The LPA receptors
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Abstract

Lysophosphatidic acid (LPA) is a growth factor-like lipid that produces many cellular responses. These responses, including actin cytoskeletal rearrangements, cell proliferation and inhibition of gap junction communication, have been documented in many cell types over the last 2 decades. Both non-receptor and receptor-mediated mechanisms had been implicated to explain these responses. A clear advance in this field was the cloning and functional identification of LPA receptors, and there are currently three high-affinity members, LP\textsubscript{A1}, LP\textsubscript{A2} and LP\textsubscript{A3} (synonymous with orphan receptor names edg-2, edg-4 and edg-7, respectively). Here we review the gene structure, expression and functions of LPA receptors. We also discuss the \textit{in vivo} roles mediated by a single LPA receptor type, based on studies of the nervous system, a major locus of LPA receptor expression. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

Lysophosphatidic acid (LPA) is a simple phospholipid, that has intercellular signaling properties. Over the last three decades, documentation of these properties has proceeded at an accelerating pace [1]. Cellular responses to LPA signaling include actin cytoskeletal rearrangements, stimulation of cell proliferation and inhibition of gap junction communica-
tion, responses which have been observed over a wide range of cell types. Despite clear documentation of these responses, one component in the field was missing; the molecular cloning of any lysophospholipid receptor that could account for the signaling properties of LPA, or the structurally related lipid with similar actions, sphingosine-1-phosphate (S1P or SPP). Good evidence supported the existence of a specific G protein coupled receptor (GPCR) for LPA [2], however the absence of a cloned receptor, as well as the absence of any specific competitive antagonists to block LPA-dependent responses, prevented further receptor-based analyses of LPA signaling properties. The alternative theory that several known chemical properties of LPA (including its detergent-like structure and Ca\(^{2+}\) binding activity) could account for the signaling properties of LPA through non-receptor mechanisms thus remained a possible explanation [3,4].

The first LPA receptor was identified during analyses of the embryonic development of the cerebral cortex [5–8]. This gene was first given the orphan receptor name “ventricular zone gene-1” or vzg-1, because of its enriched expression pattern within the neurogenic region (ventricular zone or VZ) of the embryonic cerebral cortex. Subsequent studies from our group, as well as many others, demonstrated the existence of a lysophospholipid receptor family of GPCRs that have a high degree of amino acid similarity. Within this family, two groups of receptors can be discerned based upon their amino acid similarity, genomic structures and ligand specificity [7]. One group consists of the LPA receptor subfamily (vzg-1/mrec/endothelial differentiation gene (edg)-2, edg-4 non mutant form and edg-7), while the other (edg-1, edg-3 and H218/AGR16) is the receptor subfamily for S1P [9–14].

We previously proposed a nomenclature in which LPA receptors are termed “lysophospholipid receptors (LPs)” of an “A” group consisting of three known members: LP\(_A1\), LP\(_A2\), and LP\(_A3\), and a “B” group consisting of S1P receptors termed LP\(_B1\), LP\(_B2\), LP\(_B3\) and LP\(_B4\), reflecting the four demonstrated S1P receptors [6,7,15]. Another related gene, edg-6, has been recently shown to mediate S1P responses [16]. However, based on distinct amino acid identities, this may warrant classification as a third “C” group, LP\(_C1\). Numbers indicate the order in which function was reported and underscore their molecular biological similarities. In this chapter, we focus on the molecular identification and characterization of the LP\(_A\) receptor family, including our initial cloning studies, and the possible roles for LPA signaling in nervous system development. Current progress on the characterization of S1P receptors and the biological roles for these lysophospholipid receptors are discussed in other chapters.

2. LPA receptor family

Since the first LPA receptor, LP\(_A1\)/vzg-1, was identified in 1996, two other related genes, LP\(_A2\) and LP\(_A3\), encoding GPCRs similar to LP\(_A1\) have been documented (Table 1).

