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A Growing Family of Receptor Genes for Lysophosphatidic Acid (LPA) and other Lysophospholipids (LPs)

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ABSTRACT

A missing component in the experimental analysis of cell signaling by extracellular lysophospholipids such as lysophosphatidic acid (LPA) or sphingosine-1-phosphate (S1P) has been cloned receptors. Through studies on the developing brain, the first such receptor gene (referred to as *vzg-1*) was identified, representing a member of the G-protein coupled receptor (GPCR) super family *(1)*. Here we review the neurobiological approach that led to both its cloning and identification as a receptor for LPA, along with related expression data. Summarized sequence and genomic structure analyses indicate that this first, functionally identified receptor is encoded by a member of a growing gene family that divides into at least two subgroups: genes most homologous to the high-affinity LPA receptor encoded by *vzg-1*, and those more homologous to an orphan receptor gene *edg-1* that has recently been identified as a S1P receptor. A provisional nomenclature is proposed, based on published functional ligand actions, amino acid composition and genomic structure whereby

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the receptors encoded by these genes are referred to as lysophospholipid (LP) receptors, with subgroups distinguished by letter and number subscripts (e.g., LP_{A1} for Vzg-1, and LP_{B1} for Edg-1). Presented expression data support the recently published work indicating that members of the LP_{B1} subgroup are receptors for the structurally-related molecule, S1P. The availability of cloned LP receptors will enhance the analysis of the many documented LP effects, while their prominent expression in the nervous system indicates significant but as yet unknown roles in development, normal function, and neuropathology.

Index Entries: Lysophosphatidic acid; LPA, sphinosine-1-phosphate, S1P; cannabanoid; G-protein coupled receptor; CNS development; Cerebral cortex.

INTRODUCTION

The extracellular application of lysophospholipids, most prominently lysophosphatidic acid (LPA or 1-acyl-sn-glycerol-3-phosphate) and sphingosine-1-phosphate (S1P or 1-phosphate-2-amino-4-cis-octadecene-1,3-diol; structures in Fig. 1, as compared to other compounds noted in this review) produces many cellular responses over a range of different cell types. The responses and related signaling features of these lysophospholipids have been the subject of many excellent reviews *(2–7)* and will not be pursued here, nor will the intracellular signaling roles *(8–10)* be addressed. An archetypical lipid for studying LP effects is LPA, and a missing component in the mechanistic interpretation of extracellular responses has been a cloned receptor which would allow the examination of LP signaling through molecular genetic analyses of the receptor(s). While the majority of evidence has supported the existence of a specific G-protein coupled receptor (GPCR) for LPA (and S1P), other data (e.g., the apparent lack of stereospecificity for some forms of LPA) *(11,12)* have indicated that nonreceptor mechanisms could account for some of the observed phenomena, leaving open the question of the existence of a specific, cell surface receptor.

During studies of the developing cerebral cortex *(1)*, a screen for novel GPCRs with restricted cortical expression identified a gene referred to as ventricular zone gene-*1* (*vzg-1*) because it was expressed in the neuroproliferative region called the ventricular zone *(13)* (VZ: *see* Fig. 2). Functional characterization indicated that it encoded a

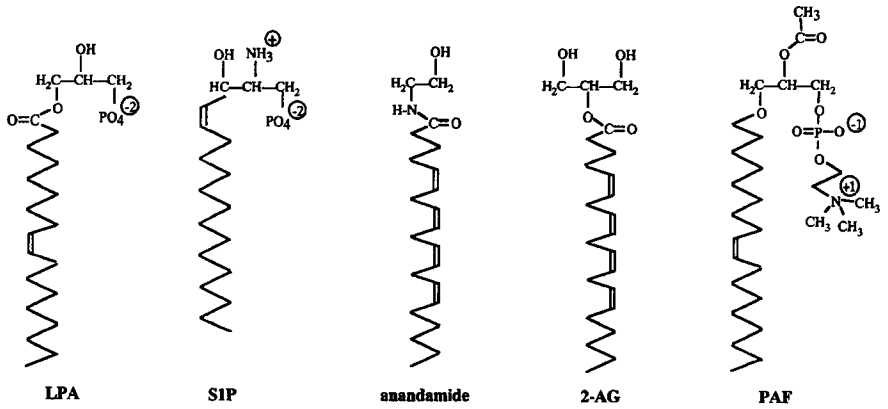

Fig. 1. Structures of endogenous lysophopholipid and related lipid ligands. Shown are 1-oleoyl lysophosphatidic acid (LPA), sphingosine 1-phosphate (S1P), anandamide, 2-arachidonyl glycerol (2-AG), and platelet-activating factor (PAF). Though only one form of LPA is shown here, the fatty acid side chain can vary in length and saturation. Biological responses are maximal when there are 16-18 carbon atoms and 2-3 degrees of unsaturation *(12,65,66)*. The side chain on S1P and PAF can also vary, while side chains on the other lipids shown are invariant. Anandamide and 2-AG have been shown to be endogenous ligands for the cannabanoid receptors CB1 and CB2 *(58,59)*.

high-affinity receptor for extracellular LPA, an identity confirmed by heterologous expression studies in yeast *(14)* and mammals *(15)*. Here we review the cloning and analysis of *vzg-1*, and discuss implications stemming from the availability of its nucleotide and translated amino acid (aa) sequence. Based on comparative sequence and genomic structure analyses, it is highly probable that related "orphan receptors" cloned by us as *vzg*-1 homologues, existing as expressed sequence tags (ESTs), or cloned by other investigators as orphan receptor genes from a variety of tissue systems and species are, in fact members of a new family within the superfamily of GPCRs that mediate extracellular LP signals, thus representing a family of LP receptors. Both aa and genomic structural motifs allow a further subdivision of these genes into two subgroups which would predict differences in ligand specificity and perhaps intracellular signaling. Consistent with this view, the two subgroups of this LP receptor family include either a demonstrated high-affinity LPA receptor, vzg-1 (renamed LP_{A1}) and a demonstrated S1P receptor *(16,17)*, encoded by endothelial differentiation gene-1, *edg-1* (renamed

