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Signaling Mechanisms and Molecular Characteristics of G Protein-Coupled Receptors for Lysophosphatidic Acid and Sphingosine 1-Phosphate

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Abstract Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are potent phospholipid mediators with diverse biological activities. Their appearance and functional properties suggest possible roles in development, wound healing, and tissue regeneration. The growth-stimulating and other complex biological activities of LPA and S1P are attributable in part to the activation of multiple G protein-mediated intracellular signaling pathways. Several heterotrimeric G proteins, as well as Ras- and Rho-dependent pathways play central roles in the cellular responses to LPA and S1P. Recently, several G protein-coupled receptors encoded by a family of endothelial differentiation genes (*edg*) have been shown to bind LPA or S1P and transduce responses of cAMP, Ca^{2+}, MAP kinases, Rho, and gene transcription. This review summarizes our current understanding of signaling pathways critical for cellular responses to LPA and S1P and of recent progress in the molecular biological analyses of the Edg receptors. J. Cell. Biochem. Suppls. 30/31:147–157, 1998. © 1998 Wiley-Liss, Inc.

Key words: LPA; S1P; G protein; intracellular signaling pathways; Edg receptors

Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are prototypes of an emerging family of phospholipid mediators with diverse biological activities. They can be generated through hydrolysis of their phospholipid precursors following cell activation [Moolenaar, 1995; Moolenaar et al., 1997; Spiegel and Merrill, 1996; Gaits et al., 1997; Goetzl and An, 1998; Igarashi, 1998]. The best documented examples of major sources of LPA and S1P are activated platelets, injured cells, and cells stimulated by protein growth factors. In serum, but not plasma, concentrations of LPA and S1P can reach the micromolar range. This and the overlapping cellular responses to LPA and S1P with that of serum suggest that LPA and S1P account for much of the biological activity of serum.

The diverse biological effects of LPA and S1P are observed in organisms throughout evolution, suggesting that LPA and S1P are important conserved biological regulators in multiple systems. LPA and S1P appear to act in different cellular systems as paracrine, autocrine, and perhaps intracellular messengers. The biological responses to LPA and S1P are diverse and can be roughly subdivided into two major categories: (1) cellular proliferation and related events, and (2) cellular activities regulated by cytoskeletal structures. The functions of LPA and S1P in the first category include stimulation or inhibition of cellular proliferation, inhibition of apoptosis, and regulation of the expression of growth factors [Moolenaar, 1995; Spiegel and Merrill, 1996]. Despite their different structures, LPA and S1P resemble polypeptide growth factors in many respects. Both types of factors modulate the proliferation of multiple

Abbreviations used: LPA, lysophosphatidic acid; S1P, sphingosine 1-phosphate; *edg*, endothelium-differentiation-gene; vzg-1, ventricular zone gene-1; G protein, guanine nucleotide-binding protein; GPCR, G protein-coupled receptor; $[Ca^{2+}]_i$, intracellular calcium concentration; PLC, phospholipase C; IP_3, inositol 1,4,5-trisphosphate; PTX, pertussis toxin; MAP kinase, mitogen-activated kinase.

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cell types by binding to high-affinity receptors and evoking multiple cellular responses in addition to those related simply to growth. The second category of activities of LPA and S1P includes aggregation of platelets, contraction of smooth muscle cells, induction of growth cone collapse and neurite retraction, regulation of cell–cell aggregation and adhesion, and chemotaxis [Moolenaar, 1995; Spiegel and Merrill, 1996; Goetzl and An, 1998; Igarashi, 1998].

These complex biological activities are attributable in part to the activation of multiple intracellular signaling pathways by LPA and S1P. Crosstalk between these signaling pathways increases the diversity of LPA- and S1P-mediated signals. Recent progress in the elucidation of cellular mechanisms for specific responses to LPA and S1P has recognized central signaling roles of several heterotrimeric G proteins, Ras, Rho and intracellular Ca^{2+}, and molecular characterization of a subfamily of G protein-coupled receptors (GPCR) for LPA and S1P. This review summarizes our knowledge of signaling pathways critical for cellular responses to LPA and S1P and describes how recent progress in molecular analysis of multiple GPCRs for LPA and S1P have enhanced our appreciation of the specificity of signaling by LPA and S1P.

SIGNALING PATHWAYS ACTIVATED BY LPA AND S1P

Ample evidence suggested that the intracellular signals of LPA and S1P are transduced by G proteins (Fig. 1). The G_q, G_i and $G_{12/13}$ types of heterotrimeric G proteins have been implicated in the initiation steps of LPA and S1P signaling. Activation of G_q stimulates phospholipase C (PLC), which generates DG and IP_3, resulting in activation of protein kinase C (PKC) and intracellular calcium mobilization. The PTX-sensitive G_i recruits mitogen-activated protein kinase (MAPK) activity and decreases the intracellular cyclic adenosine monophosphate ($cAMP_i$) level in cells. Activation of $G_{12/13}$ mobilizes the small GTPase Rho to evoke actin-based cytoskeletal rearrangement. All these pathways participate in the regulation of cell proliferation by LPA and S1P. Furthermore, crosstalk mechanisms exist between the downstream effectors of LPA and S1P signaling. Crosstalk between these signaling pathways further enhances the diversity and fine-tuning of the responses of LPA and S1P.