2.1. LP\(_A1\)/edg-2

2.1.1. Cloning and gene structure

As stated in the Introduction, experiments designed to identify novel GPCRs associated with the production of neurons in the cortex, particularly in the neuroproliferative VZ, led to
the cloning of \( lp_{A1} \) [5]. The search for novel GPCRs was based on the existence of G proteins during embryonic development and the clear role of such receptors in cell proliferation, differentiation and death in other organisms. In addition, several peptidergic growth factors, which activate receptor tyrosine kinases to modulate neuroblast proliferation, did not appear to be sufficient to account for all of the cellular events associated with cortical development [17–21]. Possible receptor types, with additional roles in cellular responses relevant to VZ neurogenesis, were GPCRs.

Immortalized cortical neuroblast cell lines were used as a source of cDNA in an effort to isolate new GPCR genes that, once cloned, could be analyzed in a cell system likely to contain relevant downstream signaling molecules [22]. GPCR gene fragments were amplified by degenerate polymerase chain reaction (PCR) of cell line cDNAs, and the resulting products were used for \textit{in situ} hybridization screening to identify genes with abundant expression within the embryonic cortex, particularly in the VZ. This approach led to the identification of “\textit{vzg-I}” [5]. Analyses of the obtained cDNA revealed that its protein product consists of 364 amino acids, a predicted molecular mass of 41 kDa, which encoded a novel 7-transmembrane domain, putative GPCR gene. It showed predicted amino acid identity to an orphan receptor derived from studies of human endothelial cells called (\textit{edg-1}; 37% amino acid identity) [23] and two known receptors, the cannibinoid and melanocortin receptor (30% and 32% amino acid identity).

Analyses of the \( lp_{A1} \) genomic clone have revealed that the gene spans over 45 kb and consists of five exons, with one intron located within the middle of transmembrane domain VI and one exon encoding the divergent 5’ sequence in another variant, \textit{mrec1.3} [24]. This isotype lacks 18 amino acids in the amino terminal region [25]. The use of different primary exons suggests the existence of alternative promoters rather than a splice variant. Whether there is a functional difference between \( LP_{A1} \) and the MREC1.3 variant remains to be determined. Another interesting feature of the \( lp_{A1} \) gene is its location on mouse Chromosome 4, as well as an additional, partial duplication in \textit{Mus spretus} on Chromosome 6 [24]. There may be a relationship between the \( lp_{A1} \) gene and one called \textit{vacillans} [26] (for more about this relationship, see below).

<table>
<thead>
<tr>
<th>Name (gene)</th>
<th>% amino acid similarity to ( LP_{A1} )</th>
<th>Synonyms</th>
<th>Chromosomal location</th>
<th>Cellular function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>( LP_{A1} ) ( (lp_{A1}) )</td>
<td>100%</td>
<td>EDG-2, MREC1.3</td>
<td>Mouse 4, close to \textit{vc} Human 9q 31.3–32</td>
<td>Adenylyl cyclase inhibition, Actomyosin stimulation, SRE activation, DNA synthesis</td>
<td>[5,8,24,62]</td>
</tr>
<tr>
<td>( LP_{A2} ) ( (lp_{A2}) )</td>
<td>60.9 (mouse), 60.4 (human)</td>
<td>EDG-4, non-mutant form</td>
<td>Mouse 8, close to \textit{kat} Human 19p12</td>
<td>Phospholipase C activation, SRE activation, Phospholipase C activation</td>
<td>[9,34,35]</td>
</tr>
<tr>
<td>( LP_{A3} ) ( (lp_{A3}) )</td>
<td>60.5 (mouse), 58.7 (human)</td>
<td>EDG-7</td>
<td>Mouse 3, Human 7</td>
<td>Adenylyl cyclase stimulation, MAP kinase activation, Phospholipase C activation</td>
<td>[10]</td>
</tr>
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2.1.2. Gene distribution

