other LPA receptors. The coexpression of LPA_4 with LPA_1 in B103 neuroblastoma cells attenuated LPA_1-driven migration, indicating functional antagonism between the two LPA receptor subtypes. This inhibitory crosstalk is likely to be essential for ensuring physiologically appropriate responses to LPA (*22*).

Vasculature Development

Embryonic vascular development consists of three continuous processes: vasculogenesis, angiogenesis, and maturation. Additionally, formation of secondary features occurs in specific regions, such as the blood brain barrier (BBB) in the central nervous system (CNS). Vasculogenesis begins with the differentiation of mesodermal cells into hemangioblasts, which are progenitors of both hematopoietic and endothelial cells. The hematopoietic cells then give rise to angioblasts which form blood islands. These structures fuse to form the primary blood vasculature. The blood vessels then undergo angiogenesis to sprout new vessels from existing vasculature, which is influenced by angiogenic factors. Angiogenic factors are secreted proteins which induce the growth of new blood vessels. This process involves the destruction of existing vasculature at specific branching locations, proliferation of endothelial cells and migration to the branching points to form new vessels (*23*). Vascular maturation then ensues with the recruitment of mural cells, such as pericytes in capillaries and vascular smooth muscle cells (VSMCs) in arteries, to ensheathe and stabilize the blood vessels (*24*). In the CNS, adjacent endothelial cells develop cell–cell adhesions through the binding of specific integral membrane proteins, hence forming tight junctions of the BBB. Three families of involved integral membrane proteins have been identified to date: occludins, junctional adhesion molecules (JAMs), and claudins (*25*). LPA influences the cells and signaling processes that mediate vascular development. This review summarizes previous studies implicating the involvement of LPA in this process (Table 1).

Many factors have been implicated in various aspects of vasculature development, such as the angiopoietins, neuropilins, and platelet-derived growth factors (PDGFs). A pivotal player in vasculature development is vascular endothelial growth factor (VEGF). Knockout studies demonstrate that deletion of one or both copies of VEGF is embryonically lethal, with homozygous mice dying between embryonic age (E) 8.5 and 9.5, and heterozygous mice dying between E11 and E12. The deficiency in VEGF impaired the differentiation of blood islands during vasculogenesis and subsequent angiogenesis. The development of endothelial cells was delayed, resulting in a defective vasculature in these knockouts (*26, 27*). On a similar note, mice deficient in VEGF receptor 2, the main mediator of VEGF signaling, died between E8.5 and E9.5 as well, with a failure to form blood islands and a severe defect in the development of hematopoietic and endothelial cells (*28*). Furthermore, VEGF has been found to be a potent mediator of angiogenesis, by inducing the proliferation, survival, and migration of endothelial cells [see review: (*29*)]. Hence, this highlights the importance of VEGF in both vasculogenesis and angiogenesis.

LPA IN VASCULOGENESIS

The first definitive study that linked LPA to vascular development was that of the autotaxin (ATX/ Enpp-2)-deficient phenotype. ATX functions as a secreted lysophospholipase D that converts lysophosphatidylcholine (LPC) into LPA (*15*). ATX-deficient mice die at E9.5 with profound vascular defects in the yolk sac and embryo, which coincides with the age of increased ATX and LPA receptor expression in normal embryos (*30*). ATX-deficient mouse embryos were found to possess severe defects, including allantois and neural tube malformations. Importantly, severe vascular defects, such as absence of yolk sac vascularization and the presence of enlarged embryonic blood vessels, were apparent by E9.5. The normal occurrence of blood islands, as well as PECAM-1 (CD31) staining, demonstrated the presence of differentiated endothelial cells from angioblasts, thus suggesting that reduced LPA concentration did not alter the capacity of endothelial progenitor cells to differentiate. ATX heterozygous mice were found to possess half the plasma LPA levels as normal littermates, which implies that ATX is the major LPA-producing enzyme *in vivo* (*31*). This strongly suggests that loss of LPA production is responsible for the observed pathological phenotype and that LPA signaling is critical for vasculature development.

Furthermore, $G\alpha_{13}$ knockout mice share a similar phenotype with ATX-deficient mice, with impaired blood vessel formation in both yolk sac and embryo and embryonic lethality at E9.5 that are, interestingly, also associated with significant brain abnormalities (*32*). Since LPA is a major upstream activator of $G\alpha_{12/13}$ (*33*), it is likely that LPA signaling mediates some of the embryonic vascular development processes through this pathway. This model is supported by the observation that individual LPA receptor ($LPA_{1\text{-}3}$) knockouts showed moderate

