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_{A1}, LP_{A2} and LP_{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 *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.

Keywords: Lysophospholipids; GPCR; SIP; SPP; edg; Lipid signalling; Growth factors

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-

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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

Table 1
Characteristics of LPA receptors

Name (gene)	% amino acid similarity to LP$_{A1}$	Synonyms	Chromosomal location	Cellular function	References
LP$_{A1}$ (lp_{A1})	100%	EDG-2 MREC1.3	Mouse 4, close to *vc* Human 9q 31.3–32	Adenylyl cyclase inhibition Actomyosin stimulation SRE activation DNA synthesis Phospholipase C activation	[5] [8] [24] [62]
LP$_{A2}$ (lp_{A2})	60.9 (mouse) 60.4 (human)	EDG-4 non-mutant form	Mouse 8, close to *kat* Human 19p12	SRE activation Phospholipase C activation	[9] [34,35]
LP$_{A3}$ (lp_{A3})	60.5 (mouse) 58.7 (human)	EDG-7	Mouse 3 Human 7	Adenylyl cyclase stimulation MAP kinase activation Phospholipase C activation	[10]

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 *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 "*vzg-1*" [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 (*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, *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 *Mus spretus* on Chromosome 6 [24]. There may be a relationship between the lp_{A1} gene and one called *vacillans* [26] (for more about this relationship, see below).

2.1.2. Gene distribution

Identification of cells expressing lp_{A1} is fundamental to understanding the functional roles of LPA signaling *in vivo*. As described previously, lp_{A1} expression was first observed within the nervous system, and this appears to be a major locus of lysophospholipid receptor expression. During embryonic life, the VZ of the mouse developing cerebral cortex shows prominent expression of lp_{A1} that diminishes by the end of cortical neurogenesis, which occurs just before birth [5]. A second locus of lp_{A1} 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 lp_{A1} expression during postnatal life revealed another cellular locus [27,28]. The lp_{A1} 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 lp_{A1}. Studies using double-label *in situ* hybridization with lp_{A1} and either the proteolipid protein gene (an oligodendrocyte marker) or glial fibrillary acidic protein gene (an astrocyte marker) identified the lp_{A1}-expressing cells as oligodendrocytes but not astrocytes. In addition, Schwann cells, the myelinating cells found in the peripheral nervous system, also express lp_{A1} with the highest level observed in the first week [29]. Thus, it is likely that lp_{A1} 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 lp_{A1} was also apparent outside of the nervous system. Northern blot analyses revealed that lp_{A1} 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 lp_{A1} as a functional LPA receptor came initially from overexpression studies using cortical neuroblast cell lines from which lp_{A1} 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 lp_{A1} 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 lp_{A1} using yeast genetics and the pheromone response pathway, and overexpression analyses of human *edg-2* (human lp_{A1} 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 $G_{i/o}$, which leads to inhibition of adenylyl cyclase and stimulation of mitogen-activated protein (MAP) kinase pathways. LPA-induced DNA synthesis and cell proliferation are mediated by the G_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} induces PLC activation through a PTX-insensitive pathway [34].

2.2. lp_{A2}/edg-4 non mutant form

Database searches revealed a human genomic sequence that encoded a GPCR with ca. 60% amino acid similarity to lp_{A1} [24]. We termed this gene lp_{A2} [35]. The obtained mouse cDNA encoded a protein consisting of 351 amino acids, yielding a predicted molecular mass of 38.9 kDa. The genomic structure of lp_{A2} included three exons with an intron inserted within a putative transmembrane domain VI, as observed for lp_{A1}, suggesting that lp_{A1} and lp_{A2} were derived from a common ancestral gene. The lp_{A2} gene is located on Chromosome 8, close to the mutations for *myodystrophy* (*myd*) and "*kidney-anemia-testis*" (*kat*), although the lp_{A2} mutation was not likely responsible for the *kat* phenotype based on additional data. Another feature of the mouse lp_{A2} gene is the existence of several variants derived from cancer cells. A surprisingly large 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_{A1} 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}.