Identification of cells expressing lpA1 is fundamental to understanding the functional roles of LPA signaling in vivo. As described previously, lpA1 expression was first observed within the nervous system, and this appears to be a major locus of lysosphospholipid receptor expression. During embryonic life, the VZ of the mouse developing cerebral cortex shows prominent expression of lpA1 that diminishes by the end of cortical neurogenesis, which occurs just before birth [5]. A second locus of lpA1 expression is present near the pial surface of the embryonic cerebral cortex, although the precise cell type responsible for this expression has not been determined. In situ hybridization analyses for lpA1 expression during postnatal life revealed another cellular locus [27,28]. The lpA1 expression reappeared in the hindbrain during the first postnatal week and subsequently expanded from caudal to rostral regions with peak expression around postnatal day 18. This expression was found within developing fiber tracts, suggesting that oligodendrocytes, the myelinating cells found in the central nervous system, expressed lpA1. Studies using double-label in situ hybridization with lpA1 and either the proteolipid protein gene (an oligodendrocyte marker) or glial fibrillary acidic protein gene (an astrocyte marker) identified the lpA1-expressing cells as oligodendrocytes but not astrocytes. In addition, Schwann cells, the myelinating cells found in the peripheral nervous system, also express lpA1 with the highest level observed in the first week [29]. Thus, it is likely that lpA1 expression correlates temporally and spatially with progression of myelination by oligodendrocytes and Schwann cells, implicating LPA signaling as a regulator of some aspect of the biology of these myelinating cells.

The expression of lpA1 was also apparent outside of the nervous system. Northern blot analyses revealed that lpA1 is expressed prominently in testis and intestine, and modestly in heart, lung, kidney, spleen, thymus, muscle and stomach [5,25,30]. Detailed cellular loci remain to be determined.

2.1.3. Function

Identification of lpA1 as a functional LPA receptor came initially from overexpression studies using cortical neuroblast cell lines from which lpA1 was originally isolated [5]. This approach was taken to ensure that the receptor interacted with biologically relevant, downstream signaling molecules present in the cells to produce a cellular response. The overexpression of lpA1 in the cortical cell lines produced increased 1) LPA binding sites, 2) receptor protein, 3) efficacy of LPA for the stimulation of cell rounding and 4) efficacy of LPA for the inhibition of cAMP production. Based on these data, we concluded that LP A1 was a functional LPA receptor. A similar conclusion was drawn from the study of the heterologous expression of lpA1 using yeast genetics and the pheromone response pathway, and overexpression analyses of human edg-2 (human lpA1 gene) in lymphoid cells [31,32].

Another important question to be addressed was whether or not LP A1 was multifunctional. LPA has been demonstrated to induce diverse cellular responses in a wide variety of mammalian cells, including fibroblasts and neuroblastoma cell lines through pertussis toxin (PTX)-sensitive and -insensitive pathways [33]. The former pathway uses G\textsubscript{i/o}, which leads to inhibition of adenyl cyclase and stimulation of mitogen-activated protein (MAP) kinase pathways. LPA-induced DNA synthesis and cell proliferation are mediated by the G\textsubscript{i}-MAP kinase pathway. Activation of the PTX-insensitive pathway results in actin rearrangement,
including stress fiber formation in fibroblasts and neurite retraction in neuroblastoma cell lines. These cellular responses are mediated by the small G protein, Rho. Another PTX-insensitive pathway is linked to phospholipase C (PLC) activation, resulting in increased inositol phosphates and intracellular Ca\(^{2+}\) concentration. The issue of whether these diverse responses, induced by LPA, were mediated by a single LPA receptor or a combination of two or more LPA receptors was uncertain.