Table 1
Summary of the effect of LPA on vasculature development

Vascular development		Evidence for role of LPA	References
Vasculogenesis	Blood island formation	No effect, blood islands are normal in ATX and LPA KO mice	(*15, 30*)
	Primary capillary plexus formation	Likely, not well-characterized	(*15*)
Angiogenesis	Proliferation of endothelial cells	LPA stimulates tritiated thymidine uptake and EC proliferation in wound healing assay	(*37, 38*)
	Migration of endothelial cells	LPA stimulates EC migration in wound healing assay in HUVECs, chemotactic response in BPAE cells, and migration in EAhy926 Ecs	(*37, 38, 41*)
	Vessel sprouting	New vessel formation in chicken CAM assay	(*43*)
	Vessel maintenance	Vessel disassembly prevented in cultured murine allantois explants	(*31*)
	Pregnancy	Elevated ATX and LPA levels during human pregnancy	(*34, 55*)
	Ovulation	High LPA levels detected in follicular fluid during formation of corpus luteum	(*55, 56, 57*)
	Cancer (pathological)	LPA found in ovarian cancer ascites and produced by cancer cells, induces upregulation of HIF-1a and VEGF-A expression in prostrate, ovarian, lymphoma, and hepatoma cancers	(*44–51*)
Vascular maturation	Recruitment of mural cells (pericytes and VSMCs)	Knockout mice of a related signaling lysophospholipid receptor, S1P1, show drastic pericyte number reduction associated with vessels; mice die at E11–12 due to failed vascular maturation and bleeding	(*79*)
	Vascular injury (pathological)	LPA1/2 mice protected from intimal hyperplasia and VSMC dedifferentiation during atherosclerosis	(*61, 62*)
	Thrombus formation	LPA can induce and prevent platelet aggregation in most humans but not mice	(*64, 65*)
BBB formation	Tight junction maintenance between endothelial cells	LPA reversibly decreased TER and sucrose paracellular flux in procine ECs	(*70, 71*)
	Astrocyte signaling	LPA induces dose-dependent increase in intracellular Ca2+	(*72, 73, 76*)
		LPA upregulates several immediate early genes and cytokines	(*76*)
		LPA increases lipid peroxidation	(*76*)
		LPA increases DNA synthesis	(*75, 76*)
		LPA can reverse astrocyte stellate morphology induced by cAMP	(*75, 76*)
	Interaction between mural and endothelial cells	Knockout mice of a related signaling lysophospholipid receptor, S1P1, show drastic pericyte number reduction associated with vessels	(*79*)

vascular defects with the presence of frontal hematomas in 2.5% of LPA_1 single knockout and 26% of $LPA_{1,2}$ double knockout embryos (*19, 20*). In addition, ~40% of $LPA_{1,2}$ double knockouts also showed blood within the lateral ventricles, suggesting that bleeding may have occurred from internal structures such as the choroid plexus (unpublished data). Also, microhematomas were noted on the developing skin and surface of the embryos (unpublished data). The lack of more severe vascular defects might be due to redundancy of the LPA receptors, as well as the continued presence of S1P lysophospholipid receptors that have proven roles in vascular development and integrity (*34–36*). It is possible that more severe disruptions of vascular development will only occur with the simultaneous deletion of additional LPA receptors and/or in conjunction with S1P receptors.

LPA IN ANGIOGENESIS

Angiogenesis is a process which involves the proliferation and migration of endothelial cells to form new sprouting blood vessels from existing vasculature. LPA has been implicated in both of these processes. In a study using adult bovine aortic and human umbilical vein endothelial cells, LPA was observed to facilitate closure of wounded endothelial monolayers of the endothelial cells *in vitro*. This was due to a stimulation of both endothelial cell migration and proliferation by LPA treatment

(*37*). In another study, LPA was shown to induce a chemotactic response in bovine pulmonary artery endothelial cells, similar in intensity to that observed with optimal levels of known endothelial cell chemoattractants such as basic fibroblast growth factor (bFGF) and VEGF (*38*). Also, LPA was shown to play a chemokinetic role in inducing the migration of several endothelial cell types, including fetal bovine heart endothelial cells and bovine aortic endothelial cells (*39*). LPA-induced migration of bovine pulmonary artery endothelial cells was found to be mediated by the recruitment of hydrogen peroxide inducible clone 5 (Hic-5), a paxillin family member, to the focal adhesions of the endothelial cells (*40*). In EAhy926 human endothelial cells, the induction of migration by LPA was found to be because of an increase in matrix metalloproteinase-2 (MMP-2), a critical player in endothelial cell migration and matrix remodeling during angiogenesis (*41*). LPA also stimulates a significant increase in the uptake of tritiated thymidine in endothelial cells, demonstrating that LPA can act as a mitogen and proliferation signal for endothelial cells (*42*). In addition, LPA was found to evoke the formation of new blood vessels in a chicken chorio-allantoic membrane (CAM) assay, confirming it as an angiogenic compound (*43*). In contrast, neither LPA nor ATX was found to be angiogenic in cultured mouse allantois explants. Rather, they were found to maintain existing vessels by preventing disassembly of the vessels (*31*).

