Lysophosphatidic acid in neural signaling

Xiaoqin Ye, Nobuyuki Fukushima,[1] Marcy A. Kingsbury and Jerold Chun[2,CA]

Department of Pharmacology, School of Medicine, University of California, San Diego, La Jolla, CA 92093-0636, USA; [1]Department of Biochemistry, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan; [2]Present address: Merck Research Laboratories, San Diego, 3535 General Atomics Court, San Diego, CA 92121, USA

[CA]Corresponding Author: jerold_chun@merck.com

The physiological and pathological importance of lysophosphatidic acid (LPA) in the nervous system is underscored by its presence, as well as the expression of its receptors in neural tissues. In fact, LPA produces responses in a broad range of cell types related to the function of the nervous system. These cell types include neural cell lines, neural progenitors, primary neurons, oligodendrocytes, Schwann cells, astrocytes, microglia, and brain endothelial cells. LPA-induced cell type-specific effects include changes in cell morphology, promotion of cell proliferation and cell survival, induction of cell death, changes in ion conductance and Ca^{2+} mobilization, induction of pain transmission, and stimulation of vasoconstriction. These effects are mediated through a number of G protein-coupled LPA receptors that activate various downstream signaling cascades. This review provides a current summary of LPA-induced effects in neural cells *in vitro* or *in vivo* in combination with our current understanding of the signaling pathways responsible for these effects. *NeuroReport* 13:2169–2175 © 2002 Lippincott Williams & Wilkins.

Key words: Cell Proliferation; LPA; LPA receptors; Morphology; Neural Progenitors; Neurons

INTRODUCTION

Lysophosphatidic acid (LPA; 1-acyl-2-*sn*-glycerol-3-phosphate) is a simple and natural phospholipid. It is a component of cell membranes produced in *de novo* biosynthesis of membrane phospholipids. It also serves as an extracellular biomediator generated via enzymatic conversion of glycerophospholipids (reviewed in [1–4]). While the signaling properties of LPA were suggested as early as the 1960s (reviewed in [1,5]), the first strong evidence was presented in 1989 when LPA was shown to induce fibroblast proliferation, an effect mediated by a speculated cell surface G protein-coupled receptor(s) (GPCRs) which was blocked by pertussis toxin (PTX) [6]. Subsequently, the exogenous application of LPA in a range of cell types has been shown to evoke various cellular responses, including Ca^{2+} mobilization, promotion of cell proliferation and survival, and changes in cell morphology and ion conductance (reviewed in [1,5,7–13]). Surprisingly, debate existed on whether these effects were receptor-mediated, or instead the result of intracellular signaling or physical-chemical perturbation of cell membranes. That many actions of LPA could be accounted for by GPCR-mediated mechanisms was not firmly established until 1996 with our cloning of the first LPA receptor gene, *lpa*$_1$, or ventricular zone gene-1 (*vzg-1*), which showed enriched expression in the embryonic cerebral cortical ventricular zone (VZ) [14]. Other names for this receptor include LP_{A1}/MREC1.3/EDG-2 [14,15]. Two additional LPA receptors, LPA_2/LP_{A2}/EDG-4 [16,17] and LP_{A3}/LPA_3/EDG-7 [18,19], have been identified in mammals. LPA_1 and LPA_2 activate the PTX-insensitive $G_{q/11/14}$ and $G_{12/13}$, and the PTX-sensitive $G_{i/o}$, whereas LPA_3 stimulates $G_{q/11/14}$ and $G_{i/o}$ but not $G_{12/13}$, to mediate the diverse cellular effects of LPA [20–28] (reviewed in [1,5,8–10,29–32]).

The potential functions of LPA in the nervous system are implied by the *in vivo* distribution of this ligand and its receptors in normal and injured neural tissues. LPA is present in the brain at relatively high levels compared to other tissues [33], and hemorrhagic brain injury result in dramatic elevations of LPA in the CSF [34–37]. Additionally, *in vivo* mapping studies demonstrate that LPA receptors are expressed in overlapping and complementary populations of cells in the nervous system and their expressions correlate with several developmental processes, including neurogenesis, neuronal migration, neuritogenesis, and myelination [9,14,24,38]. Finally, *in vitro* studies demonstrate that neural cells such as primary neurons and Schwann cells (SCs) can synthesize and secrete LPA [22,26]. Here we review the LPA effects that are relevant to the development and function of the nervous system.