To address this issue, we employed a heterologous expression system with two mammalian cell lines that do not express \(lp_{A1}\) nor responsive to LPA [8]. These cell lines were derived from two distinct lineages: hepatocytes and neuroblastoma cells. The use of an epitope-tagged \(LP_{A1}\) combined with several assays for LPA responses showed that \(lp_{A1}\) expression was sufficient to produce LPA-dependent signaling, including G-protein activation, Rho-mediated actin rearrangement, cell proliferation and stimulation of serum response elements (SREs) in these cells. These data demonstrated that \(LP_{A1}\) acts as a multifunctional LPA receptor for distinct cell types. To date, \(LP_{A1}\) has been shown to couple to at least two different G-proteins: \(G_{i/o}\) and a pertussis toxin insensitive G protein that appears to be \(G_{12}\) or \(G_{13}\). In addition, \(LP_{A1}\) may also interact with \(G_{q}\) to activate the PLC pathway, based on our data with heterologous expression of \(lp_{A1}\) suggesting that \(LP_{A1}\) inducLPA2-mediated SRE stimulation was blocked by the pretreatment of cells with PTX or C3 exoenzyme, indicating that \(LP_{A2}\) can couple to both \(G_{i}\) and Rho pathways, similar to \(LP_{A1}\). A surprising number of nucleotide variations were observed in 3’ untranslated regions (single nucleotide replacement), along with a previously reported C-terminal, G deletion present in the initially characterized human \(lp_{A2}\) (\(edg-4\)), which was cloned from an ovarian tumor cell line. The G deletion at the position of C-terminal cytoplasmic tail causes a frame shift to produce a predicted mutant protein with 4 replaced and 31 additional amino acids (382 amino acids as compared to native \(lp_{A2}\) with 351 amino acids) [35]. Because C-terminal regions of GPCR are, in general, involved in receptor desensitization and internalization, such a deletion may contribute to sustained receptor activation, leading to cellular transformation. Identification of \(LP_{A2}\) as a second LPA receptor was supported by LPA-dependent activation of SREs in Jurkat T cells [9]. The \(LP_{A2}\)-mediated SRE stimulation was blocked by the pretreatment of cells with PTX or C3 exoenzyme, indicating that \(LP_{A2}\) can couple to both \(G_{i}\) and Rho pathways, similar to \(LP_{A1}\).
of \(lp_{A2}\). However, high levels of expression were found in embryonic and neonatal brain, which additionally contained a larger transcript around 7 kb. Two species of transcripts were also observed in human lymphoid cell lines, although these transcripts differ in size (1.8 kb and 8 kb) from those found in the mouse [9], which may reflect distinct polyadenylation.

2.3. \(lp_{A3}/edg-7\)

The existence of a third LPA receptor had been suggested by studies showing that some LPA responsive cells did not express \(lp_{A1}\) or \(lp_{A2}\). RT-PCR, using degenerate primers and Jurkat T cell RNA, identified a novel human gene (\(edg-7\)) with ca. 50% amino acid similarity to human \(lp_{A1}\) and \(lp_{A2}\), and encoding a GPCR of 353 amino acids [10]. Our independent studies also isolated a homologous mouse gene, \(lp_{A3}\) (Contos et al., accepted). Based on the high degree of amino acid similarity and the conserved location of an intron inserted within transmembrane domain VI, this gene was clearly a member of the same LP_{A} family of receptors.

One anticipated function of LP_{A3} was confirmed by LPA-dependent increases in intracellular \(Ca^{2+}\) concentration in insect cells (Sf9 cells) overexpressing \(lp_{A3}\) [10]. In addition, LP_{A3} was also reported to couple to stimulatory pathways for adenylyl cyclase and MAP kinase. However, the results were obtained using an insect cell line that may not have endogenous G proteins appropriate for the coupling of exogenous GPCRs, leaving open the question of actual signaling properties for this particular receptor.

3. Neurobiology of LPA signaling

Essential questions concerning LPA signaling have now begun to move from identification of receptors, to understanding the \textit{in vivo} roles of how an individual receptor signals. A major locus of expression for LPA receptors is the nervous system. We have used the nervous system as a model for exploring the function of receptor-based LPA signaling. Analyses of \(lp_{A1}\) expression by \textit{in situ} hybridization identified three major cellular loci in the nervous system; cortical neuroblasts of the neuroproliferative VZ, oligodendrocytes and Schwann cells [5,27–29]. On the other hand, the precise expression pattern of the other \(lp_{A}\) family members in the nervous system is still unknown. However, we have accumulated several lines of evidence that \(lp_{A2}\) is expressed in the cortical plate where postmitotic or differentiating neurons are located during development (McGiffert and Chun; in preparation). We next discuss possible roles for LPA signaling in the nervous system.