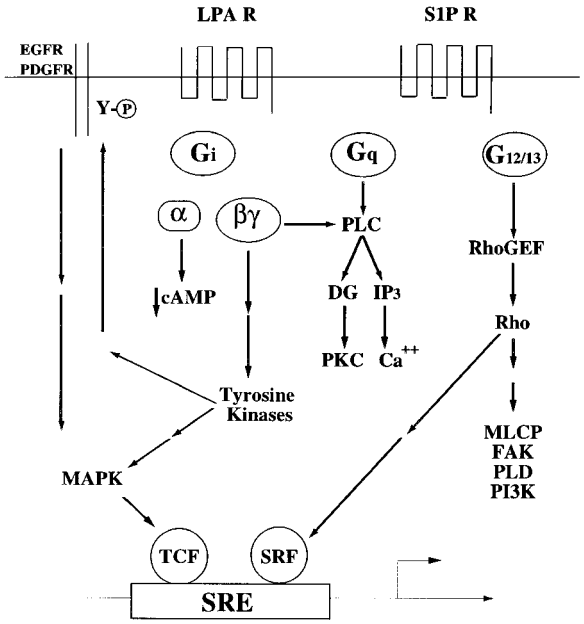

Fig. 1. Schematic outline of LPA and S1P receptor-stimulated signaling pathways leading to SRE transcriptional activation. This scheme summarizes studies of both naturally expressed and cloned receptors. It does not refer to any specific subtype of the Edg receptors. See text for further details. LPA, lysophosphatidic acid; S1P, sphingosine1-phosphate; EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptors; MAPK, mitogen-activated protein kinases; TCF, ternary complex factor; SRF, serum response factor; PLC, phospholipase C; DG, diacylglycerol; PKC, protein kinase C; IP3, inositol trisphosphate; RhoGEF, Rho guanine nucleotide exchange factor, exemplified by the p115 RhoGEF; MLCP, myosin light chain phosphatase; FAK, focal adhesion kinase; PLD, phospholipase D; PI3K, PI3 kinase.

Intracellular Ca^{2+} Mobilization

Extracellular addition of LPA results in robust increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in many cell types, including fibroblasts, platelets, vascular smooth muscle cells, neuronal cells, astrocytes, mesangial cells, many tumor cells, and *Xenopus* oocytes [Durieux et al., 1993; Jalink et al., 1995]. LPA-induced Ca^{2+} mobilization can be transduced through both pertussis toxin (PTX)-sensitive G_i and PTX-insensitive G_q proteins that activate phospholipase C (PLC).

Despite extensive observations in various cell types, the mechanism of S1P-induced mobilization of intracellular Ca^{2+} remains controversial. Some studies have demonstrated that extracellular S1P evokes Ca^{2+} responses via cell surface GPCRs and PLC activation [Okajima et al., 1996; van Koppen et al., 1996]. The recent identification of three seven-transmembrane-domain receptors for S1P supports this mode of action (see below). Other studies have proposed

an intracellular second messenger role for S1P in Ca^{2+} mobilization that is independent of inositol trisphosphate (IP_3) generation [Choi et al., 1996; Mattie et al., 1994]. The major lines of evidence supporting this mode of S1P action include high concentrations (10–100 μM) of S1P-released stored Ca^{2+} directly from the endoplasmic reticulum; heparin antagonism of IP_3 did not block this S1P-induced Ca^{2+} release; and inhibitors of sphingosine kinases also blocked FcεRI-mediated Ca^{2+} responses. However, the specific molecular target for S1P on endoplasmic reticulum (ER) membranes, presumably a Ca^{2+} channel, has not been identified.

Enhancement of the Ras-MAPK Pathway by LPA and S1P

LPA- and S1P-activated cell proliferation was susceptible to PTX inhibition, suggesting involvement of G_i protein [Goodemote et al., 1995; van Corven et al., 1989]. Further experimental results implied that the proliferative effects of LPA and S1P are dependent on MAPK activities [Howe and Marshall, 1993; Spiegel and Merrill, 1996]. Many recent reports have determined the links between GPCR and MAPK. The βγ dimeric subunits of G_i appear to be the components predominantly responsible for activation of MAPK by LPA, as contrasted with α-subunit-mediated suppression of adenylyl cyclase. The intermediates linking G_i to MAPK include Src-related tyrosine kinases [Luttrell et al., 1996] and Pyk2 [Dikic et al., 1996]. Although selective inhibitors for PI3K blocked the stimulatory effect of LPA and S1P [Hawes et al., 1996] on MAPK activity, PI3K has not been precisely positioned in the sequence. Recent reports showed that LPA induces the tyrosine phosphorylation of epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors [Daub et al., 1996], suggesting that MAPK can be indirectly activated by LPA through recruitment of peptide growth factor receptors.