NEURAL CELL LINES

LPA elicited rapid growth cone collapse, neurite retraction, and/or neuronal cell rounding in several neuronal cell lines, such as NG108-15, N1E-115, PC12, TR and TSM, and SY-SH5Y [14,20,21,27,39–46]. These shape changes involved activation of the small GTPase Rho followed by contraction of the actomyosin-based cytoskeleton. A recent study in a TR neuroblast cell line added new insight into LPA receptor-mediated actin regulation [27], in which LPA also stimulated Rho-independent actin depolymerization that was associated with the loss of membrane ruffling at the tips of neurites. This process involved the interactions between

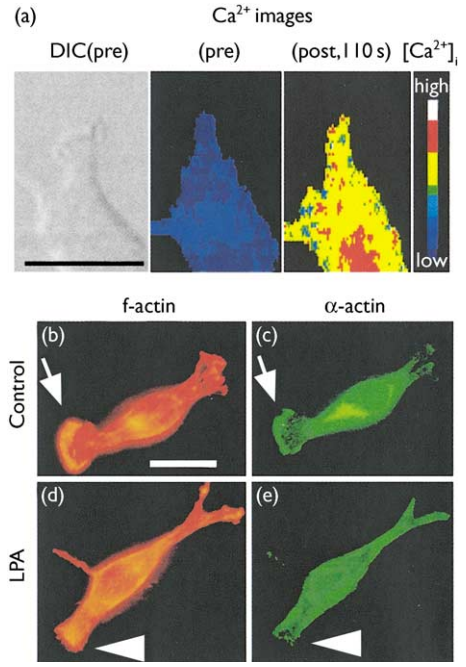

Fig. 1. LPA-induced Rho-independent actin depolymerization involved Ca^{2+} and α-actinin in a TR neuroblast cell line. (**a**) LPA-induced Ca^{2+} mobilization. DIC: Differential interference contrast image. Cells were exposed to 1 μM LPA. (**b**–**d**) LPA-caused reduction of f-actin and α-actinin labeling within membrane ruffling. Cells were treated with 0.5 μM LPA for 2 min. Arrows: co-localization of f-actin and α-actinin in control (untreated) cells. Arrowheads: reduction of f-actin and α-actinin labeling in LPA treated cells. Bars = 20 μm. (Modified from [27]).

Ca^{2+} and α-actinin, an actin cross-linking protein (Fig. 1) [27]. This was the first evidence demonstrating that one extracellular signal (LPA) regulated actin cytoskeleton through two independent pathways. This dual regulation of actin rearrangement might be effectively involved in cell shape changes of neuronal cells [27].

Interestingly, in differentiated SY-SH5Y human neuroblastoma cells, LPA-induced neurite retraction was accompanied by an increase in site-specific, Alzheimer's disease-like Tau protein phosphorylation [46]. This phosphorylation was mediated through Rho-dependent pathways with the involvement of glycogen synthase kinase-3 (GSK-3). Heterologous receptor expression in B103 neuroblastoma cells showed that LPA_1 and LPA_2, but not LPA_3, mediated LPA-induced morphological changes [20,21], implying the potential involvement of LPA_1 and LPA_2 receptors in neuronal disorders such as Alzheimer's disease.

LPA also significantly affected cell morphology and migration in glioma cells [47,48]. In C6 rat glioma cells, LPA treatment prevented cAMP-induced morphological changes from a shape resembling a fibroblast to that of an astrocyte, a process modulated by protein kinase C (PKC) [47]. In cells derived from human glioblastoma multiforme (GBM), a highly invasive malignant glioma, LPA activated intracellular machinery associated with cell migration, such as phosphorylation of the regulatory light chain (RLC) of myosin II and the formation of stress fibers and focal adhesions [48]. LPA-induced glioma cell motility was mediated by Rho/Rho-associated kinases (e.g. ROCK) and, to a much lesser extent, by mitogen-activated protein kinase (MAPK) cascades [48]. Since breakdown of the blood–brain barrier (BBB) and leakage of serum components are common features of GBM, the exposure of these tumor cells to LPA *in vivo* may contribute to their invasiveness.