Northern blot analyses showed that the 2.8 kb lp_{A2} transcript was abundant in testis, kidney, and lung in adult mice, whereas adult mouse brain did not express a detectable signal

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 *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 *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 lp_{A1} 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 LP_{A1} 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 $G_{i/o}$ 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_{A1} 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_{A1} expression in mutant mice: first, in the jimpy mouse, which shows increased oligodendrocyte apoptosis, decreased expression of lp_{A1} was found [27]; and second, a previously identified mutation, vacillans, maps to the same proximal region of Chromosome 4 as lp_{A1} [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 spatiotemporal expression pattern of lp_{A1} 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.

Acknowledgments

References

[1] Moolenaar WH. Bioactive lysophospholipids and their G protein-coupled receptors. Exp Cell Res 1999; 253:230–8.
[2] van der Bend RL, et al. Identification of a putative membrane receptor for the bioactive phospholipid, lysophosphatidic acid. Embo J 1992;11:2495–501.
[3] Simon MF, Chap H, Douste-Blazy L. Platelet aggregating activity of lysophosphatidic acids is not related to their calcium ionophore properties. FEBS Lett 1984;166:115–9.

[4] Gerrard J, Robinson P. Lysophosphatidic acid can activate platelets without increasing ^{32}P-labelling of phosphatidic acid. Biochim Biophys Acta 1984;795:487–92.

[5] Hecht JH, et al. *Ventricular zone gene-1* (*vzg-1*) encodes a lysophosphatidic acid receptor expressed in neurogenic regions of the developing cerebral cortex. J Cell Biol 1996;135:1071–83.

[6] Chun J, Contos JJA, Munroe D. A growing family of receptor genes for lysophosphatidic acid (LPA) and other lyso-phospholipids (LPs). Cell Biochem Biophys 1999;30:213–42.

[7] Chun J. Lysophospholipid receptors: implications for neural signaling. Crit Rev Neurobiol 1999;13:151–68.

[8] Fukushima N, Kimura Y, Chun J. A single receptor encoded by *vzg-1/lp$_{A1}$/edg-2* couples to G proteins and mediates multiple cellular responses to lysophosphatidic acid. Proc Natl Acad Sci USA 1998;95:6151–6.

[9] An S, et al. Characterization of a novel subtype of human G protein-coupled receptor for lysophosphatidic acid. J Biol Chem 1998;273:7906–10.

[10] Bandoh K, et al. Molecular cloning and characterization of a novel human G-protein-coupled receptor, EDG7, for lysophosphatidic acid. J Biol Chem 1999;274:27776–85.

[11] Lee MJ, et al. Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. Science 1998;279:1552–5.

[12] Zhang G, et al. Comparative analysis of three murine G-protein coupled receptors activated by sphingosine-1-phosphate. Gene 1999;227:89–99.

[13] van Brocklyn JR, et al. Sphingosine 1-phosphate-induced cell rounding and neurite retraction are mediated by the G protein-coupled receptor H218. J Biol Chem 1999;274:4626–32.

[14] Okamoto H, et al. EDG3 is a functional receptor specific for sphingosine 1-phosphate and sphingosylphosphorylcholine with signaling characteristics distinct from EDG1 and AGR16. Biochem Biophys Res Commun 1999;260:203–8.

[15] Chun J, et al. Neurobiology of receptor-mediated lysophospholipid signaling. From the first lysophospholipid receptor to roles in the nervous system function and development. Ann NY Acad Sci 2000;905:110–7.

[16] Yamazaki Y, et al. Edg-6 as a putative sphingosine 1-phosphate receptor coupling to Ca$^{(2+)}$ signaling pathway. Biochem Biophys Res Commun 2000;268:583–9.

[17] Gensburger C, Labourdette G, Sensenbrenner M. Brain basic fibroblast growth factor stimulates the proliferation of rat neuronal precursor cells in vitro. FEBS Lett 1987;217:1–5.