LPA and S1P Activate Rho-Dependent Pathways

LPA and S1P both activate Rho through a G α12/13-dependent pathway. Rho, a Ras-related small GTPase, transduces the effects of LPA and S1P on the formation of actin stress fibers, assembly of focal adhesion complexes, and neurite retraction [Ridley and Hall, 1994; Tigyi and Miledi, 1992]. In addition, Rho plays a vital role in the actions of LPA and S1P on gene transcription [Hill et al., 1995] and pH regulation [Hooley et al., 1996]. The physical linkage between heterotrimeric G protein and Rho was recently identified [Hart et al., 1998]. Lsc/p115RhoGEF has been shown to be the adapter protein that links G α13 to Rho. Activated G α13 binds directly to Lsc/p115RhoGEF, which is a GTPase activating factor, that also acts as a guanidine nucleotide exchange factor capable of enabling Rho. The downstream effectors of Rho include multiple serine/threonine kinases, phospholipase D (PLD), PI3 kinase, p125 focal adhesion kinase (FAK), myosin light chain phosphatase (MLCP), and serum response factor (SRF) [Hill et al., 1995; Malcolm et al., 1996; Moolenaar et al., 1997; Narumiya, 1996; Spiegel and Merrill, 1996]. The multiplicity of downstream targets of Rho partially explains the complexity of signaling events triggered by LPA and S1P.

Serum response element (SRE), originally identified in the c-*fos* promoter and represented in the promoters of many growth-related genes, has been used as a model system to investigate growth factor signaling of nuclear transcriptional events. LPA and S1P activate SRE, which binds both SRF and ternary complex factors (TCF) in a concerted manner to stimulate transcription [Hill et al., 1995; An et al., 1997a; An et al., 1998b]. TCF is activated by the *ras*-MAPK pathway, while SRF activity is elicited by Rho effectors. Therefore, the full induction of cell proliferation by LPA and S1P needs coordination of G_i- and $G_{12/13}$-activated pathways.

Other Cellular Functions and Signaling Pathways Regulated by LPA and S1P

In addition to the growth-related signaling mechanisms described above, LPA and S1P regulate other cellular functions, possibly through multiple signaling pathways. S1P and LPA suppress apoptosis in some types of cells. In Jurkat cells, S1P antagonized the apoptotic effect of ceramide [Cuvillier et al., 1996]. In human T lymphoblastoma cells, S1P and LPA protect against apoptosis elicited by antibodies to the T-cell receptor and to Fas [Goetzl and An, 1998]. LPA and S1P induces Cl^- influx in *Xenopus* oocytes and fibroblasts [Durieux et al., 1993; Tigyi and Miledi, 1992], which might play a role in regulating membrane potential and the fertilization process. Various types of cell–cell communication also can be regulated by LPA. Both gap junctions and tight junctions are influenced by the actions of LPA [Hill et al., 1994]. In addition, LPA and S1P augment the expression of growth factors and sensitize some growth

factor receptors [Goetzl and An, 1998]. These observations suggest that LPA and S1P might attain maximal effects in part through indirect mechanisms by modulating the generation and effects of polypeptide growth factors.

MOLECULAR BIOLOGY OF THE EDG FAMILY OF GPCRS FOR LPA AND S1P

Although the existence of cell surface receptors for LPA and S1P had long been postulated, cloning was hampered by several technical difficulties. The presence of endogenous receptors in most cell lines commonly used for recombinant protein studies, and the high nonspecific binding of LPA and S1P to cell membranes, made expression cloning and receptor purification unfeasible. Recently, genetic studies of the embryonic cortical development and sensitive methods for the assessment of LPA-induced transcriptional activation have led to the successful cloning and identification of a subfamily of GPCRs for LPA and S1P.