In addition to the morphological changes, LPA also induced increases of $[Ca^{2+}]_i$ and dopamine release in neuronal cell lines [18,37,49,50]. The LPA-induced transient increase of $[Ca^{2+}]_i$ in PC12 cells was mediated through LPA receptors concomitant with the formation of inositol triphosphate (IP_3). These changes in $[Ca^{2+}]_i$ and IP_3 production were assumed to result in LPA-stimulated dopamine release [49].

NEURAL PROGENITORS

During cerebral cortical neurogenesis in mammals, cortical progenitor cells in the VZ undergo interkinetic nuclear migration, a process in which cells translocate their nucleus from the top to the bottom of the VZ, as they alternate between fusiform and round morphologies [51,52]. The molecular mechanisms underlying the morphological changes and cell cycle progression of progenitors are unclear. However, our recent studies on LPA receptor gene expression and cellular effects of LPA indicate that LPA is involved in cortical neurogenesis [14,22,23].

A minimum requirement for a role of LPA in neurogenesis was receptor expression in the neurogenic region of the cerebral cortex. Not only did the proliferative VZ of the cerebral cortex characteristically express lpa_1 at high levels [14,38], but the temporal expression of lpa_1 was correlated with cortical neurogenesis [14]. The spatial and temporal expression pattern of lpa_1 in the proliferative VZ was consistent with the prediction of potential roles of LPA in cortical neurogenesis.

Direct demonstration of LPA effects on progenitor cell proliferation was observed in cell clusters of embryonic day 12(E12)–E13 cortical progenitor [23]. LPA treatment resulted in a 25% increase in BrdU incorporation relative to vehicle treatment. This increase was not observed in cortical progenitor cell clusters isolated from E12-13 $lpa_1^{(-/-)}$ embryos. The increase in proliferation was inhibited by pretreatment with PTX, indicating that the proliferative effect of LPA was mediated by the LPA_1 receptor and $G_{i/0}$ [21,23] (Fukushima *et al.*, unpublished observation). Interestingly, although $lpa_1^{(-/-)}$ cortical progenitor cells failed to respond to LPA, these cells showed increased proliferative responses to bFGF. Further, the bFGF response in $lpa_1^{(-/-)}$ cortical progenitor cells exceeded that in wild type cortical progenitor cells. This suggests that compensation or cross-talk may exist amongst different growth factors for cortical progenitor cell proliferation [23,53].

LPA also induced morphological changes of the cortical progenitors [22]. These changes included cell rounding, membrane retraction, formation of f-actin retraction fibers and cellular/nuclear migration in primary cortical progenitor cultures. Similar changes were also observed in explanted progenitor cultures. Comparable alterations in cell morphology are observed in the rounding-up phase of interkinetic nuclear migration. Since LPA can be produced by postmitotic neurons, LPA might serve as an extracellular signal from neurons to progenitors during the initial,

rounding-up phase of interkinetic nuclear migration that proceeds mitosis [22]. Mechanistic studies indicated the importance of the LPA_1 receptor in mediating these morphological changes. Heterologous over-expression of *lpa*$_1$ increased cell rounding in the cortical neuroblast cell lines, TSM and TR [14,20,54], whereas genetic deletion of *lpa*$_1$ severely attenuated LPA-induced morphological changes in cortical progenitors [23]. LPA receptor(s)-activated Rho-dependent pathways were involved in LPA-induced morphological changes in cortical progenitor cells [20–23].

LPA treatment also modulated the electrophysiological properties of cortical progenitors [55]. When whole-cell recordings on acutely dissociated VZ progenitors were performed, exogenous application of LPA induced increases of either chloride or non-selective cation conductance. These changes resulted in the depolarization of the cells, as early as E11 [55]. Thus, LPA was an extracellular stimulus of ionic conductance changes for cortical progenitors, developmentally proceeding or co-existing with ionotropic activation by the classical neurotransmitters GABA and L-glutamate [55,56].

