Discovery of new G protein-coupled receptors for lipid mediators

Dong-Soon Im

Laboratory of Pharmacology, College of Pharmacy, Pusan National University, San 30, Chang-Jun-dong, Keum-Jung-gu, Busan 609-735, Republic of Korea

Abstract Successful sequencing of the human genome has opened a new era in the life sciences and has greatly accelerated biomedical research. Among various research endeavors benefiting from established genomic information, one of the most fruitful areas is the research on orphan G protein-coupled receptors (GPCRs). Many intercellular mediators, including peptides, lipids, and other small molecules, have found their GPCRs in the plasma membrane, e.g., relaxin and tyramine. In the past 14 months, more than one dozen papers have been published reporting the finding of intercellular lipid mediators acting on rhodopsin family GPCRs. This review focuses primarily on intercellular lipid mediators and their recently discovered GPCRs. Im, D-S. Discovery of new G protein-coupled receptors for lipid mediators. J. Lipid Res. 2004. 45: 410–418.

Supplementary key words orphan receptor • sphingosine • fatty acid • bile acid

Molecular targets of various drugs have been found using recently developed biochemical, physiological, and pharmacological tools. For example, aspirin inhibits cyclooxygenase (1) and morphine activates the opioid receptors in the plasma membrane (2). Molecular identification of the targets at the DNA level has become possible because of the advancement of techniques in molecular biology, and G protein-coupled receptors (GPCRs) have been found to be the molecular targets of many drugs. To date, ~170 GPCRs have been found to be receptors for known intercellular mediators such as hormones and neurotransmitters, and 367 genes have been recognized as GPCRs in the human genome (3). When their ligands are not known, these GPCRs are classified as “orphan” GPCRs (3, 4). Discovery of endogenous ligands could be a springboard for the development of new therapeutic agents targeting the receptors and would help define the biological significance of the ligands (4, 5). Many potentially significant discoveries have been made in the past several years (5). The present review will focus mainly on the recent assignment of lipid mediators to GPCRs. These GPCRs include GPR3, GPR6, GPR12, GPR23, GPR40, GPR41, GPR43, GPR63, TG1019 (also known as R527), and BG37 (also known as TGR5), which have been identified as receptors for intercellular lipid messengers, i.e., sphingosine 1-phosphate (S1P), sphingosylphosphorylcholine (SPC), dioleoylphosphatidic acid (doPA), lysosphosphatidic acid (LPA), free fatty acids, eicosatetraenoic acid, and bile acids (6–19). The chemical structures of the new lipid mediators are shown in Fig. 1.

INTERCELLULAR LIPID MEDIATORS

In all multicellular organisms, including humans, there are a plethora of communication systems connecting tissues and cells. For example, endocrine cells, immune cells, and neuronal cells release chemical mediators to regulate the activities of other cells. Chemical messages are usually called hormones, autacoids, or neurotransmitters. They are categorized chemically as peptides, biogenic amines, and lipids. In this review, intercellular lipid mediators are defined as chemical transmitters that have water-insoluble hydrophobic moieties.

Intercellular lipid mediators can be divided further into two groups, according to their sites of action. Corticosteroids, testosterone, estrogen, retinoic acid, and other lipid molecules having intracellular receptors form one group; after binding their ligand, these proteins are translocated into the nucleus, where they act as transcription factors (20). The other group of lipid mediators have receptors on the plasma membrane; most of these proteins are GPCRs.

Abbreviations: doPA, dioleoylphosphatidic acid; GI, gastrointestinal; GFP, green fluorescent protein; GPCR, G protein-coupled receptor; 5-HETE, 5-hydroxy-eicosatetraenoic acid; LPA, lysosphosphatidic acid; LPC, lysosphosphatidylcholine; 5-oxo-ETE, 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid; 5(S)-HPETE, 5(S)-hydroxyperoxy-eicosatetraenoic acid; S1P, sphingosine 1-phosphate; SPC, sphingosylphosphorylcholine.
binding should be reversible; 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid; S1P, sphingosine 1-phosphate; LPA, lysophosphatidic acid; 5-oxo-ETE, doPA, dioleoylphosphatidic acid; LPA, lysophosphatidic acid; 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid; S1P, sphingosine 1-phosphate; SPC, sphingosylphosphorylcholine.