Cloning and Identification of the Edg Family of Receptors

During studies of development of the mouse cerebral cortex, Chun and colleagues used degenerate polymerase chain reaction (PCR) to isolate cDNAs encoding seven-transmembrane-domain receptors from a neocortical cell line [Hecht et al., 1996]. One such receptor showed restricted expression in the ventricular zone of the neocortex and was thus named ventricular zone gene-1 (*vzg*-1). *vzg*-1 is the mouse equivalent of a sheep orphan GPCR cDNA termed endothelial differentiation gene (*edg*)-2 [Masana et al., 1995] that is 35% identical in amino acid sequence to human *edg1* previously found to be an immediate early gene from endothelial cells [Hla and Maciag, 1990]. Therefore, *vzg-1* is also named mouse *edg2*. Chun and colleagues showed that overexpression of VZG-1/Edg2 in neocortical cells potentiated LPA-induced cell rounding and inhibition of adenylyl cyclase. Led by early findings of transcriptional activation in LPA-induced cellular proliferation [Hill et al., 1995], we developed an expression cloning scheme that used a sensitive SRE-driven reporter gene assay. In combination with degenerate PCR screening, this SRE reporter gene assay allowed us to isolate the human *edg2* cDNA that is 96% identical to mouse *vzg-1/edg2* [An et al., 1997b]. Expression of human Edg2 in HEK293 and Chinese hamster ovary (CHO) cells conferred not only LPA-induced activation of SRE-driven transcriptional activation, but also increases in specific binding to [^{3}H]-LPA. Subsequently, Erickson et al. [1998] confirmed that human Edg2 is a functional LPA receptor linked to the pheromone response pathway, when overexpressed in yeast that have no endogenous LPA receptors. These characteristics of mouse and human *edg2* strongly suggested that *edg2* encodes a functional LPA receptor. Even though the amphiphilic nature of LPA made the ligand binding parameters (K_d and B_{max}) unattainable, the functional identification of Edg2 as an LPA receptor provided a framework for subsequent revelation of the Edg subfamily of GPCRs for LPA and S1P.

When searching the GeneBank dbEST database with the human Edg2 sequence, we identified a cDNA encoding a GPCR with an amino acid sequence 46% identical to Edg2. This GPCR, dubbed Edg4, conferred LPA-inducible activation of the SRE-driven reporter gene and enhancement of [^{3}H]-LPA binding when expressed in Jurkat cells, indicating that Edg4 is another subtype of LPA receptor [An et al., 1998a].

Sequence analyses also revealed other closely related GPCR homologues. Three orphan GPCRs, human Edg1 [Hla and Maciag, 1990], Edg3 [Yamaguchi et al., 1996], and rat H218 [MacLennan et al., 1994] constitute another cluster in the Edg subfamily of receptors (Fig. 2A).

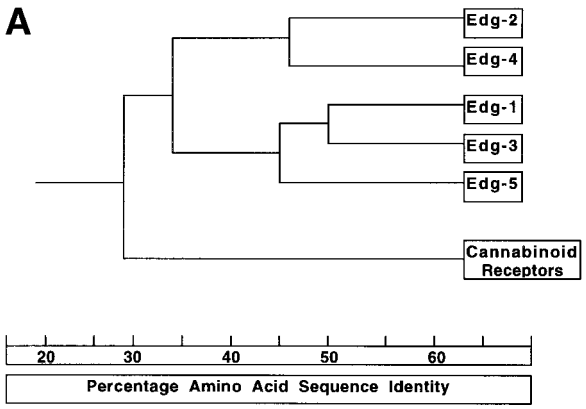

Fig. 2. **A:** Phylogenetic diagram of the Edg subfamily of G protein-coupled receptors (GPCRs) for LPA and S1P and cannabinoid receptor CB1. Percentage of amino acid sequence identity are indicated at the vertical branch point between two proteins. **B:** Multiple alignment of the predicted amino acid sequences of the Edg GPCRs for LPA and S1P. ClustalW program was used for the alignment. Residues identical in all five receptors are boxed, and residues identical to Edg2 are shaded. The seven putative transmembrane domains are indicated by a line and labeled I–VII. All sequences are from humans. See Fig. 1 for abbreviations.