[18] Ghosh A, Greenberg ME. Distinct roles for bFGF and NT-3 in the regulation of cortical neurogenesis. Neuron 1995;15:89–103.

[19] Kilpatrick TJ, Bartlett PF. Cloning and growth of multipotential neural precursors: requirements for proliferation and differentiation. Neuron 1993;10:255–65.

[20] Temple S, Qian X. bFGF, neurotrophins, and the control or cortical neurogenesis. Neuron 1995;15:249–52.

[21] Threadgill DW, et al. Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. Science 1995;269:230–4.

[22] Chun J, Jaenisch R. Clonal cell lines produced by infection of neocortical neuroblasts using multiple oncogenes transduced by retroviruses. Mol Cell Neurosci 1996;7:304–21.

[23] Hla T, Maciag T. An abundant transcript induced in differentiating human endothelial cells encodes a polypeptide with structural similarities to G-protein-coupled receptors. J Biol Chem 1990;265:9308–13.

[24] Contos JJA, Chun J. Complete cDNA sequence, genomic structure, and chromosomal localization of the LPA receptor gene *lp$_{A1}$/vzg-1/Gpcr26*. Genomics 1998;51:364–78.

[25] Macrae AD, et al. Cloning, characterization, and chromosomal localization of rec1.3, a member of the G-protein-coupled receptor family highly expressed in brain. Brain Res Mol Brain Res 1996;42:245–54.

[26] Sirlin JL. Vacillans, a neurological mutant in the house mouse linked to brown. J Genet 1956;54:42–8.

[27] Weiner JA, Hecht JH, Chun J. Lysophosphatidic acid receptor gene *vzg-1/lp$_{A1}$/edg-2* is expressed by mature oligodendrocytes during myelination in the postnatal murine brain. J Comp Neurol 1998;398:587–98.

[28] Allard J, et al. A rat G protein-coupled receptor selectively expressed in myelin-forming cells. Eur J Neurosci 1998;10:1045–53.

[29] Weiner JA, Chun J. Schwann cell survival mediated by the signaling phospholipid lysophosphatidic acid. Proc Natl Acad Sci USA 1999;96:5233–8.

[30] Contos JJA, Ishii I, Chun J. Lysophosphatidic acid receptors. Mol Pharmacol 2000;58:1188–96.

[31] Erickson JR, et al. Edg-2/Vzg-1 couples to the yeast pheromone response pathway selectively in response to lysophosphatidic acid. J Biol Chem 1998;273:1506–10.

[32] An S, et al. Molecular cloning of the human Edg2 protein and its identification as a functional cellular receptor for lysophosphatidic acid. Biochem Biophys Res Commun 1997;231:619–22.

[33] Moolenaar WH. Lysophosphatidic acid signalling. Curr Opin Cell Biol 1995;7:203–10.

[34] Ishii I, et al., Functional comparisons of the lysophosphatidic acid receptors, LP_{A1}/V2G-1/EDG-2, LP_{A2}/EDG-4, and LP_{A3}/EDG-7 in nueronal cell lines using a retrovirus expression system. Mol. Pharmacol 2000;58:895–202.

[35] Contos JJA, Chun J. Genomic characterization of the lysophosphatidic acid receptor gene, *lp_{A2}/Edg4*, and identification of a frameshift mutation in a previously characterized cDNA. Genomics 2000;64:155–69.

[36] Boulder Committee. Embryonic vertebrate central nervous system: revised terminology. Anat Rec 1970; 166:257–61.

[37] McConnell SK. Constructing the cerebral cortex: neurogenesis and fate determination. Neuron 1995;15: 761–8.

[38] Caviness VS, Takahashi T, Nowakowski RS. Numbers, time and neocortical neuronogenesis: a general developmental and evolutionary model. Trends Neurosci 1995;18:379–83.

[39] Sauer FC. Mitosis in the neural tube. J Comp Neurol 1935;62:377–405.