LPA has also been documented to be a major player in pathologic angiogenesis during the development of various cancers. Elevated LPA levels have been detected in the ascites of 98% of ovarian cancer patients, including 90% of patients with stage 1 disease, suggesting that LPA is important for early events in carcinogenesis. Also, ovarian cancer cells have been found to produce LPA, thereby maintaining an LPA-rich microenvironment (*44–46*). In ovarian cancer cells, LPA was found to upregulate expression of hypoxia-inducible factor-1-alpha (HIF1α), which plays a central role in tumor angiogenesis (*47*). Furthermore, LPA has been reported to induce VEGF-A (VEGF) expression via HIF-1α activation in a variety of cancer cells, including prostate cancer, ovarian cancer, lymphoma, and hepatoma cells (*48–50*). In ovarian cancer cells, this was inhibited by the knockdown of LPA_2 or LPA_3 receptors (*51*). VEGF is the predominant regulator of angiogenesis that stimulates vascular endothelial cell growth, survival, and proliferation. It has been shown to promote survival of existing vessels and stimulate new vessel growth (*52*). Recently, LPA was found to enhance Sp-1-mediated VEGF transcription by inducing the phosphorylation of Sp-1 and binding to the VEGF promoter via a HIF1α-independent pathway through the $G\alpha_{12/13}$-Rho-ROCK-c-Myc pathway (*53*). These results show that LPA is implicated in tumor angiogenesis.

LPA signaling has also been implicated in endometrium and placenta angiogenesis. An increase in serum lysophospholipase D activity has been noted during pregnancy in humans, suggesting that the consequential production of LPA might be important in pregnancy (*54*). LPA-conditioned medium of endometrical stromal cells was able to stimulate migration, permeability, capillary tube formation, and proliferation of human endometrial microvascular endothelial cells. This was mediated by the production of IL-8, an angiogenic factor, via a $G\alpha_i$ protein-coupled pathway (*55*). LPA was shown to influence critical steps of endometrium angiogenesis during pregnancy.

The formation of the corpus luteum during ovulation is a period of marked angiogenesis (*55*). Coincidentally, LPA was found in significant amounts in the follicular fluid of the preovulatory follicle, suggesting a possible role for LPA in ovulation (*56*). This was demonstrated in a study where the LPA-primed medium of human granulose-lutein cells was found to stimulate processes of angiogenesis, including migration, tube formation, and proliferation of umbilical vein endothelial cells. Both IL-6 and IL-8 secretion was found to be induced by LPA to mediate these processes, via a $G\alpha_i$ protein-coupled, NF-κB-dependent pathway (*57*).

Because both blood and lymphatic vascular systems are composed of vessels lined by endothelial cells, it is not surprising that LPA plays a role in lymphangiogenesis as well. In a study involving human umbilical endothelial cells, LPA up-regulated vascular endothelial growth factor-C (VEGF-C), a critical player in lymphangiogenesis *in vitro,* and subsequent endothelial cell tube formation in Matrigel assays (*58*). Furthermore, the knockdown of LPA_1 in zebrafish resulted in defects in lymphatic vessel development, such as the absence of a thoracic duct and edema in the pericardial sac. Taken together, these studies suggest that LPA plays a significant role in the lymphatic vascular system (*59*).

LPA IN VASCULAR MATURATION AND PATHOLOGICAL VASCULAR RESPONSES

LPA has also been implicated in the regulation of pathophysiologic vascular responses. LPA was found to signal through $G\alpha_q$ to promote the growth and migration of VSMCs (*60*), which are essential for the development of intimal hyperplasia after vascular injury. Mice deficient in LPA_1 and LPA_2 were protected from intimal hyperplasia (*61*), suggesting that migration induction of SMCs by LPA plays a role in this process. Furthermore, unsaturated LPA was found to stimulate VSMC dedifferentiation through the activation of ERK and p38 MAPK, which is a hallmark in the development of atherosclerosis. Thus, naturally occurring unsaturated LPAs may act as atherogenic factors (*62*). This is supported by the fact that LPA-like compounds have been found to accumulate in human atherosclerotic plaques (*63*).

LPA may be involved in thrombus formation. LPA promoted human platelet activation by inducing platelet shape change and calcium mobilization. LPA also induced the aggregation of platelets, which is essential in thrombosis (*64*). Conversely, the addition of LPA attenuated aggregation of murine platelets, thereby preventing thrombosis. Intravascular administration of LPA prolonged bleeding time and inhibited thrombosis in mice,

as well as a transgenic overexpression of autotaxin (*65*). Therefore, LPA might play dual roles as a species-specific modulator of platelet function, thrombosis, and hemostasis. This difference between the actions of LPA on murine and human platelets may be attributed to a difference in expression of LPA receptors, resulting in differential activation of signaling pathways.