In addition to the effects in cortical progenitor cells, LPA also induced Ca^{2+} mobilization, changes in ion currents, and cell proliferation in embryonic chick retina and human retinal pigment epithelial (RPE) cells, both of which possess neural progenitor properties [57–59]. The LPA-induced Ca^{2+} mobilization, a process that involved both PTX-sensitive and insensitive G proteins, was coincident with the neurogenesis of retinal cells, whose mitotic activity peaks at E3 and declines toward E8, indicating that LPA could influence retinal cell proliferation through Ca^{2+} mobilization [57]. In cultured human RPE cells, LPA triggered an inward non-selective cation current, which was mediated by the LPA receptor(s) and PTX-sensitive G proteins. LPA also stimulated an outward current carried predominantly by K^+, which was mediated by PTX-insensitive G protein(s) [58]. In addition, LPA promoted RPE cell proliferation through activation of LPA receptor(s) and PTX-sensitive G protein(s) [59], an effect that may also be regulated by Ca^{2+} mobilization [60]. These effects suggest that LPA may play significant roles in normal retinal development.

PRIMARY NEURONS

LPA induced morphological changes, such as neurite retraction and growth cone collapse in several types of primary neurons [28,61,62]. Cultures enriched with post-mitotic young cortical neurons responded to LPA within 15 min with retraction of the neurites or lamella structures through Rho activation, resulting in retraction fiber caps (Fig. 2) [28]. These structures may represent growing axons, dendrites of differentiating neurons or leading processes of migrating neurons. LPA-induced retraction was transient, implicating that LPA may control the timing of outgrowth. As these neurons matured, LPA-induced retraction decreased while growth cone collapse, a process that occurs during neuronal migration or axonal pathfinding, became detectable. LPA-induced growth cone collapse was at least partially mediated through actin depolymerization via Ca^{2+} and α-actinin interactions [27,28]. These findings indicated

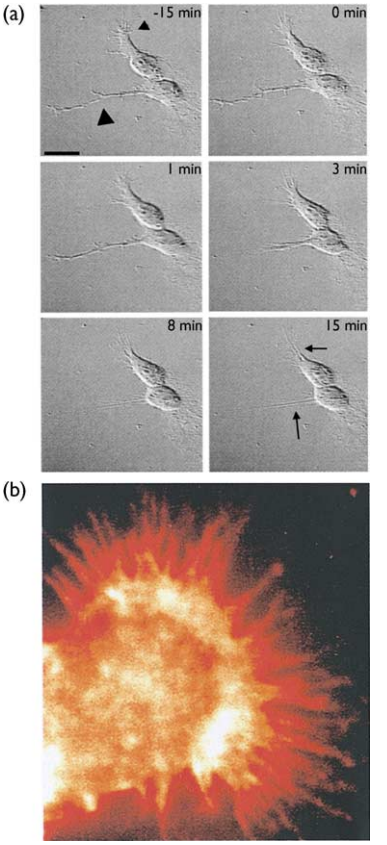

Fig. 2. LPA-induced Rho-dependent membrane retraction and formation of retraction fiber cap in isolated cortical cells. (**a**) Time-lapse recording of DIC images. E12 cortical cells were cultured for 24 h before vehicle treatment (−15 to 0 min) and subsequent 0.5 μM LPA treatment (0–15 min). Large arrowhead: process. Small arrowhead: extending membrane. Arrows: retraction fibers. Bar = 10 μm. (**b**) a retraction fiber cap labeled for f-actin. (Modified from [28]).

that the response of cortical neurons to LPA evolved during their neuronal maturation.

