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LPA in Neural Cell Development

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Abstract Lysophosphatidic acid (LPA) elicits diverse cellular responses through cell surface LPA receptors in nervous system-derived cells and cell lines. The developing nervous system is one of the major loci for LPA receptor expression. Recent studies have also revealed that metabolic pathways of LPA are present in the nervous system. A growing body of literature suggests a crucial role for LPA in neuronal development processes, including neurogenesis, neuronal migration, neuritogenesis, and myelination. J. Cell. Biochem. 92: 993–1003, 2004. © 2004 Wiley-Liss, Inc.

Key words: LPA; neuron; glia; development

Lysophosphatidic acid (LPA) has been shown to produce many cellular and biochemical responses in a range of different nervous system-derived cells [Moolenaar, 1995; Moolenaar et al., 1997]. These include Ca^{2+} mobilization, stimulation of cell proliferation, inhibition of cell survival, changes in cell morphology and ion conductance, and activation of various signaling cascades, all of which are also observed in non-neural cells. LPA-evoked responses are primarily mediated by cell surface G protein-coupled receptors. The LPA receptor family currently consists of four subtypes, lpa_{1-4}, although lpa_4 is molecularly distinct from the other three subtypes and closer to platelet-activating factor receptor [Fukushima et al., 2001; Noguchi et al., 2003]. LPA_1 and LPA_2 utilize similar intracellular signaling pathways, pertussis toxin (PTX)-sensitive $G_{i/o}$, and PTX-insensitive $G_{q/11/14}$ and $G_{12/13}$, while LPA_3 stimulates $G_{i/o}$ and $G_{q/11/14}$, but probably not $G_{12/13}$, in mediating the diverse cellular effects of LPA [Ishii et al., 2000]. The coupling profile of LPA_4 to G proteins is still unknown.

The nervous system is a major locus of LPA receptor (lpa_{1-3}) expression [Contos et al., 2000b; Fukushima et al., 2001]. The expression profiles are correlated with many developmental processes, including neurogenesis, neuronal migration, neuritogenesis, and myelination [Chun, 1999; Fukushima and Chun, 2001; Fukushima et al., 2001]. LPA-induced cellular responses are relevant to the function and development of the nervous system. Consistent with being an endogenous signaling factor, LPA and its metabolic pathways are present in the brain [Lee et al., 1996; Kai et al., 1997; Hooks et al., 1998; Sugiura et al., 1999]. Extracellular LPA has also been shown to be produced by cultured postmitotic neurons and Schwann cells (SCs) [Fukushima et al., 2000; Weiner et al., 2001]. Here, I review the recent progress in elucidating the cellular and biochemical effects of LPA and the intracellular signaling mechanisms in different neural cell types, and discuss the potential physiological and pathological roles of LPA signaling in the nervous system.

HISTORY OF LPA IN THE NERVOUS SYSTEM

The first indication of LPA signaling in the nervous system came from pioneering studies using neural cell lines, including N1E-115, NG108-15, and PC12 cells [Tigyi and Miledi, 1992; Jalink et al., 1993]. These peripheral nervous system-derived cells were shown to

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extend processes under serum-free conditions or nerve growth factor treatment, and respond to LPA with a rapid retraction of their processes, resulting in cell rounding mediated by rearrangement of the actin cytoskeleton. These morphological changes were reminiscent of the process of neuronal degeneration. Other interesting findings were that LPA was produced by activated platelets and was present in serum at approximately micromolar concentrations [Eichholtz et al., 1993], and that its levels could be increased following insults to the brain [Das and Hajra, 1989; Tigyi et al., 1995]. Taken together, these observations suggested the possibility that LPA may directly cause neuronal degeneration under pathological conditions where the blood–brain barrier is damaged and serum components leak into the brain [Moolenaar, 1995; Moolenaar et al., 1997]. In contrast, the physiological roles of LPA signaling remained unclear, although neuronal morphological changes were also features observed in the developing brain. This is primarily because LPA metabolism and receptor-mediated cell activation mechanisms had not been clarified in organs including the brain.