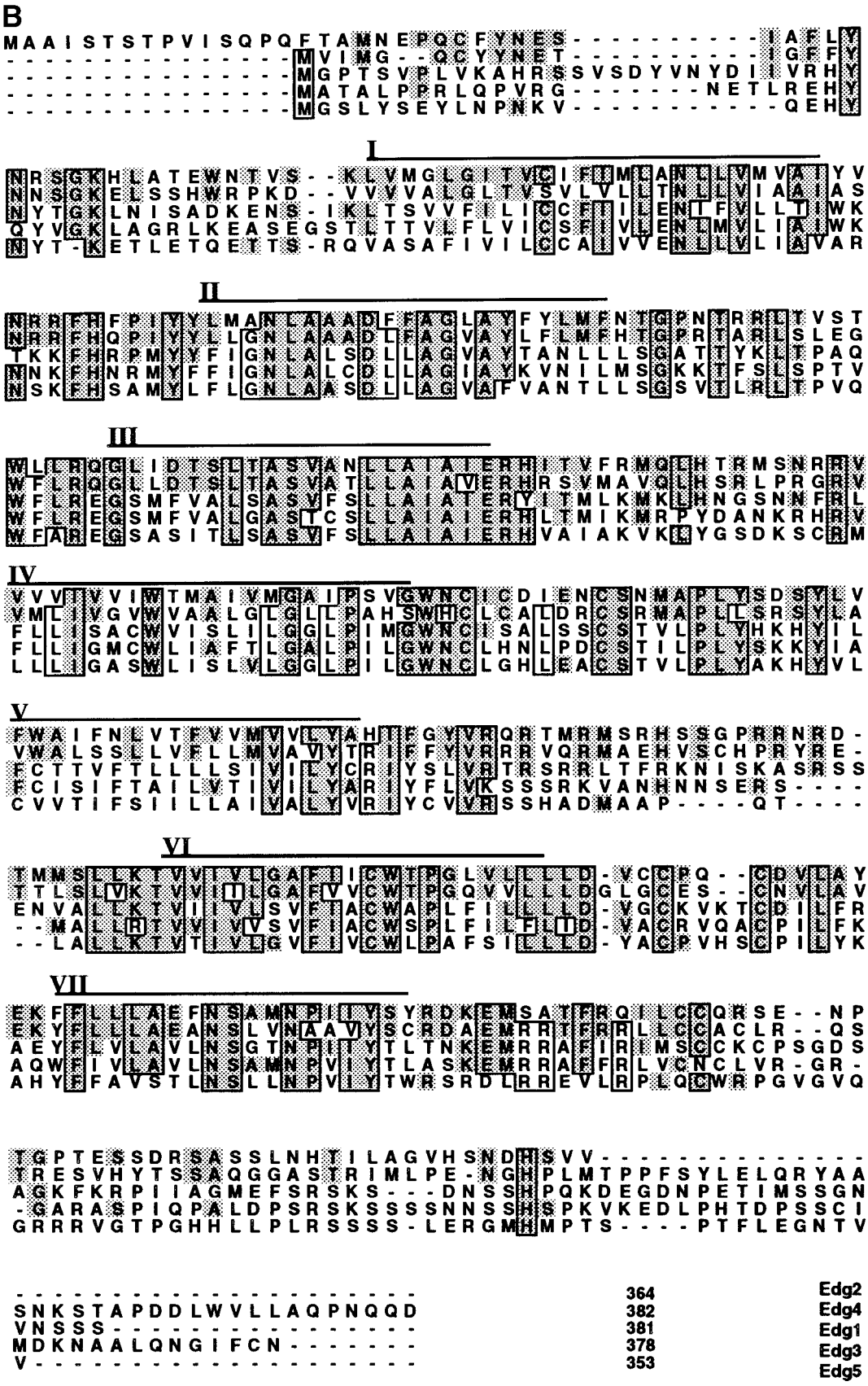

Figure 2. (Continued.)

Their sequence similarity to Edg2 and Edg4 suggested that these GPCRs might recognize LPA or LPA-related bioactive lipids, such as S1P. To test this hypothesis, we expressed Edg1, Edg3, and rat H218 in Jurkat cells and *Xenopus* oocytes and examined biochemical responses to S1P and related sphingolipids. In both the SRE-driven reporter gene assay and the oocyte Ca^{2+} efflux assay, Edg3 and H218 responded to S1P with EC_{50} values within the nanomolar range. Edg1 showed a weaker, although significant, activity in the SRE-driven reporter gene assay [An et al., 1997a]. Subsequently, other laboratories demonstrated that S1P indeed activated Edg1 to inhibit adenylyl cyclase, activate MAPK, and induce cell–cell aggregation through enhanced cadherin expression [Lee et al., 1998; Zondag et al., 1998].

A different LPA receptor named PSP24 was isolated from *Xenopus* oocytes by its potentiation of LPA-evoked oscillatory Cl^- current [Guo et al., 1996]. This receptor does not belong to the Edg subfamily based on low sequence homology and therefore is not discussed further in this review.

Nomenclature, Classification, and Sequence Alignment of the Edg Receptors

A working nomenclature is used tentatively by most researchers in the field. This operational nomenclature, although currently convenient to individual laboratories, is likely to be replaced by a formal consensus following the guidelines of the IUPHAR. For the convenience of the readers, this review adopts the term Edg to describe *edg* gene-encoded GPCRs with the references mentioned above. We recently isolated a human version of rat H218 and named it human Edg5 following this particular nomenclature system.

The five currently known Edg receptors are divided into two clusters (Fig. 1A). Edg2 and Edg4 (46% identical in amino acid sequence) are 30–34% identical to the second cluster of Edg1, Edg3, and Edg5. Within the second cluster, Edg1 is 50% and 44% identical to Edg3 and Edg5, respectively. This classification, based on sequence identity, correlates well with their ligand recognition, as Edg2 and Edg4 are LPA receptors, and Edg1, Edg3, and Edg5 are S1P receptors. Among members of GPCR superfamily, the Edg family receptors are most closely related to the cannabinoid receptors, with 28% sequence identity. Hence, it is not surprising that the endogenous ligands for cannabinoid receptors, anandamide and 2-arachidonylglycerol, are similar to LPA and S1P in structure.