LPA-induced growth cone collapse was also observed in *Xenopus* retinal neurons and three types of primary cultured chick neurons: dorsal root ganglion neurons, retinal neurons, and sympathetic ganglion cells [61]. LPA-induced growth cone collapse in *Xenopus* retinal neurons was dependent on proteasome-mediated proteolysis [62], whereas that in chick root ganglion neurons was mediated through Rho-kinase and collapsin response mediator protein-2 (CRMP-2), a Rho-kinase substrate [63]. A recent study indicated that CRMP-2 inhibited neuronal phospholipase D_2 (PLD_2) activity by direct interactions [64]. Since PLD activation was required for LPA-induced actin cytoskeleton rearrangement [65,66], the inhibition of PLD_2 by CRMP-2 may indicate feedback regulation of LPA-induced growth cone collapse. Unlike cortical progenitors, which mainly express *lpa*$_1$ [14], the differentiating cortical neurons mainly express *lpa*$_2$ [28], suggesting that LPA_2 may mediate LPA-dependent neurite retraction and growth cone collapse, thereby playing a role in axonal growth and pathfinding.

Besides the effects on morphology, LPA induced cell death in hippocampal neurons but promoted cell survival in early post-mitotic neurons [67] (Kingsbury *et al.*, unpublished data). An increase in extracellular glutamate concentration and subsequent elevation of $[Ca^{2+}]_i$ may contribute to LPA-induced cell death in hippocampal neurons [68]. Oxidative stress, an increase of nitric oxide and a decrease in mitochondrial membrane potential could also lead to apoptosis through caspase activation [67]. In contrast, LPA was a survival factor for early post-mitotic neurons in the VZ, a region characterized by lpa_1 expression [14] (Kingsbury *et al.*, unpublished data). The opposite effects of LPA in neuronal cells imply that the effects of LPA are cell type-specific, which may be related to different expression profiles of the LPA receptors.

In addition to morphological changes and cell death in hippocampal neurons, LPA may modify NMDA receptor functions in these cells [69]. LPA treatment enhanced the NMDA-evoked currents (I_p) and blocked the phorbol-ester-induced potentiation of I_p in hippocampal neurons isolated from postnatal rats. These responses were abolished by pretreatment with chelerythrine (a selective PKC inhibitor), genistein or Src peptide (40–58) (tyrosine kinase inhibitors), suggesting that LPA receptors regulated NMDA receptor functions through a PKC-dependent activation of the non-receptor tyrosine kinase (e.g. Src) signaling cascade [69]. Since tyrosine phosphorylation is a key modulator of NMDA receptor activity [70–73], LPA may serve as a physiological regulator of NMDA receptor-mediated responses. This regulation may be involved in neuronal development associated with NMDA receptors [74–77].

In vivo studies demonstrated that LPA had nociception-producing activity on nociceptor endings of primary afferent neurons [78,79]. Intraplantar injection of LPA into the hind limb of mice induced a dose-dependent nociceptive flexor response, which was markedly blocked by the application of lpa_1 antisense oligodeoxynucleotide, revealing the involvement of LPA_1 in this process [78,79]. Two distinct pathways were proposed for mediating this LPA signaling. The first was the activation of PTX-sensitive $G_{i/o}$ proteins presynaptically with the subsequent release of substance P from nociceptor endings [78] while the second was the activation of PTX-insensitive G-proteins and H1-type histamine receptors [79]. These studies suggest that LPA and its receptor(s) are involved in peripheral pain transmission [79] (Table 1).

Additionally, LPA could play a role in modulating synaptic function. LPA inhibited Na^+,K^+-ATPase activity in rat cerebral cortical synaptosomes [80] and might be involved in synaptic vesicle formation [81]. Whether LPA receptors were involved in these processes remains unclear.

OLIGODENDROCYTES

The expression of lpa_1 in mature oligodendrocytes suggested a role of LPA in CNS myelination [24,82]. *In situ* hybridization revealed that the expression of lpa_1 in the postnatal brain was correlated temporally and spatially with myelination. Furthermore, the cells expressing lpa_1 were identified to be oligodendrocytes [24]. These cells started to express lpa_1 during the first postnatal week, showed a peak at postnatal day 18–21, and maintained a relatively high level of lpa_1 expression throughout adulthood [24]. Consistently, mature, myelin-forming oligodentrocytes, but not oligodendrocyte precursors, responded to LPA stimulation with an increase in $[Ca^{2+}]_i$, a process mediated by LPA receptor(s)-coupled PTX-sensitive G proteins [82]. In support of LPA_1-mediated LPA function in oligodendrocytes, *jimpy* mice were observed to have increased cell death of oligodendrocytes, and correspondingly, decreased expression of lpa_1 [8,24]. This observation suggest that LPA could be a survival factor for oligodendrocytes, as shown in Schwann cells [25].