**Fig. 1.** Chemical structures of new lipid mediators. doPA, dioleoylphosphatidic acid; LPA, lysophosphatidic acid; 5-oxo-ETE, 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid; S1P, sphingosine 1-phosphate; SPC, sphingosylphosphorylcholine.

**GPCRs**

GPCRs have seven transmembrane helical domains (4). The N terminus faces the extracellular space, and the C terminus is located in the cytosol. The exofacial aspects of the proteins, including the N terminus, extracellular domains, and outer segment of helices, recognize intercellular mediators (ligands), and subsequent correct docking of the mediator with the receptor results in changes of receptor conformation (4). Consequently, the conformational change of the C terminus, intracellular domains, and inner helical segments leads to the activation of G proteins, which is followed by the modulation of effector functions. Effectors include enzymes such as adenylyl cyclase and phospholipases C and D as well as ion channels (4). GPCRs are encoded by a large family of genes and continue to be pursued avidly as major drug targets (21). Indeed, more than half of the medicines currently available act either positively (agonists) or negatively (antagonists) on the GPCRs (22). As described above, finding natural ligands of orphan GPCRs is not only crucial to elucidate their physiological and pathological significance but important in developing new therapeutic agents.

Recently, 10 orphan GPCRs have been suggested as receptors for lipid mediators (6–19). However, particular caution is advised in interpreting experimental results in the case of lipid ligands because of the unique molecular characteristics of lipids. Therefore, the aims of this review are to summarize the recent assignment of GPCRs as lipid receptors, to evaluate the findings using pharmacological criteria, and finally to speculate on the future directions of lipid mediator research. Pharmacological criteria for identifying endogenous ligands of orphan GPCRs are as follows: 1) a candidate ligand should bind to the GPCR directly and specifically (or selectively); 2) a candidate ligand should bind to GPCR with high affinity; 3) the binding should be reversible; 4) the binding of the candidate ligand to GPCR should activate the GPCR; 5) the candidate ligand should be present in the relevant cellular and biological systems; and 6) the concentration of the candidate ligand around the target cells should change under a variety of physiological conditions (23). Among these six criteria, the activation of the GPCR is satisfied most readily, because the functional analysis is usually the basis of ligand screening protocols. Reports on lipid mediator research, however, usually do not show binding data (failing to fulfill criteria 1–3). This failure is often the result of the lack of a suitable radioligand. Criteria 5 and 6 are also problematic because the concentration of “free” lipid ligand is difficult to ascertain. However, biochemists and physiologists, who characterize enzymes involved in the formation and degradation of ligands, sometimes are able to satisfy the last two criteria.

**A BRIEF HISTORY OF THE DISCOVERY OF LIPID MEDIATORS AND THEIR RECEPTORS**

In the past, lipid molecules were considered mainly to be components of the membrane or intermediary metabolites. However, elucidation of cyclooxygenase as the target of nonsteroidal anti-inflammatory drugs, including aspirin (1), and recognition of the important pathophysiological roles played by prostaglandins and leukotrienes drastically changed attitudes toward recognizing lipid molecules as intercellular signaling messengers (24). The first cannabinoi receptor cDNA was cloned in 1990 as the first GPCR for lipid mediators (25), even though endogenous cannabinomimetic ligands (anandamide and arachidonyl glycerol) were recognized subsequently (26, 27). During the decade since this initial discovery, GPCRs for long-established eicosanoids such as the prostanoids [prostaglandin E2 receptor, prostaglandin F2α receptor (FP), prostaglandin D2 receptor (DP), and IP] and leuckotrienes [leugotriene B4 receptor (BLT1), leugotriene C4 receptor (BLT2)] have been cloned (28, 29).