[40] Blaschke AJ, Staley K, Chun J. Widespread programmed cell death in proliferative and postmitotic regions of the fetal cerebral cortex. Development 1996;122:1165–74.

[41] Blaschke AJ, Weiner JA, Chun J. Programmed cell death is a universal feature of embryonic and postnatal neuroproliferative regions throughout the central nervous system. J Comp Neurol 1998;396:39–50.

[42] Seymour RM, Berry M. Scanning and transmission electron microscope studies of interkinetic nuclear migration in the cerebral vesicles of the rat. J Comp Neurol 1975;160:105–25.

[43] Fukushima N, Weiner JA, Chun J. Lysophosphatidic acid (LPA) is a novel extracellular regulator of cortical neuroblast morphology. Dev Biol 2000;228:6–18.

[44] Contos JJA et al., Requirement for the LP_{A5} lysophosphatidic acid receptor gene in normal suckling behaviour. Proc. Natl Acad Sci USA 2000;97:13384–9.

[45] Dubin A, et al. Lysophosphatidic acid (LPA) stimulates neurotransmitter-like conductance changes that precede GABA and L-glutamate in early, presumptive cortical neuroblasts. J Neurosci 1999;19:1371–81.

[46] LoTurco JJ, et al. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. Neuron 1995;15:1287–98.

[47] Cameron HA, Hazel TG, McKay RDG. Regulation of neurogenesis by growth factors and neurotransmitters. J Neurobiol 1998;36:287–306.

[48] Daub H, et al. Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. Nature 1996;379:557–60.

[49] Brose K, Tessier-Lavigne M. Slit proteins: key regulators of axon guidance, axonal branching, and cell migration. Curr Opin Neurobiol 2000;10:95–102.

[50] Culotti JG, Merz DC. DCC and netrins. Curr Opin Cell Biol 1998;10:609–13.

[51] Jalink K, et al. Inhibition of lysophosphatidate- and thrombin-induced neurite retraction and neuronal cell rounding by ADP ribosylation of the small GTP-binding protein Rho. J Cell Biol 1994;126:801–10.

[52] Tigyi G, et al. Lysophosphatidic acid-induced neurite retraction in PC12 cells: control by phosphoinositide-Ca^{2+} signaling and Rho. J Neurochem 1996;66:537–48.

[53] Sakai T, de la Pena JM, Mosher DF. Synergism among lysophosphatidic acid, beta1A integrins, and epidermal growth factor or platelet-derived growth factor in mediation of cell migration. J Biol Chem 1999;274:15480–6.

[54] O'Connor KL, Shaw LM, Mercurio AM. Release of cAMP gating by the alpha6beta4 integrin stimulates lamellae formation and the chemotactic migration of invasive carcinoma cells. J Cell Biol 1998;143:1749–60.

[55] Yamamura S, et al. Sphingosine 1-phosphate regulates melanoma cell motility through a receptor-coupled extracellular action and in a pertussis toxin-insensitive manner. Biochemistry 1997;36:10751–9.

[56] Saito S. Effects of lysophosphatidic acid on primary cultured chick neurons. Neurosci Lett 1997;229:73–6.

[57] Jin Z, Strittmatter SM. Rac1 mediates collapsin-1-induced growth cone collapse. J Neurosci 1997;17:6256–63.

[58] Syroid DE, et al. Cell death in the Schwann cell lineage and its regulation by neuregulin. Proc Natl Acad Sci USA 1996;93:9229–34.

[59] Bologa L, et al. Serum contains inducers and repressors of oligodendrocyte differentiation. J Neurosci Res 1988;20:182–8.

[60] Eichholtz T, et al. The bioactive phospholipid lysophosphatidic acid is released from activated platelets. Biochem J 1993;291:677–80.

[61] An S, et al. Recombinant human G protein-coupled lysophosphatidic acid receptors mediate intracellular calcium mobilization. Mol Pharmacol 1998;54:881–8.