SCHWANN CELLS

The involvement of LPA in the development and function of peripheral nervous system myelination was supported by the following observations. lpa_1 was highly expressed in SCs throughout sciatic nerve development [25,26], LPA-specific activity was detected in the conditioned medium of *in vitro* SC culture [26], and more directly, LPA promoted SC survival and regulated SC morphology and adhesion [23,25,26].

LPA was a potent survival factor for SCs [25]. LPA at maximal effective concentrations could reduce to ~50% teh level of apoptosis induced by serum withdrawal, and its efficacy was comparable to that of neuregulin-β, a potent peptide SC survival factor [25]. The effect of LPA on SC survival was at least partially mediated by LPA_1, since exogenous over-expression of LPA_1 in SCs significantly reduced apoptosis upon serum deprivation, while deletion of lpa_1 in mice resulted in an 80% increase of sciatic nerve SC apoptosis compared to wild-type mice [23,25]. The survival signal of LPA was mediated by PTX sensitive $G_{i/o}$/phosphoinositide 3-kinase (PI3 K)/Akt signaling pathways and possibly enhanced by the activation of PTX-insensitive Rho-dependent pathways [25,26] (reviewed in [12]).

LPA also induced actin cytoskeleton-based morphological changes in SCs [26]. LPA treatment caused f-actin rearrangements resulting in a wreath-like structure, with actin loops bundled peripherally by short orthogonal filaments. It also stimulated the formation of extensive cell–cell junctions containing N-cadherin, resulting in cell clustering [26]. Since SCs cultured from $lpa_1^{(-/-)}$ mice had a severe reduction in morphological responses to LPA, indicating that these changes were mediated by LPA receptor(s). These morphological changes were further mediated by the activation of PTX−insensitive G proteins and Rho pathways [26].

ASTROCYTES

The expression profiles of LPA receptor(s) in astrocytes were different *in vivo* and *in vitro* [24,83]. Lpa_1 was not detectable in astrocytes in the brain by *in situ* hybridization [24], whereas significant levels of lpa_1 were detected in cultured astrocytes by Northern analysis [83]. This discrepancy may suggest the induction of lpa_1 expression during astrocyte culture. It is not known whether lpa_2 and lpa_3 are also expressed in these cells.

Astrocytes responded to LPA with a rapid reversal of astrocyte stellation induced by serum withdrawal or

Table I. LPA signaling in cells related to the nervous system.