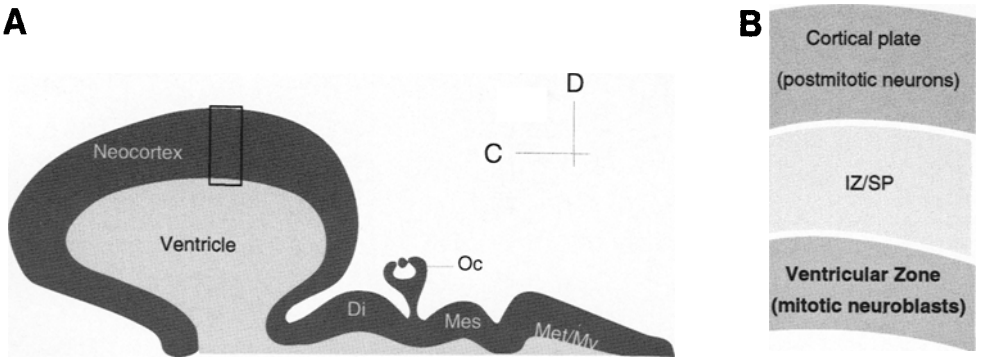

Fig. 2. Schematic diagram of the embryonic neural tube, and embryonic layers of the cerebral wall indicating the ventricular zone. The neural tube is presented in parasagittal view, with the proximal portion of the tube indicated as an enlarged cerebral cortex (neocortex). Within the lumen of the tube is the ventricle that contains cererbrospinal fluid. Enlargement of the cerebral wall (rectangle in **A**) is shown in **B**. Adjacent to the ventricle is the region of cell proliferation, the ventricular zone. The vast majority of cerebral cortical neurons are generated here before birth. Newly postmitotic neurons migrate superficially from this zone, through the intermediate zone/subplate (IZ/SP), then locate within the cortical plate (which become the cortical layers of the adult). The ventricular zone is expanded in greater detail in Fig. 3. Abbreviations: Di, diencephalon; Oc, optic cup; Mes, mesencephalon; Met/My, metencephalon/myelencephalon; C, cephalad (head); D, dorsal.

LP_{B1}). Further support for the grouping is presented in experimental expression data. The existence of multiple LP receptors with different tissue distributions, ligands/ligand affinities and intracellular signaling pathways can account for a variety of seemingly disparate or contradictory observations in the literature. It is further likely that this family of receptors has both additional members and additional subgroups that could be receptors for other LPs. The prominent expression of several of these LP receptors in the developing and mature nervous system indicates that they likely have hitherto unanticipated roles in nervous system biology and pathology.

NEUROBIOLOGICAL STUDIES LED TO CLONING OF VENTRICULAR ZONE GENE-1 (*VZG-1*)

The first LP receptor gene, *vzg-1*, was identified in studies of the embryonic cerebral cortex as a receptor for LPA. This receptor gene

name reflects the basic neuroanatomy of the system (Fig. 2). Arising from the proximal portion of the neural tube, the cerebral cortex undergoes marked cell proliferation during embryonic life, with the vast majority of neurons existing in the adult cortex arising from a proliferative region that surrounds the ventricles (the ventricles being filled with cerebrospinal fluid), thus named the ventricular zone (VZ). There are three major cellular outcomes that occur within this zone. These are

1) proliferation *(18,22)*,
2) differentiation *(18–23)*, and
3) death *(24–27)*.

The molecular mechanisms controlling these activities are not known in detail.

A striking feature known through many decades of morphological study is that, within the VZ during the period of neurogenesis, cortical blasts undergo a peculiar motion that is somehow associated with the three cell fates. Called interkinetic nuclear migration or simply described as a to-and-fro motion that was first reported over 60 yr ago *(28,29)*, proliferating blasts have a fusiform or bipolar shape during S-phase, then retract their pial (also called basal or superficial) process to form a rounded (spherical) morphology at the ventricular surface. At this point the rounded cell undergoes mitosis, and the daughter cells regain their fusiform shape to continue the process. Alternatively, if the cell becomes postmitotic, it then migrates out of the VZ to locate in a superficial compartment of postmitotic neurons. The to-and-fro motion is shown schematically in Fig. 3. Thus, morphological cell rounding changes are part of the normal processes occurring within the VZ, and through these motions and outcomes, the vast majority of cerebral cortical neurons are generated during embryonic life.

The embryonic brain provides numerous challenges to molecular approaches, not the least of which is the limiting amount of tissue in an embryonic VZ, and the lack of an easily accessible, analytical system to study the function of VZ genes. To overcome some of these difficulties, cell lines were produced from this region in the mouse. It should be noted that, with very few exceptions, neuronal cell lines in common use (e.g., neuroblastoma *[30,31]* cells) are derived from the neural crest that itself gives rise to the peripheral nervous system. By contrast, demonstrated central nervous system

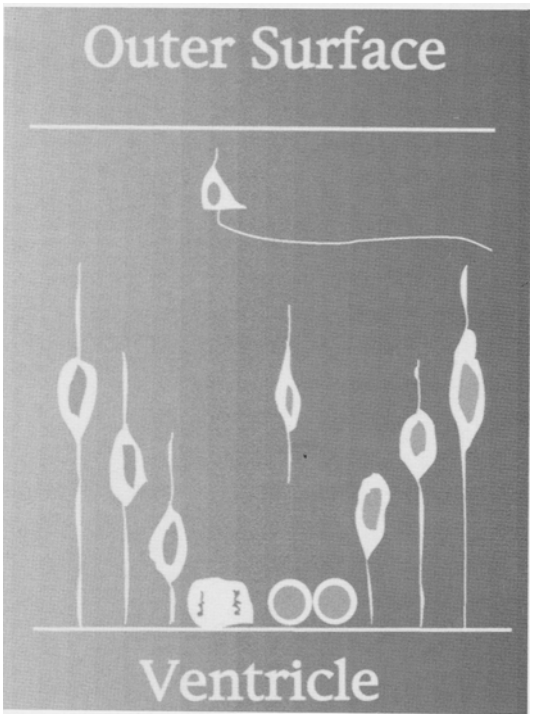

Fig. 3. Schematic diagram of cell motions within the ventricular zone. Within the ventricular zone, proliferating blasts do not simply divide like a tumor but rather undergo a regular motion whereby a fusiform cell (left part of diagram) undergoes DNA synthesis, then migrates to the ventricular surface where it becomes "round," retracting its pial (superficial) process, undergoes mitosis, then either again becomes fusiform to repeat the process or migrates away as a postmitotic neuron. This motion has been called "interkinetic nuclear migration" or a "to-and-fro" motion. The motion is linked to cell proliferation, differentiation and death of cortical neuroblasts.

(CNS) cell lines with neuronal characteristics such as those observed within the cerebral cortex, are rare. To develop CNS neuronal cell lines, a novel, 2-hit approach utilizing sequential delivery of different, oncogenic retroviruses allowed the generation of clonal lines expressing markers that were consistent with cells of a cortical VZ origin *(34)*. The cell lines (called TR and TSM for Large T, Ras and Large T, Src-Myc, respectively) provided a simplified source of cDNA from which genes of interest could be identified and analyzed *(1,23,25)*.

One gene family of particular interest was that encoding GPCRs. Thus, GPCR gene fragments were amplified from TR and TSM cell line cDNA by degenerate PCR strategies, as had been used successfully in other systems *(1,36,37)*. The rationale for this approach was the unknown existence of GPCR interactions within the VZ despite the clear role of such receptors in cell proliferation, differentiation and death in other tissue systems. The GPCR family is both large and has documented roles in normal CNS function (e.g., adrenergic and muscarinic receptors). Moreover, studies on a variety of polypeptide growth factors, mediated by receptor tyrosine-kinases that functioned within the embryonic cortex, did not appear to be sufficient to account for all of the signaling phenomena associated with cortical development *(38–41)*, raising the possibility that GPCRs could have a relevant role.