The amino acid sequence alignment of the Edg GPCRs shows features that are both common and unique to the GPCR superfamily (Fig. 2B). Features most common to all GPCRs, i.e., seven stretches of hydrophobic residues that presumably span the membrane, N-linked glycosylation sites in the N-terminus, and multiple phosphorylation sites in the intracellular regions are all conserved in the Edg receptors. In most other GPCRs, a cysteine residue in the first extracellular loop forms a disulfide bond with another cysteine residue in the second extracellular loop. However, this disulfide bond may be formed between the second and the third extracellular loops of the Edg receptors. In Edg4, an alanine residue replaces a proline residue conserved in most other GPCRs in the NPXXY sequence of the seventh transmembrane domain. In Edg5, a Src homology-2 (SH2) domain may exist in the intracellular face [MacLennan et al., 1994]. However, its interaction with phosphotyrosine-containing proteins has not been demonstrated. The structure–function relationship of the Edg receptors in ligand binding, G protein coupling, and interaction with other signaling molecules also remain to be determined.

Genomic Structure and Gene Expression of the Edg Receptors

Genomic structures of some of the *edg* genes have begun to be unveiled. The mouse *edg2* gene contains multiple exons and introns and has been localized to the centromeric region of mouse chromosome 4 [Macrae et al., 1996]. A region on human chromosome 19p12 encompassing most of the human *edg4* gene with multiple exons and introns has been sequenced by the Human Genome Project (GeneBank accession number AC002306). The mouse *edg1* gene has two exons and one intron that separates the transcriptional start site from the entire open reading frame [Liu and Hla, 1997]. The human *edg3* gene was mapped to human chromosome 9q22.1–q22.2 [Yamaguchi et al., 1996]. One of the characteristics of mRNAs for the *edg* receptors is the presence of the AU-rich sequence AUUUA in their 3′-UTR. This sequence motif commonly found in growth-re-

lated immediate early genes influences the stability of their mRNAs.

The expression of *edg* genes in tissues has been studied by using Northern blot analyses and in situ hybridization. Patterns of high expression in numerous adult and embryonic tissues suggest functional roles in multiple systems and in embryonic development. Mouse *edg2* is widely distributed with the highest expression in the brain, where it is localized in the hindbrain, midbrain, and hypothalamus [Macrae et al., 1996]. In the neocortex of the E12 to E18 mouse brain, *edg2* shows a restricted expression in the ventricular zone [Hecht et al., 1996]. Interestingly, *edg4* has an expression pattern very different from that of *edg2* [An et al., 1998]. The two *edg4* transcripts were almost undetectable in brain, heart, placenta, and digestive tract, where *edg2* transcripts were abundant, but were found instead in leukocytes where *edg2* was undetectable. The 8-kb *edg4* transcript was detected in peripheral blood leukocytes, thymus, and spleen, whereas the 1.8-kb transcript was in the leukocytes, testis, prostate, and pancreas. We also found that *edg4* is expressed in embryonic tissues and cultured tumor cells derived from tissue types that do not normally express *edg4* [An et al., 1998a].

In general, mRNAs for *edg1, edg3,* and *edg5/h218* are widely distributed throughout various organs (Fig. 3). Human *edg1* is abundantly and uniformly expressed in all adult tissues examined (Fig. 3). During embryogenesis, high levels of *edg1* expression was observed from E15.5 and thereafter in the liver, lung, the pulmonary trunk, and skeletal structures undergoing ossification [Liu and Hla, 1997]. The mRNA for rat *h218/edg5* is most abundant in the nervous system during embryogenesis, and its protein is preferentially expressed in young, differentiating neuronal cell bodies and axons [Maclennan et al., 1997].

Signal Transduction Properties of the Cloned Edg Receptors

It is difficult to equate cloned receptors with endogenously expressed receptors because there is no selective ligand available for each Edg receptor. Receptor-G protein coupling and downstream signaling pathways are also likely to be cell-type specific. Nevertheless, the observed signaling properties of the recombinant receptors overexpressed in heterologous systems are mostly consistent with those expressed in natural cells (Fig. 1).

When expressed in neuroblasts and hepatoma cells, mouse, and human Edg2 directly coupled to G_i protein to inhibit adenylyl cyclase [An et al., 1998b; Fukushima et al., 1998; Hecht et al., 1996]. Edg2-G_i coupling also leads to PLC-mediated increases in $[Ca^{2+}]_i$ in Jurkat and HTC4 cells [An et al., 1998b], although it has not been observed in SF9 insect cells or *Xenopus* oocytes [Hooks et al., 1998; Zondag et al., 1998]. Edg2 expression in hepatoma and neuroblast cells also conferred LPA-induced stress fiber formation and neurite retraction in a Rho-dependent fashion [Fukushima et al., 1998]. Human Edg4 couples to both G_i and G_q to elicit adenylyl cyclase inhibition and PLC-mediated increases in $[Ca^{2+}]_i$ [An et al., 1998b]. In Jurkat and HTC4 cells, both Edg2 and Edg4 activate ERKs and SRF, which were blocked by PTX and C3 exoenzyme, respectively [An et al., 1998a] (An et al., unpublished observations). These observations indicated the involvement of both G_i-ERKs and $G_{12/13}$-Rho pathways that converge to activate SRE-driven transcription and mitogenesis by Edg2 and Edg4.