In 1996, the first LPA receptor gene, lpa_1, was identified and shown to be expressed in the neurogenic region of the developing cerebral cortex [Hecht et al., 1996]. This was followed by the demonstration that two other LPA receptor genes, lpa_2 and lpa_3, were also expressed in pre- and post-natal brains [Chun, 1999; Fukushima et al., 2001]. Recent important progress has included the identification of two enzymes involved in LPA metabolism and their presence in the brain: lysophospholipase D (lysoPLD), an LPA-producing enzyme, and lipid phosphate phosphohydrolase (LPP), an LPA-degrading enzyme. LysoPLD was shown to be identical to autotaxin, an enzyme having pyrophosphatase and phosphodiesterase activities [Lee et al., 1996; Umezu-Goto et al., 2002]. This enzyme is associated with or secreted from plasma membranes, and can effectively produce more LPA from lysophosphatidylcholine by means of its ecto-lipase activity. LPP is an integral membrane protein with six potential transmembrane domains and catalytic sites within the extracellular regions that dephosphohydrolase extracellular LPA [Brindley and Waggoner, 1998]. The LPP family primarily consists of three isoforms (LPP1-3) and is expressed in brain [Kai et al., 1997; Hooks et al., 1998].

CELLULAR EFFECTS OF LPA ON NEURAL CELLS

Neuronal Cell Lines

As described above, many neuronal cell lines respond to LPA with cell shape changes accompanied by rearrangement of the actin cytoskeleton. Studies on these cell lines have provided many important observations regarding the signaling mechanisms through which LPA-induced neuritic process retraction occurs (Fig. 1). LPA_1 and LPA_2, but not LPA_3, can mediate LPA-induced process retraction when overexpressed in neuronal cells [Ishii et al., 2000]. The intracellular signaling pathways for neurite retraction involve G_{12} family proteins, the small GTPase Rho and Rho kinase. Although both G_{12} and G_{13} are members of the G_{12} family and mediate similar biological responses, G_{13} is likely to be a signal amplifier for LPA receptors in fibroblasts, and also perhaps in neurons [Gohla et al., 1998; Sayas et al., 2002]. However, the identity of the LPA receptor subtype coupled to G_{13} remains to be determined.

G_{13} activation is subsequently transmitted to the Rho pathway via the action of the GDP/GTP exchange factor specific for Rho, RhoGEF [Kozasa et al., 1998]. Many types of RhoGEF have been identified as molecules with additional amino acid sequence motifs, such as the pleckstrin homology (PH), dbl homology (DH), and/or PDZ [Sah et al., 2000] domains. The presence of these domains suggests that, in addition to the G_{13}-Rho interaction, other types of protein interactions are present in the LPA-activated intracellular signaling cascade.

Rho stimulation and the subsequent Rho kinase activation lead to the phosphorylation of the myosin light chain (MLC) and myosin phosphatase, which removes phosphate residues from phosphorylated MLC [Amano et al., 1996; Kimura et al., 1996]. Since phosphorylated myosin phosphatase is inactive, the Rho pathway activation results in the accumulation of phosphorylated myosin (possibly mainly myosin II) [Amano et al., 1998], and consequently increases actin polymerization and cell contraction. Although this signaling paradigm has been established in fibroblasts, it has also been shown to fit well with LPA-induced neurite retraction. However, the LPA-induced phenotype of fibroblasts involves the formation of stress fibers consisting of polymerized actin,