GPCR genes were amplified at several different stringencies and in all combinations of multiple primer pairs. The hundreds of resulting cDNA fragments from the initial screens revealed a number of species with enriched expression within embryonic cortex by *in situ* hybridization. One fragment, represented by multiple, independent clones, showed restricted VZ expression (Fig. 4) and was thus named *vzg-1* in 1993 *(70[1])*. These fragments were used to identify a full-length cDNA that encoded a novel GPCR gene that possessed highest and similar degrees of homology to a cannabanoid receptor and a human orphan receptor called endothelial differentiation gene-1 *(edg-1)(46)*. Northern blot analysis revealed a single mRNA band of 3.8 kb in the CNS. This raised the question of the biological function–particularly the identity of a ligand–for this putative receptor. To address this issue, mammalian expression constructs were made with coding regions in either the sense (coding) or antisense (orientation control) orientations compared to the empty expression vector (vector control) and transfected into the cell lines from which *vzg-1*-dependent morphological changes might be observed (with limiting *lacZ* vector cotransfection to visualize *vzg-1*-transfected cells). Morphological changes were monitored as the initial screen because of its ease and speed, and the possibility that one of the three known VZ outcomes and/or morphological changes like the to-and-fro motion might be observed.

Following *vzg-1* transfection and overexpression (as determined by Northern blot), a clear difference in the morphology of the cells

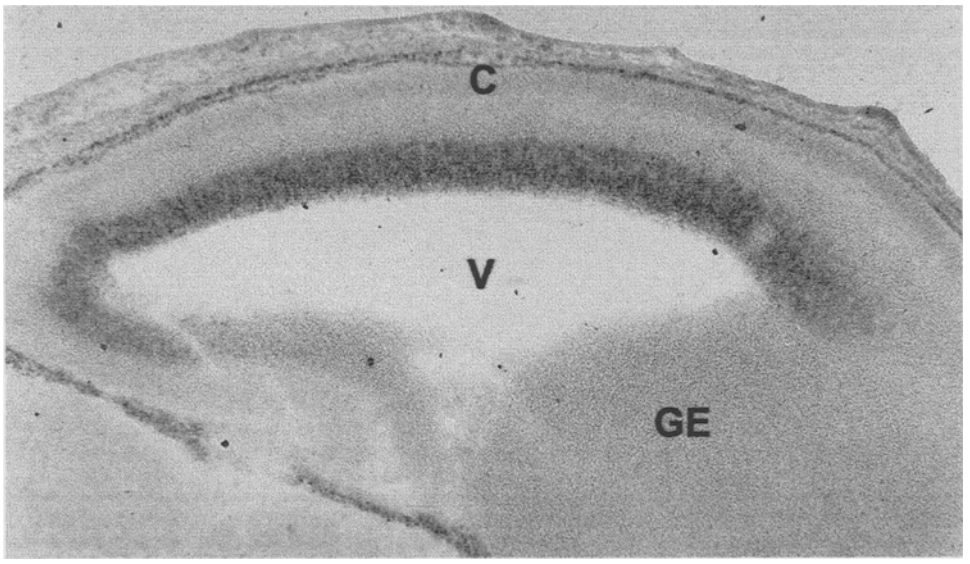

Fig. 4. *In situ* hybridization with *vzg-1* labels the ventricular zone of the cerebral cortex. An embryonic day 16 brain was sectioned in the parasagittal plane and processed for *in situ* hybridization. Note the dark band that partially encircles the ventricles (V). Labeling is not seen in the cortical plate (C) nor is it present in noncortical, neuroproliferative regions of the brain (GE is the ganglionic eminence that predominantly gives rise to neurons of the basal ganglia). The nose of the animal is to the right. Additional signal can be seen in the outer covering of the brain, but the cells responsible for this have not been identified. Thickness of the *vzg-1* band is about 100 μM.

was observed: their shape went from fusiform (or pyramidal) to round, appearing as spheres by light microscopy (Fig. 5, bright round cell). Overexpression of the *vzg-1* cDNA only in the sense orientation (not antisense or empty vector) was found to produce this cell rounding. Importantly, rounding was further found to require the presence of serum, and the active component(s) was also found to be heat-stable. These experimental observations were consistent with the known and biologically relevant to-and-fro motions of VZ neuroblasts. Moreover, detailed studies on the behavior of dissociated cortical blasts in culture indicated that their growth and survival depended not only on peptidergic growth factors, but also required unknown factors that were present in serum or cell membrane fractions *(39,41,42)*. From these data, the ligand had proper-

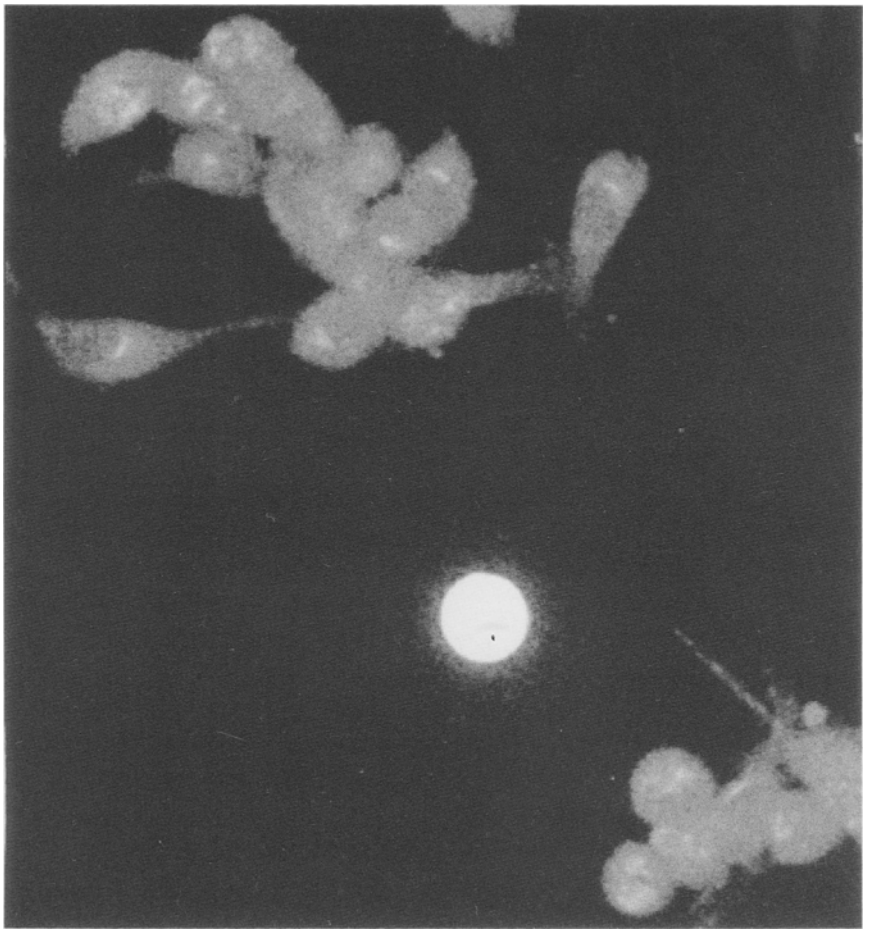

Fig. 5. Cell rounding produced by overexpression of the *vzg-1* sense construct with limiting amounts of *lacZ*, as revealed by anti-β-galactosidase immunofluorescence (single bright round cell). Rounding occurs within 15 min and is clearly distinguishable from the morphology of untransfected cells. Rounding requires the expression of the sense construct and can be observed with nM concentrations of LPA. Cell diameter is approximately 12 μm.

ties of being a heat-stable component of serum and perhaps membranes that participated in morphological changes in neuroblasts, and could also be involved in proliferation, differentiation and/or death. This profile pointed to a well-documented class of cellular signaling molecules, the LPs, which led in turn to the identification of LPA as a ligand for the *vzg-1* gene product.