Cell type	LPA-induced effects	Signaling elements/pathways	References
Neuronal cell lines (NG108-15, NIE115, PC12, TR and TSM, Sy-SH5Y)			
	1. Growth cone collapse; neurite retraction; transient cell rounding;	1. LPA_1 and LPA_2/Rho; Ca^{2+} and α-actinin	[27,37,39–43,46,49,50,97]
	2. Tau phosphorylation;	2. Rho; GSK-3	
	3. Increases in $[Ca^{2+}]_i$ and dopamine release	3. LPA receptor(s)/IP_3	
	Neural cell line (C6 rat glioma)Morphological changes	PKC	[47]
	Neural cell line (Human GBM) cell migration	Rho/ROCK; $G_{i/o}$/MAPK	[48]
Cortical progenitors	1. Increases of Cl-/cation conductances;	1. Unknown	[14,22,23,55,21]
	2. Increase of cell proliferation;	2. $LPA_1/G_{i/o}$	
	3. Morphological changes	3. LPA_1/PTX-insensitive G proteins/Rho	
Embryonic chick retina	Increase in $[Ca^{2+}]_i$	PTX-sensitive/insensitive G proteins	[57]
Human retinal pigment epithelial (RPE) cells	1. Inward non-selective cation current;	1. LPA receptor(s)/PTX-sensitive G proteins	
	2. Outward K^+ current;	2. LPA receptor(s)/PTX-insensitive G proteins	
	3. Cell proliferation	3. LPA receptor(s)/PTX-sensitive G proteins	[58,59]
Cortical neurons	1. Neurite retraction and growth cone collapse;	1. Rho; Ca^{2+} and α-actinin	
	2. Survival	2. LPA_1 and LPA_2?	[28]; Kingsbury *et al.*, unpublished data
Hippocampal neurons	1. Cell death;	1. Glutamate; $[Ca^{2+}]_i$; oxidative stress; nitric oxide; mitochondrial membrane potential; caspases	[67–69,98]
	2. Modification the functions of NMDA receptors	2. LPA receptors/NMDA receptors; PKC-dependent activation of non-receptor tyrosine kinase signaling cascade	
Xenopus retinal neurons	Growth cone collapse	Proteasome-mediated proteolysis	[62]
Primary chick neurons	Growth cone collapse	Rho/Rho-kinase/CRMP-2 (DRG neurons)	[61,63,99]
Sensory neurons	Nociception	LPA_1; PTX-sensitve/substance P receptor; PTX-insensitive G proteins/H1-type histamine receptor	[78,79]
Cortical synaptosomes/synaptic vesicle	1. Inhibition of Na^+, K^+-ATPase activity;	Unknown	[80,81]
	2. Synaptic vesicle formation		
Oligodendrocytes	Increase in $[Ca^{2+}]$i	LPA receptor(s)/PTX-sensitive G proteins	[82]
Schwann cells	1. Survival;	1. $LPA_1/G_{i/o}$/PI3K/Akt; LPA_1/PTX-insensitive G proteins/Rho	
	2. Morphological changes	2. LPA_1/PTX-insensitive G proteins/Rho	[25,26]
Astrocytes	1. Reversal of stellate morphology;	1. Rho; phosphorylation of RLC	[20,36,83–89]
	2. Increases in $[Ca^{2+}]_i$;	2. PTX-sensitive/insensitive G proteins	
	3. Cell proliferation;	3. PTX-sensitive G proteins/ $[Ca^{2+}]_i$/ROS/DNA synthesis	
	4. Inhibition of glutamate and glucose uptake	4. PTX-insensitive G proteins	
Mouse microglia	1. Increase of $[Ca^{2+}]_i$;	1. LPA_1?/PTX-sensitive G proteins	
	2. Increase of metabolic activity/cell proliferation	2. LPA_1	[91]
Brain endothelial cells	1. Increases of tight junction permeability and production of endothelin-1;	1. PKC	[34,92,93]
	2. Vasoconstriction	2. PTX-sensitive G proteins	

GSK-3: glycogen synthase kinase-3. CRMP-2: collapsin response mediator protein-2. DRG: dorsal root ganglion. PI3K: phosphoinositide 3-kinase. ERK: extracellular signal-regulated kinase. GBM: glioblastoma multiforme. RLC: regulation light chain. ROS: reactive oxygen species. IP_3: inositol triphosphate.

intracellular cAMP elevation [36,84,85]. This reversal resulted from the activation of PTX-insensitive Rho pathways and consequent assembly of stress fibers and focal adhesions [36,84,85]. LPA-induced stabilization of stress fibers was accompanied by increases in phosphorylation of the RLC of myosin, suggesting that LPA-induced signaling cascades in astrocytes can regulate actin/myosin interactions [36].

Astrocytes also responded to LPA with an increase in $[Ca^{2+}]_i$ [83,84,86]. In astrocytes from neonatal rat brains, the Ca^{2+}-mobilizing effect of LPA was PTX sensitive [86], while in astrocytes from embryonic rat brains, both PTX-sensitive and -insensitive G proteins were involved [83]. This difference may reflect the involvement of varying signaling cascades at different stages of astrocyte development. LPA-induced Ca^{2+} response may also vary depending on culture conditions.

LPA also induced astrocyte proliferation [83,86,87]. LPA-stimulated mitogenesis, a process mediated by PTX-sensitive G proteins, was closely associated with increases in $[Ca^{2+}]_i$, production of reactive oxygen species (ROS), and DNA synthesis [86,88]. It was thus suggested that the activation of PTX-sensitive G proteins could lead to an increase in $[Ca^{2+}]_i$, which may result in an increase in ROS that then led to an increase in DNA synthesis [86]. However, some other studies indicated that LPA had no effect on astrocyte cell proliferation [50,89].