3.1. Cortical neuroblasts

The neurons of the mammalian cerebral cortex are generated in the embryonic telencephalon by neuroblasts that proliferate in the VZ, a zone overlying the lateral ventricle [36]. Cortical neurogenesis is a highly regulated event in which the phases of the cell cycle, cell morphology, and cell fate decisions are spatio-temporally coordinated [37,38]. As the cell cycle proceeds, neuroblasts show a “to-and-fro movement” which has been termed “interki-
netic nuclear migration” [39]. During S-phase of the cell cycle, neuroblasts are fusiform in shape (i.e. bipolar processes oriented towards the ventricular and superficial (pial) surfaces of the cerebral wall), and the nuclei are positioned at the superficial border of the VZ. During the next G2 phase, the nucleus descends to the ventricular surface, the pial process is retracted, and the cell rounds up. The cell then enters M-phase and divides, after which daughter cells either migrate away as postmitotic neurons, undergo programmed cell death [40,41] or re-extend their bipolar processes to enter the cell cycle again [39,42].

In addition to the restricted $l_{PA_1}$ expression in the cortical VZ, LPA-induced cellular effects, including cytoskeletal effects and cell proliferation, are reminiscent of VZ neuroblast behavior [6]. Thus, we have examined what roles LPA plays in cortical neurogenesis [43]. Whole brains from mouse embryos were cultured and VZ neuroblast morphology was histologically examined. Cresyl violet and 4,6-diamino-2-phenylindole (DAPI) staining of tissue sections prepared after culturing showed fusiform structures of cortical VZ neuroblasts and their nuclei in control brains. This morphology was quite similar to that observed in vivo. In contrast, adding LPA to the culture resulted in clear morphological changes of cortical neuroblasts. Most neuroblasts responded to LPA with cellular and nuclear rounding. To examine more closely the LPA-induced morphological changes in neuroblasts, we carried out real-time recording using cluster cultures, consisting of cortical VZ neuroblasts. Bath application of LPA resulted in rapid cellular and nuclear migration towards the center of clusters, accompanied by cell rounding and the formation of fine retraction fibers. These LPA-induced morphological changes in neuroblasts resembled those observed during interkinetic nuclear migration. The intracellular signaling pathways (Rho and actomyosin pathways) activated in response to LPA-induced morphological changes were similar to those mediated by $L_{PA_1}$ in neuroblastoma cell lines [8].

Another prominent feature of VZ neuroblasts, which may be related to LPA signaling, was cell proliferation. We therefore examined the proliferative effects of LPA on VZ neuroblasts [44]. Treatment of clusters with LPA for one day resulted in the increase in the proliferative population, as determined by 5-bromo-2′-deoxyuridine-5′-monophosphate (BrdU) incorporation. This increase was inhibited by the pretreatment with PTX suggesting the involvement of Gi/o pathway.

In addition to these morphological and proliferative effects of LPA, we also observed that LPA alters ionic conductance in the VZ neuroblasts [45]. When clusters of cortical cells were examined by using whole-cell patch clamp techniques, LPA was found to induce two clear types of ionic changes within VZ cells. These changes consisted of increases in both chloride and nonselective cation conductances, resulting in a depolarization of the cells, based on the resting potential. Interestingly, the ionic responses developmentally preceded or existed simultaneously with the previously identified GABA and/or L-glutamate ionotropic conductances [46]. The LPA-dependent ionic changes might influence DNA synthesis or cell proliferation described previously.

Collectively, these data demonstrate multiple functions for LPA in proliferative cortical neuroblasts and implicate receptor-mediated LPA signaling as a biologically relevant mechanism during cortical neurogenesis. Many peptide growth factors or transmitters are present during the same embryonic period [47] suggesting that they are unlikely to function independently of one another. Because LPA-induced activation of intracellular signaling
pathways may cooperate with those activated by other stimuli via receptor tyrosine kinases (e.g. epidermal growth factor receptor [48]), the interaction between LPA and other stimuli in regulating neurogenesis is of great interest.