VZG-1 ENCODES A FUNCTIONAL RECEPTOR FOR LPA

From this initial identification of *vzg-1* as a putative receptor for LPA, a range of criteria were examined using both transient and stable cell lines transfected with the sense construct (as well as with the antisense and empty vector constructs), and analyzed for LPA responsivity. In all these experiments, expression of the transfected gene was ascertained by Northern blot, and the gene product ascertained by Western blot *(1)*; these expression controls are essential in any rigorous experiment that examines the role of a putative receptor since, without these controls, proposed receptor mechanisms remain in question. It should be noted that the lipophilic nature of LPA makes rigorous ligand binding, at least to date, unachievable, as high levels of nonspecific binding are observed. Nevertheless, overexpression of *vzg*-1 always produced an overall increase in ^{3}H-LPA membrane binding (*1*, Table 1). Thus, using a range of independent techniques to examine the identity of the *vzg-1* gene product, the independent biology of the VZ neuroblasts, and compared to the prior literature, *vzg-1* encoded a high-affinity receptor for LPA with the properties listed in Table 1.

A prediction from these overexpression experiments was that heterologous expression of the same receptor in a receptor-null cell line should produce essentially the same responses. This was demonstrated in cell lines from two distinct lineages that neither expressed *vzg-1* nor responded to LPA *(15)*. Using epitope-tagged *vzg-1/lp*$_{A1}$ (along with other constructs), combined with assays for expected LPA responses (*see* Table 1), *vzg-1/lp*$_{A1}$ was found to couple directly to Gi, stimulate pertussis toxin insensitive activation of Rho, and was necessary and sufficient for mediating LPA-dependent responses in a dose-dependent manner. Morever, *vzg-1/lp*$_{A1}$ was able to activate multiple responses in cell lines from distinct embryologic lineages (neuronal and nonneuronal), demonstrating that a single receptor was capable of mediating mutiple responses to LPA.

This identification provided a rational framework for testable hypotheses, and related to this, independent confirmation has been reported by at least two groups in overexpression analyses of the human *vzg-1/lp*$_{A1}$ homologue referred to as *edg-2* (*43*; *see* Tables 1–3 for relevant data; the human sequence has 97% aa identity to mouse) and by heterologous analyses in yeast—which do not respond to LPA—utilizing the pheromone response pathway to demonstrate

Table 1

Properties of the Vzg-1/lp_{A1} LPA Receptor[a,b]

Structure:	7-transmembrane (predicted) GPCR
Genomic data:	5 exons on murine Chr. 4 (partial duplication on 6 in *m. spretus*)[c]
mRNA size:	3.7–3.8 kb
Number of amino acids:	364
Predicted Mol. Mass:	41 kda
Observed Mol Mass:	41–42 kda (Western blot of cell lines and brain)
Effects on ^{3}H-LPA binding:	Heterologous expression and homologous over expression increases specific membrane binding
Effects on ^{35}S-GTPγS binding:	Heterologous expression increases ^{35}S-GTPγS binding to G-proteins (Gi, other) that is dose-dependent on LPA
Effects on cell rounding:	Heterologous expression is necessary and sufficient for, and homologous overexpression increases, cell rounding in neuroblast cell lines following LPA exposure (EC_{50}=1.3 n*M*), pertussis toxin insensitive, C3-exoenzyme sensitive
Effects on stress-fibers:	Heterologous expression is necessary and sufficient for LPA-dependent formation of stress fibers (fibroblast cell lines), pertussis toxin insensitive, C3-exoenzyme sensitive
Effects on cAMP levels:	Overexpression reduces cAMP concentrations following LPA exposure (EC_{50}=5.9 n*M*), pertussis toxin sensitive SRE-activation: Heterologous expression activates exogenous *cfos* SREs dose-dependent on LPA.
BrdU incorporation:	Heterologous expression increases incorporation
Ligand specificity:	Oleoyl LPA > Stearoyl LPA; not stimulated by related compounds at 1 μ*M* (oleyl LPE, LPG, LPC; PA)
Tissue distribution:	High levels in CNS, undetectable levels in liver, intermediate levels in other tissues (lung>kidney>spleen)
Orphan receptor synonyms:	Rec1.3[d], EDG-2 (sheep[e], human[f]) (ligand not identified)

[a]Data from Hecht et al. (1996) *J Cell Biol* **135**, 1071–1083.

[b]Data from Fukushima, Kimura and Chun, (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 6151–6156. A single receptor encoded by *vzg-1/lp_{A1}/edg-2* couples to G-proteins and mediates multiple cellular responses to lysophosphatidic acid (LPA).

[c]Contos and Chun, Complete cDNA sequence, genomic structure and chromosomal localization of the LPA receptor gene, *vzg-1/lp_{A1}, (73).*

[d]Rec 1.3–Macrae, A.D (1996) *Mol Brain Res* **42**, 245–254. mRec is a probable splice variant of *vzg-1*, differing in the extracellular amino terminus of the receptor.

[e]Masana et al. (1995) *Receptors Channels* **3**, 255–262.

[f]Incyte Pharmaceuticals (Palo Alto) patent filing (1995), other independent clonings.

Table 2

Characteristics of LP Receptorsa

Name (gene)	% Identity to *vzg-1*	Synonyms	Mr	Chromosomal location	Isolation methods	Expression	Refs.
LP_{A1} (lp_{A1})	100%	*vzg-1*	41109	mouse 4	PCR from cortical lines	Embryonic: cerebral cortex	Hecht et al. (1996)
	see text	*mrecl.3*	39265	(by *D4Mit44*)	PCR from taste papillae	Adult: brain, testes, muscle	Macrae et al. (1996)
	97%	*edg-2*	41119		PCR from pars tuberalis	Lung, spleen, heart	Masana et al. (1995)
LP_{A2} (lp_{A2})	54%		39060	human 19p12	Human chromosome 10 genomic sequence (cosmid R33799)	Embryonic (?) Adult kidney, testes, lung	Genbank accession # AC002306
LP_{B1} (lp_{B1})	36%	*edg-1*	42695		Differential library screen for endothelial differentiation genes	Widely in embryo >E15 adult brain, liver, spleen, lung, heart	Hla and Maciag (1990)
LP_{B2} (lp_{B2})	32%	*H218*	38735	mouse 9 (by *Epor*)	Low stringency screen w/D2 receptor	Embryonic brain	MacLennan et al. (1994)
		AGR16			PCR from rat aortic smooth muscle	Postnatal brain, heart, lung, stomach, intestine, adrenal	Okazaki et al. (1993)
LP_{B3} (lp_{b3})	35%	*edg-3*	42294	human 9q22.1–2	PCR from genomic DNA using cannabinoid receptor primers	Embryonic (?) Adult: heart, placenta, lung, liver, muscle, kidney, pancreas	Yamaguchi et al. (1996)

[a]Molecular mass (Mr) is predicted based on translated sequence but does not include possible glycosylation differences. The chromosomal location of *edg-1* has not been reported.