Edg1 predominantly couples to G_i and ras-raf-ERK pathways. While overexpression of Edg1 in HEK293 cells also induced morphogenetic differentiation in a C3 exoenzyme-sensitive manner [Lee et al., 1998], Edg1-mediated SRE-driven transcriptional activation is relatively weak in Jurkat and HTC4 cells [An et al., 1997a]. Edg1 did not elicit an increase in $[Ca^{2+}]_i$ when overexpressed in *Xenopus* oocytes, SF9, Jurkat, or HTC4 cells [An et al., 1997a; Zondag et al., 1998] (An et al., unpublished observations). In contrast, Edg3 and Edg5 transduce Ca^{2+} signals in *Xenopus* oocytes, Jurkat or HTC4 cells predominantly via the G_q-PLC pathway [An et al., 1997a] (An et al., unpublished observations). In contrast to Edg1, Edg3, and Edg5 markedly activate SRE-driven transcription [An et al., 1997a]. This activity is inhibited by both PTX and C3 exoenzyme, suggesting involvement of both Gi and Rho GTPases (An et al., unpublished observations). As for LPA receptors, transactivation of SRE-driven immediate early genes via Edg3 and Edg5 provide the basis for a GPCR-mediated mechanism of S1P-induced cell proliferation.

Identification of the GPCRs for S1P and their G protein-mediated signaling pathways conclu-

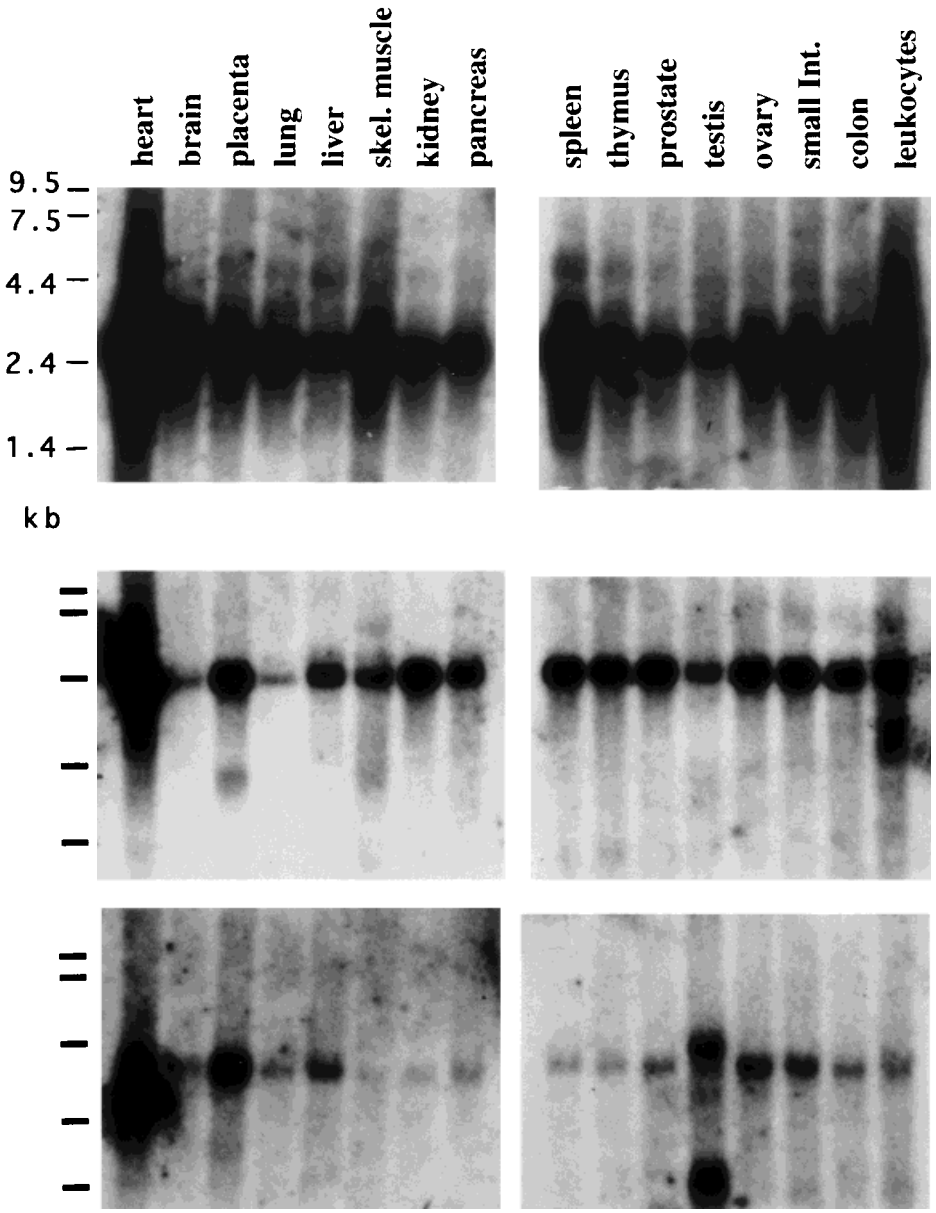

Fig. 3. Northern blot analyses of mRNAs for *edg1* (*top*), *edg3* (*middle*), and *edg5* (*bottom*) in human tissues. The multiple tissue Northern blots were obtained from Clontech, which contain 2 µg of poly $(A)^+$ RNA from indicated adult human tissues in each lane. The blots were hybridized with full-length human cDNAs for *edg1, edg3,* or *edg5* under high stringency conditions and were then exposed for 24 h. The molecular mass markers are indicated on the left (in kilobases).