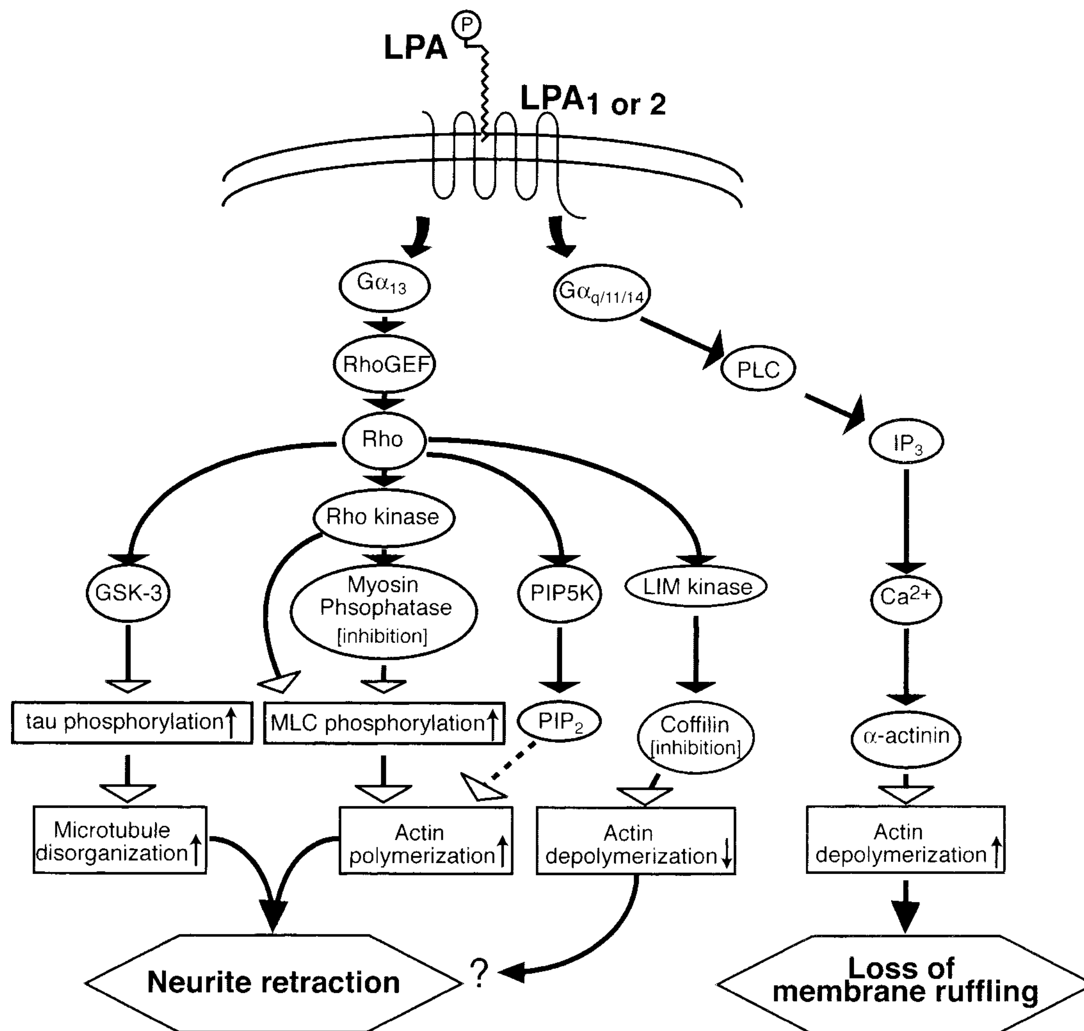

Fig. 1. Possible intracellular signaling pathways for cytoskeletal rearrangement in neuronal cells. LPA_1 and LPA_2 activate the G_{13}-Rho pathway leading to actin polymerization, which is affected by the PIP5K pathway branch of the Rho pathways. Another branch, the GSK-3-mediated pathway, regulates microtubule rearrangement. These cooperative cytoskeletal rearrangements produce neurite retraction. The role of LIM kinase signaling in neurite retraction is unknown. On the other hand, the Ca^{2+}-alpha-actinin route, perhaps downstream of G_q, stimulates actin depolymerization, which contributes to the loss of membrane ruffling.

quite distinct from that of neuronal cells that have few stress fibers and remarkable cortical actin at rounding. This might be due to different interactions between actin and other cytoskeletal-associated proteins, such as focal adhesion proteins.

Other lines of evidence have also demonstrated the involvement of distinct kinases, including glycogen synthase kinase-3 (GSK-3), phosphatidylinositol 4-phosphate 5-kinase (PIP5K), LIM kinase (Fig. 1), and unknown tyrosine kinases. LPA induces GSK-3 activation in a Rho-dependent manner, leading to hyperphosphorylation of the microtubule-associated protein tau in human neuroblastoma and cerebellar granule neurons [Sayas et al., 1999, 2002]. This phosphorylation of tau is thought to induce microtubule disorganization, and thereby cause neurite retraction.

Involvement of PIP5K in the LPA-induced neurite retraction has been shown by using a mutant PIP5K lacking its kinase activity [van Horck et al., 2002; Yamazaki et al., 2002]. Overexpression of this mutant in N1E-115 cells promotes neurite outgrowth that shows no retraction response to LPA, while the introduction of active PIP5K results in cell rounding that is not inhibited by a Rho kinase inhibitor, Y27632. Therefore, PIP5K and its product, phosphatidylinositol 4,5-bisphosphate (PIP_2),

appear to act downstream of Rho kinase during LPA-induced neurite retraction. From the view of the regulation of actin rearrangement, this idea is rational because PIP_2 is a key molecule that regulates actin rearrangement through binding to actin-associated proteins. Another target of Rho kinase is LIM kinase. Rho kinase activates LIM kinase, which in turn phosphorylates coffilin, an actin-depolymerizing protein, and inhibits its activity during LPA-induced neurite retraction in N1E-115 cells [Maekawa et al., 1999]. The mechanism for how this inhibition coordinates actomyosin-derived contractility is still unclear.