Moreover, LPA inhibited glutamate and glucose uptake by astrocytes through PTX-insensitive pathways [86,88]. The impairment of glutamate uptake could result in an increase of extracellular glutamate, thus leading to neuronal cell death [90]. Since the brain is a rich source of LPA and LPA levels can significantly increase following brain trauma [33–37], LPA may exacerbate excitotoxic processes in various neurodegenerative conditions [88].

MICROGLIA

Two types of LPA-induced responses were detected in microglia; increases in $[Ca^{2+}]_i$ and promotion of metabolic activity/cell proliferation, a common feature of activated microglial cells [91]. Microglia are phagocytes that reside in the CNS and are mobilized after injury, infection, or disease. Hence, LPA-mediated microglial activation might play an important role in response to brain damage, especially hemorrhagic brain injury. It was observed that there was species specificity in LPA-induced cellular responses in microglia. LPA increased $[Ca^{2+}]_i$ in mouse microglia through the release of Ca^{2+} from intracellular stores, and through Ca^{2+} influx only at high LPA concentrations ($\sim 3\,\mu M$), whereas increases in rat microglia were primarily through Ca^{2+} influx [91]. In addition, LPA-induced Ca^{2+} mobilization in mouse microglia was completely blocked by PTX pretreatment whereas that in rat microglia did not respond to PTX pretreatment [91]. An LPA-induced increase in metabolic activity/cell proliferation was detected in mouse, but not rat microglia [91]. This discrepancy may reflect the different roles of LPA receptors in mediating the cellular effects of LPA; mouse microglia predominantly expressed lpa_1 while rat microglia predominantly expressed lpa_3 [91]. However, one study reported that LPA-induced Ca^{2+} mobilization was not observed in rat microglia, despite a low level expression of lpa_1 [83].

BRAIN ENDOTHELIAL CELLS AND CEREBROVASCULAR BED

LPA treatment increased tight junction permeability of the BBB [92]. The BBB is mainly formed by brain capillary endothelial cells, which are coupled with a continuous belt of complex high electrical-resistance tight junctions. LPA caused a rapid, reversible, and dose-dependent decrease in transcellular electrical resistance in cultured brain endothelial cells, as well as an increased paracellular flux of sucrose. These effects were related to increased tight junction permeability of the BBB and were attenuated by the activation of PKC [92]. LPA also stimulated the formation of stress fibers, the recruitment of focal adhesion contacts, and the appearance of tyrosine phosphorylated protein at focal contacts in these cells [92].

LPA also caused a dose-dependent vasoconstriction that was shown to be PTX-sensitive in piglet cerebrovascular bed [34]. The vasoconstriction may result from LPA-stimulated production of endothelin-1 (ET-1) [93]. ET-1 is a peptide released from the endothelial cells and is a potent vasoconstrictor that has been implicated in the pathogenesis of stroke, hypertension, cerebral hemorrhage, and atherosclerosis. Altered vascular reactivity is often found in posthemorrhagic conditions, suggesting that LPA might play a role in these pathophysiological states [34,93]. The frontal hematomas observed in $lpa_1^{(-/-)}$ and $lpa_1^{(-/-)}lpa_2^{(-/-)}$ embryos may reflect defects in LPA-induced endothelial dysfunction and/or altered vascular reactivity [23,94–96]. An intriguing concept is that LPA may be a critical regulator of BBB permeability and/or vascular reactivity under both physiological as well as pathophysiological conditions.

CLOSING REMARKS

The LPA-induced effects reviewed above strongly implicate the involvement of receptor-mediated LPA signaling in the nervous system. LPA potentially has physiological functions in many aspects of the nervous system, such as neurogenesis, neuronal migration, myelination, vision development, and neurotransmitter transmission. LPA could also be involved in pathological processes in the nervous system, such as tumor cell invasion, aggravation of neurodegenerative conditions, peripheral pain transmission, and cerebral hemorrhage. Further investigations, such as those using LPA receptor(s)-deficient mice, will both add important details and identify new processes relevant to the physiological, pathological and therapeutic roles of LPA in the nervous system.

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