Table 3

RT-PCR Detection of *lp* Members in Various Cell Lines Compared to LPA or S1P Responsivity[a]

Cell Line	B103	CHO	293	Swiss 3T3	COS
lp member					
lp_{A1}	-	+	++	+	+
lp_{A2}	-	-	+/-	-	+/-
lp_{B1}	-	-	++	+++	+
lp_{B2}	+/-	+	?	+	?
lp_{B3}	++	-	++	+	+++
LPA responsive	No	Yes	Yes	Yes	Yes
S1P responsive	Yes	Yes	Yes	Yes	Yes

[a]Cell lines commonly used in the analysis of lysophospholipid signaling, along with a neural cell line (B103 *[15]*) were examined for expression of receptor mRNA using reverse-transcriptase-PCR, and the resulting PCR products (after 30 cycles) detected by gel electrophoresis and and ethidium bromide staining. -, No detectable signal; +/-, barely detectable signal; +, a clear signal; ++ to +++, strong to very strong signals; ?, reflects lack of primate PCR primers for the analysis. LPA and S1P responsivity was determined using a combination of cAMP and cytoskeletal-change measurements.

the LPA-dependent coupling of human *vzg-1* to the yeast heterotrimeric G-protein in stimulation of the MAP kinase pathway *(14)*. Based on this identity as an LPA receptor, clear comparisons can be made between *vzg-1* and homologous genes, which led to obvious predictions about the receptor identity in view of the molecular structure of LPA and the prior literature. Before exploring these issues, it is worth considering where *vzg-1* is normally expressed.

mRNA EXPRESSION PATTERN OF *VZG-1* WITHIN THE NERVOUS SYSTEM

Expression of *vzg-1* was first observed in the brain *(1)* and was present from at least embryonic d 12 (gestation in the mouse is about 20 d) through adulthood. By Northern blot, a single band was observed with an apparent size of 3.8 kb (although more than one, similar-sized species may also be present within the single band; discussed below). *In situ* hybridization revealed two, general patterns of expression during development. In embryonic life, the majority of labeling was found associated with neuroblasts within the ven-

tricular zone (VZ), with a smaller but clearly detectable signal present near the pial surface (most superficial portion) of the cortex that is associated with other cell types of unknown lineage. The embryonic period of cortical neurogenesis extends from around E11-E18 and *vzg-1* expression appeared most robust in the VZ during this period, but gradually decreased to undetectable levels from the VZ around the same time as the last cortical neurons are being produced *(71)*.

The second major *in situ* hybridization expression pattern (Weiner, Hecht and Chun, Soc. Neurosci. Abstr. 23: 1689 and submitted) *(70,72)* became evident during the first postnatal week where it is most prominent in developing fiber tracts. Use of double-labeling *in situ* hybridization techniques indicated that a major locus of expression is within developing oligodendrocyte glial cells. This expression pattern correlates with the process of myelination and is maintained during the first few postnatal weeks, then decreases to lower levels that continue to be observed during adulthood. The precise locus of expression in the mature adult CNS is not entirely certain but appears to be primarily glial with much lower expression amongst neuronal constituents. Expression in the peripheral nervous system has also been observed.

mRNA EXPRESSION PATTERN OF *VZG-1* OUTSIDE OF THE NERVOUS SYSTEM

In addition to expression of *vzg-1* within the embryonic cortex, expression in other parts of the embryo were apparent, most notably within cells that appeared to be of fibroblast lineage based on *in situ* hybridization *(1)*. During adult life, Northern blots revealed *vzg-1* expression to be most prominent within the CNS and it was also detectable within the lung, kidney and spleen, but was not detected in the liver *(1)*. Expression has also been reported in the testes *(44)*. A more comprehensive tissue survey by *in situ* hybridization is currently being pursued.

GENOMIC STRUCTURE AND CHROMOSOMAL LOCATION OF *VZG-1* REVEALS MULTIPLE EXONS ON CHROMOSOME 4: COMPARISON TO *EDG-1*

Analysis of genomic clones for *vzg-1* has revealed that the gene spans over 45 kb and is separated into at least five separate exons on

murine chromosome 4 (*73*; see Table 1). The chromosomal location generally agrees with the location of mrec1.3 *(44)*, although the physical position on the chromosome appears to be distant from the reported centromeric location. The difference in the amino terminus sequence between Vzg-1 and mrec1.3 can be attributed to alternate 5' exons found in divergent cDNA clones that are located upstream from a common exon sequence encoding most of the open reading frame (ORF), but ending at an intron located within the middle of transmembrane domain VI. Such a genomic structure is not observed with any other GPCR genes. The most similar are the oxytocin and vasopressin receptor genes, which contain an intron between transmembrane domain VI and VII, not within it. Thus, *vzg-1* has a novel genomic structure consisting of multiple exons in the coding region and further undergoes mRNA splicing that produces two, similar-sized gene products. By contrast, a mouse genomic clone for *edg-1* contains two exons, with a single coding exon preceded by a 1.6 kb intron, located 172 bp upstream of the initiation codon *(45)*. There is no intron located within transmembrane domain VI.

Vzg-1 and edg-1: Basis of a Proposed Nomenclature for LP Receptors

Based on the initial characterization of *vzg-1* and subsequent confirmatory studies, it is clear that *vzg-1* encodes a receptor for LPA. This does not, however, imply that only a single receptor exists, and indeed, it would be surprising for a GPCR super-family member to exist alone. At the time of its initial identification in 1993, *vzg-1* had highest database homology (37% aa) to the orphan receptor gene *edg-1 (46,47)*. Hla and colleagues also identified other *edg* members that are not structurally homologous to *edg-1*, including *edg-2*, a possible transcription factor *(45)* and *edg-3*, which is IkB/MAD-2 *(49)*. These *edg* members have identical names with other genes that encode homologues of *vzg-1* and *edg-1* (discussed further below, and see Table 2) that have been referred to as *edg*s, as well as by numerous other designations. The identity of the ligand for the *edg-1* gene product has been recently identified as S1P *(16,17)*, however there may also be low-affinity (LPA) receptor characteristics (EC_{50} in the μM range, *[74]*). As previously noted, *vzg-1* has a distinct genomic structure compared to *edg-1 (45)*. This difference in genomic structure, relatively lower aa sequence homology (compared to other receptor

genes; *see* below) and different high-affinity ligand specificities that may nonetheless show lower affinity similarities suggest that *vzg-1* and *edg-1* have a common evolutionary origin in functioning with LP ligands, that subsequently diverged to produce functionally distinct receptors.