It has long been known that tyrosine kinases play an important role upstream and/or downstream of Rho in LPA-induced cytoskeletal changes [Moolenaar, 1995; Moolenaar et al., 1997; Kranenburg et al., 1999; Sayas et al., 1999]. Pharmacological approaches using fibroblasts have implicated epidermal growth factor receptor in LPA-induced stress fiber formation [Gohla et al., 1998]. However, molecular identification of the kinase(s) involved and its substrate in neuronal cells remains to be accomplished.

Our recent research has identified an earlier temporal response in the LPA-responsive immortalized cortical neuroblast cell lines, TR and TSM. These cells express lpa_1 and lpa_2 and show process retraction responses to LPA exposure through Rho activation and actomyosin interactions [Hecht et al., 1996; Ishii et al., 2000]. Prior to the process retraction, LPA induces loss of membrane ruffling, concomitant with Ca^{2+} mobilization, in growth cone-like structures at the tips of the processes [Fukushima et al., 2002b]. This event involves actin depolymerization, independent of Rho kinase activation, and requires interaction between Ca^{2+} and alpha-actinin, an actin-cross-linking protein, since a mutant of alpha-actinin lacking Ca^{2+} sensitivity attenuates the LPA-induced loss of membrane ruffling when expressed in TR cells (Fig. 1). These cellular phenomena may reflect collapse and retraction of the basal processes of neuroblasts, or leading processes or axons of immature neurons that are perhaps involved in the proper reorganization of the actin cytoskeleton following LPA exposure.

Neuroblasts

A neurogenic region of the mammalian cerebral cortex consists of neuroblasts, neural progenitors that predominantly express lpa_1 correlated with neurogenesis. We have proposed that LPA signaling between neuroblasts and neurons, potential LPA-producing cells, may play an important role in the regulation of neuroblast morphology and growth, and thus neurogenesis [Fukushima et al., 2001]. Further details are described in the section by Kingsbury et al. [2003] in this issue, together with their recent observations.

Neurons

Developing neurons generated from neuroblasts are highly active in their motility and morphology. They migrate to their final destination by using their leading processes. Thereafter, they commence to extend axons to make synaptic connections through the exploring and navigating actions of growth cones. These changes in the cell dynamics require intrinsic and extrinsic signals for pathfinding of the leading processes and/or growing neurites. Many attractive and repellant factors have been proposed as potential extrinsic signals in different systems [Culotti and Merz, 1998; Song and Poo, 1999; Brose and Tessier-Lavigne, 2000]. Repellant signals influence the direction and/or timing of the extension of the growth cones or growing tips of neurites, by inducing collapse or turning their structures. LPA-induced cellular effects on neuronal cell lines have implicated LPA as a repellant signal.

lpa_2 is expressed in the cortical plate which mainly consists of differentiating neurons [Fukushima et al., 2002b; McGiffert et al., 2002]. We have examined how LPA influences young differentiating cortical neurons. Time-lapse analyses have shown that LPA rapidly induces neurite retraction through activation of the Rho pathway [Fukushima et al., 2002b], as previously noted in neuronal cell lines. The maximal response is observed 15 min after LPA exposure. This retraction response is always accompanied by the formation of fine retraction fibers (Fig. 2), which are hardly observed in neuronal cell lines. At 20 min after LPA exposure, neurons begin to reextend neurites by growth of membranes and cytoplasm from the base of the retraction fibers (i.e., the base of the original neurites) toward the top of the fibers (Fig. 2). These findings suggest that LPA-induced neurite retraction is temporary and that LPA may refine neurite shape and/or regulate the timing of neurite outgrowth.