LPA causes a dose-dependent increase in the cell death of both HUVECs and porcine cerebral microvascular endothelial cells. Brain explants and retinas exposed to LPA also exhibited diminished vasculature (*66*). When endothelial cell monolayers were incubated with LPA, a loss of confluence due to cellular detachment was noted, which indicated a loss in vascular integrity. Also, a gain in hydraulic permeability in rat mesenteric venules was observed *in vivo* when exposed to LPA, showing endothelial dysfunction and loss of vascular integrity (*41, 67*). Therefore, LPA may be involved in vascular injury by causing endothelial cell death and vascular degeneration. The topical application of synthetic LPA was also found to induce dose-dependent vasoconstriction in the cerebral circulation of newborn pigs. Because LPA-like bioactive mediators were found to be generated in an intracranial hematoma model (*68*), this suggests that LPA might contribute to the development of posthemorrhagic vasoconstriction.

LPA IN BLOOD BRAIN BARRIER (BBB) FORMATION

The BBB is a structural component specific to the vasculature of the CNS, which acts as a selective barrier to the paracellular flux of molecules between the bloodstream and surrounding neural tissue. It is composed of three cellular components: endothelial cells with associated tight junctions, end feet of astrocytes ensheathing the vessels, and pericytes embedded in the capillary basement membrane (*69*). LPA has been implicated in the maintenance of each of these components.

One of the most distinctive features of the BBB is the presence of tight junctions, which are sites of fusion between the plasma membrane of adjacent endothelial cells. These tight junctions exhibit high electrical resistance and limited transcellular flux, and greatly limit the permeability of the BBB to hydrophilic solutes and ions. In porcine brain endothelial cells, LPA was found to cause both a rapid, reversible, and dose-dependent decrease in transcellular electrical resistance (TER) and an increase in the paracellular flux of sucrose, indicating increased tight junction permeability. This was attributed to formation of stress fibers, focal contacts, and focal contact-associated phosphorylation rather than relocalization or phosphorylation of adherens junction- or tight junction-associated proteins (*70, 71*). Currently, there is no evidence of a direct effect of LPA on tight junction proteins.

Astrocytes have been shown to communicate with endothelial cells through calcium (Ca^{2+}) signaling [(*72*) which has been reported to affect BBB permeability (*73*)]. LPA signaling may be involved in this interaction since the addition of LPA has been shown to induce a dose-dependent increase in intracellular calcium concentrations in several studies involving rat astrocytes. Higher LPA concentrations (>10 μM) usually elicit sustained increases in Ca^{2+} concentrations, whereas lower concentrations stimulate Ca^{2+} transients (*74–76*). In addition, LPA is capable of inducing many other responses in astrocytes, which might influence the role of astrocytes in maintaining the BBB. LPA was found to mediate the up-regulation of various immediate early genes and cytokines (*77*). In addition, LPA has been shown to stimulate lipid peroxidation and the rate of DNA synthesis in astrocytes as well through pertussis toxin sensitive G protein(s). LPA also inhibits the uptake of glutamate and is capable of reversing the stellate morphology of astrocytes induced by cyclic adenosine monophosphate (cAMP) (*74, 76*). Actomyosin contraction of astrocytes can be stimulated by LPA, which is part of the wound healing process that occurs following the disruption of the BBB (*77*). Conditioned medium from LPA-primed astrocytes was found to increase neural differentiation of neural progenitor cells, suggesting that the presence of an LPA-induced, astrocyte-derived soluble factor (*78*). It is possible that astrocytes could influence endothelial cells in a similar manner via indirect, as well as direct, actions of receptor-mediated LPA signaling.

Although little is known of the effect of LPA on pericytes, a related family of lysophospholipid receptors, sphingosine 1-phosphate receptors ($S1P_{1\text{-}5}$), have been reported to play a significant role in vascular maturation propagated by pericytes. Knockout mice deficient in $S1P_1$ die at Ẽ11-12 because of a failure in vascular maturation and subsequent bleeding. This follows a drastic reduction of pericytes associated with the developing blood vessels, a key process in vascular maturation (*79*). Because LPA and S1P signaling have been reported to signal through similar pathways and exert overlapping effects on various cell types, it is likely that LPA signaling is involved in the interaction between pericytes and endothelial cells.

CONCLUSION

Although no severe vascular defects are reported in single deletions of LPA receptors, the importance of LPA signaling might be under-appreciated because of the functional redundancy within this family of receptors that also are homologous to and share signaling with the S1P receptors which have a proven role in the vasculature. LPA has been shown to invoke a myriad of responses in a variety of endothelial and mural cells. Collectively, the studies described here indicate that LPA signaling plays a significant role in the development of the vasculature, and requires further investigation in this context toward understanding how receptor-mediated LPA signaling affects this system.

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