To acknowledge the functional identity of members of this receptor family and integrate aa similarities along with differences in genomic structure, we propose the following nomenclature. As the first demonstrated lysophospholipid receptor, Vzg-1 would be renamed Lysophospholipid receptor A1 ($\mathbf{LP_{A1}}$) whereas Edg-1 would be renamed Lysophospholipid receptor B1 ($\mathbf{LP_{B1}}$). Similarly, their gene names would reflect this designation (lp_{A1}, lp_{B1}, respectively). This nomenclature also leaves open any number of other receptors within each subgrouping, and it can also accommodate distinct receptor subgroups (e.g., $LP_{C,D}$, etc.). An advantage of this nomenclature is that it leaves open the possibility of more than one biolgically relevent ligand, a point underscored by the fact that a more distant but nonetheless related receptor, the cannabanoid receptor CB1 (*see* Figs. 5–7) acts not only with anandamide but also with 2-arachadonyl glycerol *(50)*. This nomenclature also eliminates confusion over the names of the receptors and their genes, especially in view of published *edg* members encoding nonreceptor proteins, and allows consolidation of the many non-edg names as well (e.g., EST postings, H218, AGR16, rec1.3, etc.; *see* below).

ORPHAN RECEPTORS CAN BE GROUPED AS LP_A OR LP_B BASED ON PREDICTED AA SEQUENCE AND GENOMIC STRUCTURE

In this light, a number of other receptor genes with unknown ligand identity but with predicted aa sequence homology to LP_{A1} (Vzg-1) and LP_{B1} (Edg-1) receptors have also been reported or entered into sequence databases under a variety of different names (summarized in Table 2 *(44,46,51–56)*, and various unpublished database entries including public domain patent filings). Based solely on their aa similarity/identity (Fig. 6, Fig. 7), these putative receptors, while significantly homologous to one another, have highest homology to distinct members which divides them into two subgroups. The two members of the LP_A subgroup have aa similarity of over 60% while those of LP_B also have approx 60% aa similarity to

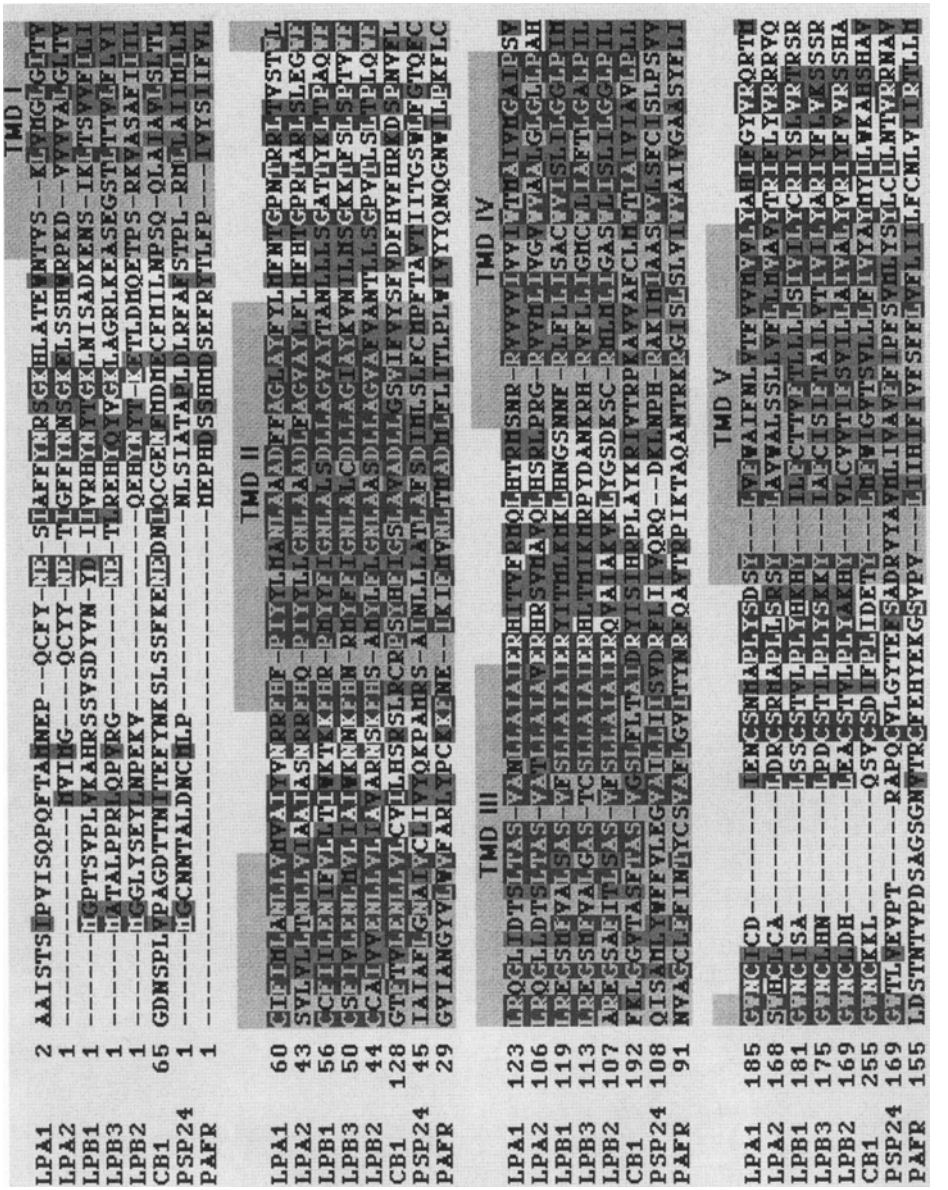

Fig. 6. Part 1.