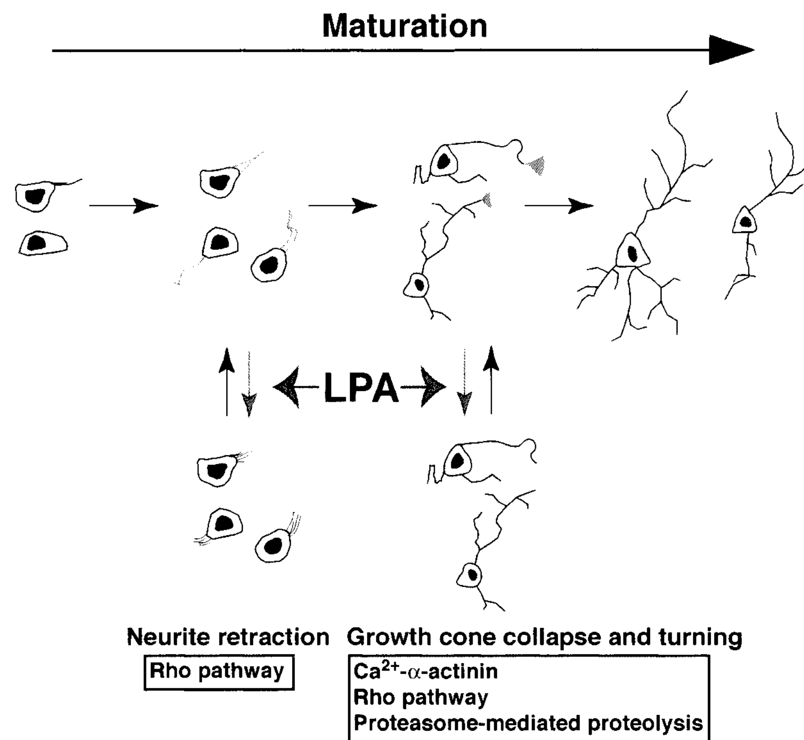

Fig. 2. Potential roles of LPA in developing postmitotic neurons. Young postmitotic neurons undergoing neuritogenesis show neurite retraction and cell rounding in response to LPA, while older neurons show growth cone collapse or turning. These responses are transient and reversible.

As neuronal maturation progresses, retraction responses are decreased whereas growth cone collapse is observed in a certain population of cortical neurons following LPA exposure (Fig. 2) [Fukushima et al., 2002a,b]. This second response is inhibited by overexpression of a Ca^{2+}-insensitive alpha-actinin mutant. This suggests interactions between Ca^{2+} and alpha-actinin in the LPA-induced growth cone collapse, as observed at the growing tips of neuroblast cell lines. Therefore, with neuronal maturation, primary cortical neurons probably show different cellular responses by activating distinct signaling pathways in response to LPA.

Recent reports have also shown that other mechanisms are involved in the actions of LPA on growth cone dynamics. A low concentration of LPA can induce repulsive turning, not collapse, of the extending growth cone of *Xenopus* spinal cord neurons [Yuan et al., 2003]. This response utilizes Rho and Rho kinase-mediated signaling. A study based on observation of the cytoskeleton has revealed a novel type of actin regulation by LPA in the growth cones of *Aplysia* neurons [Zhang et al., 2003]. Growth cones contain two types of actin-based structures: an actin arc present in the T (transition) zone and moving into the C (central) domain interacting with microtubules, and actin bundles in filopodia in the P (peripheral) domain affecting rearward transport of microtubules. LPA increases the actin arc density and its centripetal movement into the C domain, contributing to the retraction and collapse. This process also uses the Rho pathways. Another mechanism is ubiquitin-dependent proteolysis via proteasomes during LPA-induced growth cone collapse in retinal neurons [Campbell and Holt, 2001, 2003]. This involves the apoptotic pathway including p38 kinase and caspase-3. These observations indicate an intriguing relationship between cytoskeletal rearrangement and protein degradation in growth cone collapse and turning.

Besides the effects on cell morphology, LPA induces other cellular effects relevant to neural cell development: enhancement of *N*-methyl-D-aspartate (NMDA)—evoked currents and stimulation of neuronal apoptosis or survival [Holtsberg et al., 1998; Lu et al., 1999]. Regulation of NMDA signaling by LPA seems to be mediated through tyrosine kinase activation

and may be involved in neuronal development associated with NMDA receptors [Scheetz and Constantine-Paton, 1994]. LPA-induced stimulation of apoptosis in hippocampal neurons likely involves Ca^{2+} mobilization. However, recent reports have demonstrated the opposite effects of LPA on neuronal survival. LPA and cyclic phosphatidic acid (cPA), an LPA-like lipid mediator present in the brain, have been shown to elicit neurotrophin-like effects, including cell survival and neurite-promoting effects in cultured hippocampal neurons [Fujiwara et al., 2003]. A study using an ex vivo culture system has also shown that LPA promotes cell survival of early postmitotic, cortical neurons [Kingsbury et al., 2003]. The apoptotic effects of LPA are observed in neurons cultured for 7–9 days, while the survival effects are seen in neurons cultured for 1–2 days. Moreover, the survival effects of LPA and cPA in hippocampal neurons are detectable in a narrow range of cell densities in culture, similar to that of neurotrophins [Fujiwara et al., 2003]. Thus, the effects of LPA on neuronal survival might be dependent on cell maturation and/or cell density.