sively affirmed an extracellular messenger role of S1P. On the contrary, specific molecular targets and pathways for the proposed intracellular action of S1P have not been identified. It remains to be determined whether S1P can indeed function as a dual extracellular and intracellular messenger in physiological settings.

It should be noted that reservations remain despite positive identification of Edg proteins as LPA or S1P receptors by several laboratories. We believe that part of the skepticism is rooted in the difficulties detecting the activities of the cloned Edg receptors in heterologous expression systems. First, reliable radioligand binding data are virtually unattainable due to the extreme amphiphilicity of LPA and S1P. Second, most cultured cells commonly used in recombinant protein characterization exhibit endogenous LPA or S1P responsiveness. Third, specific antibodies against Edg proteins are not

readily available, which makes it impossible to assess the cell surface expression of Edg proteins. Finally, expression of recombinant Edg proteins and their signaling responses may be down-regulated in cells cultured in the presence of serum that contains high concentrations of LPA and S1P. While reliable binding studies await the future development of high-affinity hydrophilic radioligands, functional characterization of the recombinant Edg receptors requires careful design and proper controls.

SUMMARY AND FUTURE DIRECTIONS

The Edg family of GPCRs encompasses multiple subtype receptors for LPA or S1P (Table I). As in other GPCR subfamilies, this multiplicity has important biological significance. Diverse subtypes of Edg receptors may provide functional redundancy for physiological effects of LPA and S1P that may be important to the organism. Different receptor subtypes for the same ligand may have different functions. Diverse intracellular signaling pathways may elicit distinct cellular functions that result in specific physiological effects. Different subtype receptors may also have quantitative differences in tempo or EC_{50} that may translate to subtle differences in responsiveness to the available ligands under certain physiological or pathological conditions. Additionally, multiple subtype receptors provide diversity in functions and flexibility in their controls, which can be achieved by tissue-specific expression and independent regulation on transcription or translation levels in a subtype-specific manner.

The availability of these cloned Edg receptors has now provided useful tools to elucidate the molecular mechanisms of diverse biological effects of LPA and S1P. Nonetheless, numerous challenges still confront investigators in the field. First, more receptors for LPA and S1P may be cloned, which may or may not belong to the Edg receptor family. Second, LPA and S1P may only be the prototypes of an emerging family of bioactive lysophospholipids. New natural ligands may be discovered, which may or may not act on the Edg receptors. Third, the intracellular signaling mechanisms of the Edg receptors, especially those in native cells, have not been fully characterized. Understanding the diverse signaling pathways and their crosstalk in specific cell types will reveal the important cellular functions of these mediators. Knowledge from these in vitro studies should help us identify integrated biological systems in which the functions of these mediators predominate and thus can be studied. Finally, the physiological and pathological roles of LPA and S1P are unknown, although their properties suggest possible functions in cell growth during development, wound healing, and tissue regeneration. Knock-out mice with deletions in specific *edg* genes are being created to study their roles in neural development. Subtype-specific pharmacological compounds are needed to study the in vivo functions of LPA and S1P. The recombinant Edg receptors and sensitive cell-based signaling assays should provide high throughput screenings to obtain specific agonists or antagonists for distinct Edg receptors.

TABLE I. List of Edg Family of LPA and S1P Receptors, Their Nomenclature, Synonyms, GeneBank Accession Numbers, Chromosomal Locations, Major Sites of Tissue Expression, and Major G Proteins Coupled, Associations

Ligand	Human receptor name (GeneBank no.)	Other synonyms (species)	Chromosome location	Tissue expression (enriched in)	Major G proteins
LPA	Edg2 (U80811)	VZG-1 (mouse)	Mouse 4	Ubiquitous (brain, embryo)	G_i, $G_{12/13}$
	Edg4 (AF011466)		Human 19p12	WBC, testis (embryo, tumor)	G_i, G_q, $G_{12/13}$
S1P	Edg1 (AF022137, M31210)			Ubiquitous	G_i
	Edg3 (AF022139, X83864)		Human 9q22.1–2	Ubiquitous (heart)	G_i, G_q, $G_{12/13}$
	Edg5 (AF034780)	H218, ARG16 (rat)		Ubiquitous (heart, embryo)	G_i, G_q, $G_{12/13}$

LPA, Lysophosphatidic acid; S1P, sphingosine1-phosphate; WBC, white blood cells.

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