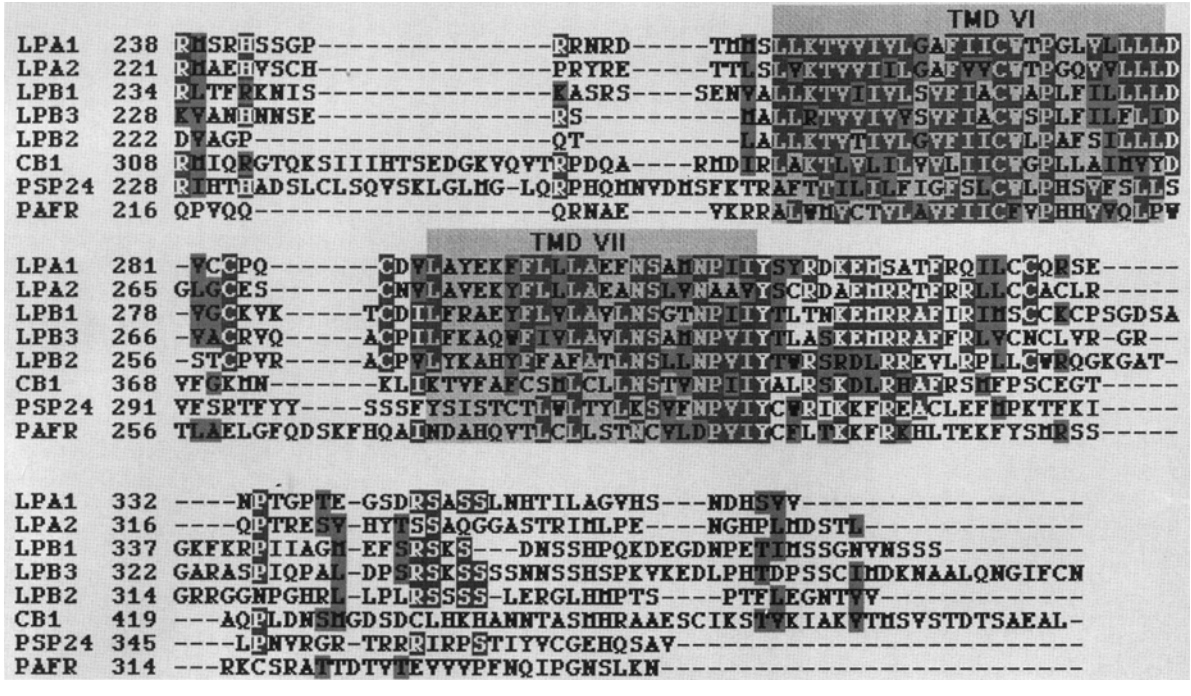

Fig. 6. Alignment of LP, CB1 cannabinoid, PSP24, and PAF receptor proteins, based on predicted amino acid sequences. Amino acid residues identical in 50% or more of the aligned sequences are indicated by white letters, while conservative amino acid substitutions are indicated by a darker grey surround. Putative transmembrane domains are shaded light grey. LP_{A1}, LP_{A2}, LP_{B1}, LP_{B3}, and PAF-R are human sequences, while LP_{B2} is rat, CB1 is mouse, and PSP24 is *Xenopus*. Progressive alignment was done with the CLUSTAL W program *(67,68)*.

% AMINO ACID IDENTITY

	LP_{A1}	LP_{A2}	LP_{B1}	LP_{B2}	LP_{B3}	CB1	PSP24	PAF-R
LP_{A1}		53.5	35.6	31.9	35.3	29.7	23.8*	11.8*
LP_{A2}	60.4		32.8	34.2	32.1	25.4	21.8*	15.1*
LP_{B1}	45.5	41.9		47.1	51.1	26.9	16.1*	14.1*
LP_{B2}	41.3	41.5	58.6		46.3	27.1	19.0*	14.1*
LP_{B3}	43.9	41.9	60.9	54.9		25.3	13.7*	15.3*
CB1	37.8	35.3	36.4	35.3	34.4		21.4*	20.4*
PSP24	*	*	*	*	*	*		17.8
PAF-R	*	*	*	*	*	*	28.0	

% AMINO ACID SIMILARITY

Fig. 7. Comparison of the LP receptor family based on predicted amino acid identity and similarity. Percentage amino acid identity is shown in the upper right part of the figure, while percentage amino acid similarity (referring to conservative amino acid changes) is shown in the lower left part of the figure. Pairwise alignments were conducted with full-length polypeptide sequences using the version 9.1 GCG GAP program (Genetics and Computer Group, University of Wisconsin). Asterisks (*) indicate pairwise sequences that are too dissimilar to align using the default parameters in the program (i.e. gap creation penalty of 12, gap extension penalty of 4, and the Blosum62 substitution matrix). To obtain percentage identities for these sequences, comparisons were made using the Feng-Doolittle software package *(69)*. For sequence pairs where both programs could be used, % identites were always within two percentage points. This program does not give % similarity scores, so these have been left blank.

LP_{B1} (Edg-1). By contrast, the similarity between the two subgroups drops significantly (e.g., LP_{A1} vs. LP_B ranges from 41–45% aa similarity; Fig. 7). This subgrouping receives independent support from analysis of gene structure, as noted for lp_{A1} (*vzg-1*) and lp_{B1} (*edg-1*) previously. In general, the more homologous receptors are, the more likely that their genes will have a similar genomic structure. This conservation is strongest in the coding region, where small nucleotide changes near intron/exon boundaries can cause out-of-frame mutations. Examination of the genomic structure for the genes in

Table 2 indicates that lp_{A1} and lp_{A2} have coding regions represented by 2–3 exons, whereas lp_{B1}, lp_{B2}, and lp_{B3} are each encoded by a single exon *(75)*. The latter structure of the lp_B subgroup is similar to the CB1 cannabinoid receptor gene, which is also encoded by a single exon preceded by an intron *(57)*. This suggests that the transmembrane domain VI intron observed in the lp_A subgroup arose after divergence from an ancestral gene common to all LP receptors; moreover, an even earlier ancestor is likely shared by both the LP and cannabanoid receptor families (*see* Fig. 8). Interestingly, the relationship between LP and cannabanoid receptors appears to extend beyond this aa homology and genomic structure, based on the recent discovery of a second endogenous cannabanoid ligand *(50,58,59)* that is sn-2 arachidonylglycerol (*see* Fig. 1); the molecular similarity amongst the LP ligands LPA and S1P and this cannabanoid ligand are consistent with the structural similarity amongst these receptors.

LP_B RECEPTORS INCLUDE HIGH-AFFINITY RECEPTORS FOR S1P

The existence of at least two distinct branches in the lysophospholipid receptor family raises the question of the identity of high-affinity ligands for the subgroups. The prototypical LP_A member, LP_{A1}, is a high affinity receptor for lysophosphatidic acid but could conceivably act as a low-affinity receptor for one or more members of the LP family. By comparison, LP_B members, as epitomized by LP_{B1}, might act as low affinity receptors for lysophosphatidic acid, but also appear to serve as a high-affinity receptor for some other LP. Prior observations have reported that LPA and the structurally-related molecule sphingosine-1-phosphate (S1P; *see* Fig. 1) can act on the same *(60)* or different *(61)* receptors. In light of the existence of multiple LP receptors, an explanation for these differences is that the cellular responses that are assayed—be they calcium or chloride conductances, G_i-linked or cytoskeletal changes—will depend on 1) the concentration of the ligand, and 2) the single or multiple receptors that are expressed by the examined cell. Studies in which the same receptor was postulated to mediate effects of both LPA and S1P were assayed using micromolar concentrations of these ligands *(60)* whereas nanomolar concentrations were used in studies where different receptors were postulated *(61)*. Moreover, the different cell