As mentioned above, evidence is accumulating regarding regulation of intracellular pathways and cytoskeletal dynamics by LPA in developing neurons. The results obtained are similar to those observed with neuronal cell lines. For example, utilization of the Rho pathways in neurite retraction is common between primary neurons and neuronal cell lines. However, retraction fiber formation, which was unexpected from the studies with neuronal cell lines, has been observed in developing neurons and the underlying mechanisms involving cytoskeletal rearrangement are currently unknown. Thus, how the signaling mechanisms illustrated by studies using cell lines (Fig. 1) explain the effects of LPA in developing neurons remains to be fully elucidated. Furthermore, the LPA receptor subtype involved in these actions of LPA has not been determined. Together with a role for LPA-metabolizing enzymes in the developing brain, these issues need to be fully addressed in the future.

Oligodendrocytes

Oligodendrocytes, the myelin-forming cells, are the second locus of lpa_1 expression in the central nervous system. The lpa_1 expression profile in oligodendrocytes is correlated with maturation and myelination in the brain [Allard et al., 1998; Weiner et al., 1998]. Consistent with this finding, in vitro studies have revealed that mature oligodendrocytes, but not oligodendrocyte precursors, express lpa_1 and respond to LPA stimulation with an increase in the intracellular Ca^{2+} concentration [Moller et al., 1999]. This signal is generated by Ca^{2+} influx via the plasma membrane through PTX-sensitive G-proteins.

From the analogy between oligodendrocytes and SCs, the myelinating cells of the peripheral nervous system, LPA signaling may play an important role in oligodendrocyte survival. SCs respond to LPA by increased cell survival (see below) [Weiner and Chun, 1999]. This survival effect of LPA is mediated through the PI3K-Akt pathway. Oligodendrocytes have been shown to utilize the same intracellular signaling pathway for their survival in response to extracellular stimuli [Vemuri and McMorris, 1996]. The jimpy mouse, a mutant mouse with increased oligodendrocyte apoptosis and decreased lpa_1 expression, may provide a hint for understanding the in vivo role of LPA signaling in oligodendrocyte survival [Weiner et al., 1998].

However, contradictory results have been recently reported. These demonstrate that oligodendrocyte precursors express lpa_1, that oligodendrocytes show only a restricted response to LPA (phosphorylation of MAP kinase but not the Ca^{2+} response and survival), and that the Ca^{2+} responses are PTX-insensitive [Stankoff et al., 2002; Dawson et al., 2003; Yu et al., 2004]. Thus, the cellular effects of LPA in oligodendrocytes and their precursors are still unclear. However, from a developmental viewpoint, it is possible that LPA signaling may directly or indirectly affect oligodendrocyte differentiation as well as neurogenesis; based on the expression of lpa_1 in neuroblasts which are also potential glial progenitors. Further investigation is awaited for a full understanding of the physiological roles of LPA signaling in oligodendrocyte development.

Astrocytes

Astrocytes show no lpa_1 expression in vivo [Weiner et al., 1998], but express significant levels of lpa_{1-3} in culture when prepared from the cerebral cortex [Tabuchi et al., 2000; Rao et al., 2003; Sorensen et al., 2003]. In cultured astrocytes, LPA can induce various biochemical and cellular responses. The LPA-evoked biochemical responses are inhibition of forskolin-

stimulated cAMP production, ERK activation, Ca^{2+} mobilization, arachidonic acid production, phosphoinositide metabolism, and serum-responsive element (SRE)-mediated transcription [Manning and Sontheimer, 1997; Pebay et al., 1999; Tabuchi et al., 2000; Rao et al., 2003; Sorensen et al., 2003]. All of these responses are consistent with previous results obtained using cells heterologously expressing each LPA receptor subtype, or fibroblasts from mice lacking lpa_1 and lpa_2 [Fukushima et al., 1998; Ishii et al., 2000; Contos et al., 2002].

The LPA-induced cellular responses include morphological changes, stimulation of cell proliferation and inhibition of glucose and glutamate uptake. LPA stimulates the transition of astrocyte morphology from a stellate shape to a flat shape [Manning and Sontheimer, 1997; Suidan et al., 1997; Manning et al., 1998]. The stellate shape of astrocytes is induced by dibutyryl cAMP treatment and reversed by LPA treatment. This morphological reversal requires the activation of a Rho pathway, but not a G_i nor G_q pathway [Manning and Sontheimer, 1997; Suidan et al., 1997; Manning et al., 1998]. Although LPA-stimulated cell proliferation has been demonstrated in many reports, there are also contradictory results regarding the signaling pathways utilized and the responses. For instance, the PTX-sensitivity of LPA-induced DNA synthesis differs between studies [Keller et al., 1997; Tabuchi et al., 2000; Sorensen et al., 2003]. Another example is that striatal astrocytes fail to respond to LPA by stimulation of DNA synthesis [Pebay et al., 1999]. Some of these discrepancies may be due to differences in the origins, species, developmental stages and/or culture conditions of the astrocytes. Inhibition of glutamate and glucose uptake by LPA is mediated through PTX-insensitive pathways [Keller et al., 1996, 1997]. The impairment of glutamate uptake could result in an increase in the extracellular glutamate level, which might exert neuronal toxicity [Huang et al., 1997].