3.2. Differentiating neurons

After neuroblasts commit to becoming postmitotic neurons and exit the cell cycle, these differentiating neurons leave the VZ and migrate towards the subplate, intermediate zone and cortical plate. Once these cells arrive at their final destination, the formation and elongation of axonal and dendritic neurites begins. Both cell migration and neurite formation are dynamic events that require cytoskeletal rearrangement, similar to interkinetic nuclear migration, of proliferative VZ neuroblasts. Soluble regulatory factors, such as chemorepellants or chemoattractants, have been shown to play guidance roles in migration and neurite formation of these differentiating neurons [49,50]. In addition to the retraction effects of LPA in neuroblastoma cell lines [51,52], LPA is also known to regulate cell migration of cancer cells and possibly cause growth cone collapse in neurons in culture [53–57].

These effects of LPA on the cytoskeleton allowed us to examine whether LPA could regulate both the morphology and the migration of differentiating neurons (Fukushima and Chun; in preparation). This regulation by LPA was examined by using dissociated cortical cultures where most of the single cells were differentiating neurons, which underwent neurite formation. Within 15 minutes of LPA addition, the neurites or lamella structures retracted and the fine retraction fibers, characterized by f-actin filaments, formed. Other cytoskeletal components, including nestin or β-tubulin, also responded to LPA with a transition from bundled to collapsed structures. In addition to these observations, LPA was found to inhibit cell migration of differentiating neurons from cortical tissues. These findings, taken together, suggest that LPA signaling may exert a significant, extracellular influence on neurite formation and migration of differentiating neurons.

3.3. Myelinating cells: oligodendrocytes and Schwann cells

The expression of \( lp_{Al} \) in myelinating cells in the peripheral and central nervous systems suggests that LPA signaling is important in the biology function of these cells, including cell survival and myelination. Additionally, there were two interesting observations related to \( lp_{Al} \) expression in mutant mice: first, in the jimpy mouse, which shows increased oligodendrocyte apoptosis, decreased expression of \( lp_{Al} \) was found [27]; and second, a previously identified mutation, vacillans, maps to the same proximal region of Chromosome 4 as \( lp_{Al} \) [24]. This extinct mutant mouse exhibited signs of peripheral neuropathy consistent with a defect in nerve function that can accompany demyelination [26]. Although the expression of other LPA receptor genes remains to be clarified in these myelinating cells, the spatio-temporal expression pattern of \( lp_{Al} \) suggests the possibility that LPA signaling could influence the basic neurobiology of myelinating cells.

This possibility has been examined in Schwann cells by addressing the biological functions of LPA signaling [29]. Previous reports have demonstrated that serum markedly affects the differentiation and survival of Schwann cells as well as oligodendrocytes [58,59]. These
results suggest that LPA might be involved in Schwann cell survival, because this lipid is present in serum at micromolar concentrations [60]. Using primary cultures of rat Schwann cells under serum-free conditions, we found that LPA could protect these cells from apoptosis after serum withdrawal without affecting cell proliferation [29]. The potency was equivalent to or even higher than that of neuregulins, which are peptidergic survival factors for Schwann cells [54]. Of particular note was that LPA-induced survival effects were mediated by LP_A1/G_i/PI-3 kinase/Akt pathways, while the effects of neuregulin involve the activation of their receptor tyrosine kinases and PI-3 kinase/Akt downstream. Additionally, it was also striking that S1P showed no effect on Schwann cell survival, in spite of the relatively abundant expression of the cognate GPCR, LP_B3, indicating a non-redundant role for LP receptors in this system. These results demonstrate a role for LP_A1-mediated LPA signaling in the Schwann cells. Because similar intracellular signaling pathways are involved in oligodendrocyte survival, LP_A1-mediated signaling may play an important role in the survival of myelinating cells.

4. Conclusion and future prospects

With the identification of the LP receptor families, LP_A and LP_B, perhaps other subfamilies, our understanding of extracellular LPA and S1P signaling has rapidly advanced. However, the lack of receptor-specific agonists and antagonists still prevents traditional pharmacological analyses of LPA both in vitro and in vivo. Molecular genetic approaches based on overexpressing, misexpressing or mutating the receptors (e.g. the generation of receptor-null mice) offer a clear opportunity to understand new aspects of LP signaling in organismal development, function and pathology. Combined with the further characterization of how signaling LPA is synthesized, released, degraded and regulated, the coming years should see important advances in the complex roles of this simple phospholipid.

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References