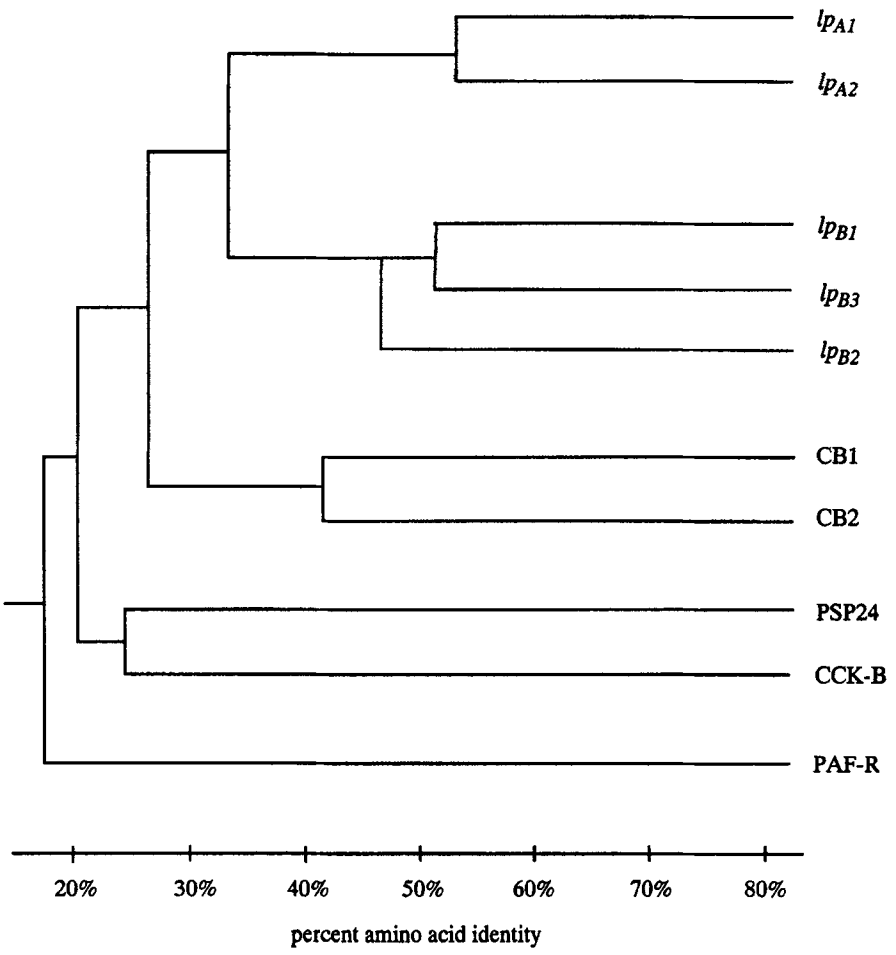

Fig. 8. Proposed evolutionary divergence of the *lp* receptor genes based on amino acid identities. The farthest branch point connecting any pair of genes indicates the approximate % amino acid identity between the two. Bootstrap analysis conducted using either the CLUSTAL W *(67,68)* or Feng-Doolittle *(69)* software packages results in the same relative groupings and subgroupings of genes. The most homologous known receptors in Genbank to the putative *Xenopus* LPA receptor PSP24 are the cholecystokinin (CCK) receptors (shown for the B subtype) that interact with polypeptide ligands. Other abbreviations: CB1 and CB2 (cannabinoid receptors 1 and 2), PAF-R (platelet activating factor receptor)

systems examined—platelets vs N1E-115 neuroblastoma cell lines—could easily explain the differences observed between the two studies as it would not be surprising to find a range in the number of receptors expressed by a given cell type. Based on the aa similarites

between LP_A and LP_B receptor subgroups, it was highly likely that a ligand for the B group should be similar in structure to LPA itself yet distinct, based on the apparent low affinity interactions of LP_{B1} with LPA, observed by Hla and colleagues. The existence of S1P receptors in the LP_B subbranch has recently received strong support by studies *(16,17,62,75)*, and this ligand identity is entirely consistent with the aa homology between the LP_A and LP_B receptor subfamilies.

ENDOGENOUS EXPRESSION OF LP RECEPTORS IN ROUTINELY USED CELL LINES

One of the difficulties encountered in the analysis of LP receptor functions is the presence of endogenous LPA responses. The use of heterologous expression in mammalian cells used to characterized *vzg-1/lp$_{A1}$ (15)* was made possible by knowledge of where this gene was not expressed, based on *in situ* hybridization studies *(1)*; two tissue loci that did not have detectable expression were the liver and embryonic CNS regions outside of the cerebral cortex. This led to a search of cell lines from these tissues that in turn, allowed identification of cells that neither expressed *vzg-1/lp$_{A1}$* nor responded to LPA *(15)*. With the availability of LP receptor nucleotide sequences, it is a simple matter to examine the profile of known LP genes expressed in a given cell line and to compare this expression profile to the responsivity of these lines to submicromolar concentrations of LPA or S1P using cAMP and/or cytoskeletal changes as a measure of cellular response (Table 3).

Based on these analyses, two major conclusions can be drawn. First, common cell lines used in the study of LPA/S1P signaling *(7,63)* contain multiple LP receptors (at least at the mRNA level), which obfuscates the issue of how a single LP receptor actually signals and raises the potentially major analytical problem of heterologous desensitization of an assayed signaling pathway by an endogenously expressed receptor. Second, it is clear that LP_B expression correlates with S1P responsivity, whereas LP_A members correlate with high-affinity LPA responsivity. This identity assignment has received further support from overexpression transfection studies and ^{35}S-GTPγS labeling of G-proteins in ligand-dependent manner *(75)*. Note that this does not mean that the LP receptors *only* interact with these ligands, and the referral to these as lysophospholipid receptors leaves open the possibility that multiple ligands

can interact at a single receptor, albeit with discernible differences in apparent affinity.

NON-HOMOLOGOUS LP RECEPTORS?

An issue that remains unresolved is that of a GPCR with markedly dissimilar aa sequence that has been reported to be a *Xenopus* high affinity receptor for LPA *(64)*. This receptor, called PSP24, was initially identified based on studies using the platelet activating factor (PAF) receptor nucleotide squences in antisense strategies to block electrophysiologically identified LPA responses in oocytes. PSP24 is remarkable for its lack of similarity to either LP_A or LP_B receptor subfamilies, or for that matter, similarity to the PAF receptor (Figs. 7,8). Indeed, beyond conserved residues common to most GPCR family members, PSP24 is not related to either the LP or PAF receptor subfamilies, but does have signficant homology to the GPCR for the polypeptide hormone cholecystokinin (CCK-B; Fig. 8). In view of the aa similarities amongst LP and CB1 receptors, and a consistent structural similarity of their respective cognate ligands—the LPs and 2-arachidonyl glycerol— it is unclear how PSP24 functions as a receptor for LPA. Either PSP24 interacts in a fundamentally different way with LPA as compared to the LP receptor family, or it is not a *bona fide* LPA receptor. Further studies should resolve this issue.

FUTURE DIRECTIONS

The availability of cloned LP receptors settles debate over their existence, and will allow the systematic categorization of ligand interactions, and receptor-dependent repsonses for each of the LP receptors, along with the identification of new receptors and related ligands. The existence of multiple receptors for a single ligand likely underlies differences in expression pattern—with relevence to different tissues or developmental stages—and/or differences in the recruited signaling paths following ligand stimulation. Molecular genetic approaches will allow a detailed analysis of these LP receptors using approaches ranging from site-directed mutagenesis, through the creation of receptor-null mice. This latter approach is necessary and will be especially informative since there are currently no truly specific competitive antagonists with which to block LP receptor function, which has hampered an understanding of the bio-

logical significance of this signaling system. Of special note will be the role of LP receptors in the nervous system, where their prominent expression likely underscores important though as yet undefined roles in development, normal function and pathology.

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