Although neural cells seem to be one of the sources of brain LPA, platelets are known to produce high levels of LPA in serum. If platelet-derived LPA penetrates into the brain due to impairment of the blood–brain barrier following brain injury [Eichholtz et al., 1993; Tigyi et al., 1995], LPA-induced astrocyte responses, such as glutamate uptake inhibition and gliosis, may induce neuronal cell death or accelerate neurodegeneration triggered by other stimuli. Interestingly, injection of LPA into the striatum of adult mice induces astrogliosis, reflecting astrocyte proliferation, and is responsible for the formation of glial scars that resemble those observed in neurodegenerative diseases [Sorensen et al., 2003]. Therefore, LPA-related signaling, including LPA receptors, could be a drug target in various brain diseases.

Microglial Cells

Microglia show species differences in LPA receptor expression and Ca^{2+}-mobilizing machinery [Moller et al., 2001]. In culture, mouse microglia predominantly express lpa_1, while rat microglia predominantly express lpa_3. LPA can induce Ca^{2+} mobilization in both types of microglial cells. However, mouse microglial responses appear to originate from intracellular Ca^{2+} stores, whereas those in rat microglia are primarily attributable to the influx of extracellular Ca^{2+}. LPA also stimulates proliferation activity, a response related to microglial activation, in mouse but not rat microglial cells [Moller et al., 2001]. Whether Ca^{2+} mobilization is related to proliferation remains to be determined. These different profiles for the expressions of LPA receptor subtypes and cellular responses suggest different roles for LPA receptor subtypes in microglial cells originating from different species.

Two possibilities have been raised for roles of activated microglia in neurodegenerative diseases: the removal of degenerated myelin and dead neurons by phagocytosis, and the production of reactive oxygen species and/or cytokines that stimulate neuronal cell death [Hickey, 1999; Schubert et al., 2001]. Whether microglia activated by LPA are involved in these processes remains to be determined.

SCs

LPA has been shown to be a potent survival factor for rat SCs, that predominantly express lpa_1 [Weiner and Chun, 1999]. LPA can prevent SCs from undergoing apoptotic cell death after serum withdrawal. Its potency was comparable to that of neuregulin-β, a potent peptidergic growth factor for SC survival and proliferation. This survival effect of LPA is mediated by LPA_1 and PTX-sensitive $G_{i/o}$/PI3K/Akt signaling pathways and possibly enhanced by activation of PTX-insensitive Rho-dependent pathways [Weiner and Chun, 1999; Weiner et al., 2001].

Consistent with these data, an increase in sciatic nerve SC apoptosis is observed in $lpa_1^{(-/-)}$ mice compared to wild type mice [Contos et al., 2000a].

LPA also induces morphological changes in cultured SCs [Weiner et al., 2001]; SCs have a spindle shape and LPA treatment results in cell rounding. These morphological changes are accompanied by rearrangement of the actin cytoskeleton; from bundles of filamentous actin arranged parallel to the cell axis to a "wreath"-like structure with actin loops devoid of a nucleus and bundled peripherally by short orthogonal filaments. The assembly of focal adhesions that precedes wreath formation is also observed at the outer edge of the actin filaments. Both responses require Rho and Rho kinase activation. LPA also stimulates the formation of extensive cell–cell junctions containing complexes of N-cadherin and ß-catenin, resulting in Ca^{2+}-dependent cell clustering. These responses are mediated by LPA receptor(s) since SCs cultured from $lpa_1^{(-/-)}$ mice show a severe reduction in the morphological responses to LPA.

SC precursors migrate along developing nerves between embryonic day 12 (E12) and E15, when lpa_1 expression can already be detected [Weiner and Chun, 1999; Weiner et al., 2001], after which they differentiate into mature SCs that contact neuronal axons and myelinate them. During this period, SCs undergo changes in both their adhesive properties and morphology. The cellular effects of LPA on SC survival, morphology, and adhesion might be biologically relevant to SC development. However, $lpa_1^{(-/-)}$ and $lpa_1^{(-/-)}/lpa_2^{(-/-)}$ mice show no obvious defects in peripheral nerves [Contos et al., 2000a, 2002], while mice lacking neuregulin receptors show loss of SC precursors and peripheral neurons [Adlkofer and Lai, 2000]. Because the survival effect of LPA appears to be closely associated with cell-substratum adhesion [Weiner et al., 2001], LPA may play a regulatory role in SC survival that is maintained by other signals, such as neuregulins. This might explain the relatively low levels of SC apoptosis in $lpa_1^{(-/-)}$ mice. Alternatively, LPA may play a different, as yet unidentified, role in SC development in $lpa_1^{(-/-)}$ and $lpa_1^{(-/-)}/lpa_2^{(-/-)}$ mice. To better understand the biology of LPA in SCs, the combination of two types of analysis would be necessary: loss-of-function (i.e., analyses of receptor knockout mice) and gain-of-function (analyses of receptor overexpression or activation) analyses, as performed by Kingsbury et al. [2003].

LPA METABOLISM IN THE NERVOUS SYSTEM

For a more comprehensive understanding of LPA signaling in the nervous system, the source of LPA and its metabolic pathway need to be determined. Studies on non-nervous systems have suggested that the cellular sources of LPA include activated platelets and adipocytes [Eichholtz et al., 1993; Valet et al., 1998]. Although LPA from these non-neuronal cells may enter the nervous system under physiological and pathological conditions, based on several lines of evidence, LPA in the nervous system appears to originate from cells intrinsic to the nervous system. First, functionally active LPA is present in adult brain tissues [Das and Hajra, 1989; Sugiura et al., 1999]. Second, active LPA is detected in the conditioned medium of neuron-enriched cultures or SC cultures [Fukushima et al., 2000; Weiner et al., 2001]. Third, developing brains express lysoPLD and other potential LPA-producing enzymes, such as phospholipases A_1 and A_2 [Thomson and Clark, 1995; Lee et al., 1996; Higgs et al., 1998].

LPPs are very likely to be important for the degradation of signaling LPA and in this role may regulate LPA receptor-mediated cellular responses. All LPP isoforms have been demonstrated to be present in the brain [Kai et al., 1997; Hooks et al., 1998]. A recent study identified *PRG-1* as a plasticity-related gene facilitating axonal development and regeneration, and interestingly demonstrated that this is a member of the LPP family [Brauer et al., 2003]. However, *PRG-1* and its related genes (*PRG2-4*) lack the conserved phosphatase domains that are present in not only LPPs but also other types of phosphatases [Brindley and Waggoner, 1998; Brauer et al., 2003; Savaskan et al., 2004]. The precise mechanisms by which PRGs regulate LPA-mediated signaling await further investigation.

Together, an autocrine and/or paracrine loop of LPA signaling is likely to be present in developing and mature nervous systems. However, these key enzymes are involved in the metabolism of not only LPA but also other phospholipids, such as sphingosine 1-phosphate,

another type of signaling lysolipid affecting cell dynamics and growth [Brindley and Waggoner, 1998; Clair et al., 2003]. Further analyses of these components should provide us with a mechanism for LPA signaling in neuronal development.

FUTURE PROSPECTS

In recent years, major efforts have identified genes involved in LPA signaling, including LPA receptors and LPA-metabolizing enzymes. Our knowledge of LPA signaling in the nervous system at molecular and cellular levels has rapidly progressed. The major unresolved issues are the determination of the LPA receptor subtypes that mediate the cellular effects of LPA relevant to biological phenomena in the nervous system, and the signaling mechanisms that regulate the morphology of developing neurons. However, the production of genetically-engineered mice lacking LPA receptors, and the development of authentic LPA receptor agonists/antagonists and lysoPLD and LPP inhibitors have progressed and more work in these areas is currently underway. Although no severe phenotype related to survival or behavior has been identified in the brains of these mice, closer analyses may identify some structural or functional differences during development of the nervous system. Another benefit these mice bring is that researchers can investigate and confirm LPA receptor subtype-specific effects [Contos et al., 2000a, 2002; Fukushima et al., 2002b; Kingsbury et al., 2003]. In addition, many novel techniques are being applied to investigate cytoskeletal rearrangements, including actin–microtubule interactions, in developing neurons. Such useful tools and techniques should reveal these presently unelucidated mechanisms and improve our understanding of the roles of LPA at the organ level in nervous system development, function and disease; also giving us insights into any medicinal potential. Although we are still in the early stages of exploring LPA signaling in the nervous system, research over the next decade will most certainly increase our understanding of the important features and the scope of LPA signaling.

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