A subfamily of GPCRs has been identified as receptors for lysophospholipids (30, 31). These receptors recognize either LPA or S1P. LPA and S1P modulate a large number of cellular events such as cell growth, differentiation, and apoptosis (31–36). The recognition of these lysosphospholipid mediators as regulators of cellular functions originated with observations of the LPA-induced modulation of blood pressure and platelet aggregation (37, 38). Later, Moolenaar’s group (39) identified LPA as a growth-stimulating factor in serum and suggested the involvement of G protein signaling. The accumulation of information on LPA-induced actions in many cell types finally led to the discovery of the first LPA receptor gene in 1996 (40), and this was followed by identifications of structurally related receptor genes (41–47). In 1998, S1P was identified as the endogenous ligand of Edg-1 (now named S1P) GPCR (41–46). Subsequently, four GPCRs have been characterized as S1P receptors (41–46, 48, 49).
A more contentious subset of GPCRs are named OGR1 (GPR68), GPR4, TDAG8 (GPR65), and G2A and are distinct from the LPA and S1P receptors (Fig. 2). In 2000, Xu et al. (50) reported that OGR1 (GPR68) receptor is an SPC receptor. Likewise GPR4 and G2A have been reported to recognize SPC and lysophosphatidylcholine (LPC), albeit with different affinities (51, 52). TDAG8 was identified as a target of psychosine (galactosyl sphingosine), and its involvement in cytokinesis was demonstrated (53). The identification of GPCRs for LPA and S1P has been verified by many scientists in numerous studies on receptor-mediated signaling and pathophysiological implications (54–58), and receptor type-specific agonists and antagonists are now being developed (59–64). In contrast, the original OGR1 receptor family reports have not been verified independently. The ligands for this group are putatively LPC, SPC, and the glycolipid psychosine. The lack of strong support for the ligand-GPCR relationship from independent studies may derive from the fact that this subfamily of GPCRs displays high constitutive activities in many cellular systems (65). Further studies to explore this issue and to determine whether there are additional ligands for these receptors are critically important. Indeed, the identification of OGR1 and GPR4 as proton-sensing receptors increases the uncertainty regarding their status as lysophospholipid receptors (66). Furthermore, Bektas et al. (67) suggested that SPC and LPC are not the ligands for GPR4. The observation of TDAG8 receptor as a mediator of psychosine-induced uncoupling of cytokinesis provided a good clue for research on cytokinesis and Krabbe’s disease, although it did not satisfy the pharmacological criteria of specific binding with high affinity and reversible activation (53). When considering the cases of *Xenopus* PSP24 and LPA1 (formerly Edg2) receptors, the importance of binding data is easily understandable (40, 68). PSP24 was originally reported as an LPA receptor, but without binding data (68). Later, mammalian homologs (e.g., GPR45 and GPR63) were reported to be without LPA receptor activity (69, 70). Recently, another group claimed the mammalian PSP24 homolog, GPR63, as a receptor for doPA and S1P, but this report also lacked radioligand binding analyses (8). The uncertainty regarding claims for these receptors emphasizes the need to satisfy the aforementioned pharmacological criteria as fully as possible.

**GPR3, GPR6, GPR12, GPR23, AND GPR63 RECEPTORS FOR S1P, LPA, SPC, AND DOPA?**

In 2002, Uhlenbrock, Gassenhuber, and Kostenis (6) reported that a subfamily of GPCRs, composed of GPR3, GPR6, and GPR12, is constitutively activated in HEK293 cells and modulated in Ca2+ mobilization and cAMP production by S1P. These three receptors share high amino acid sequence identity (>60%), and are closely related to Edg receptors for S1P and LPA and to the cannabinoid receptors (Fig. 2) (6). GPR3 was originally named hACCA (human adenylate cyclase constitutive activator) because significant increases in cAMP levels were found in a variety of cell lines after forced overexpression of GPR3 (71). The presence of GPR3, GPR6, and GPR12 close to the other receptors for lipid mediators on the phylogenetic tree of GPCRs and the constitutive activation of adenylyl cyclase by GPR3 led researchers to predict that a lipid mediator(s) might activate the receptors via Gs protein coupling (71). Uhlenbrock, Gassenhuber, and Kostenis (6) confirmed this prediction experimentally by observing that the constitutive activation of adenylyl cyclase by GPCR occurred not only in GPR3-expressing cells but also in GPR6- and GPR12-expressing HEK293 cells. Enhanced Ca2+ mobilization by S1P was blocked by pertussis toxin treatment in HEK293 cells that express these receptors (6).

Different magnitudes of responses were found in each receptor-expressing cell. For example, the effect of the polyanion suramin on GPR3-mediated Ca2+ response was quite different from the effects on GPR6- and GPR12-mediated responses (6). However, the conclusion appears to be supported by limited experimental evidence. First, the evidence for direct interaction between S1P and the receptors is lacking. As mentioned above, to meet the pharmacological criteria for receptor identification, the binding must be specific with high affinity and be reversible. In the case of lipid mediators, however, it is not easy to prove experimentally because of nonspecific binding of lipids to the plasma membrane. In such cases, guanosine 5’-[γ-35S]thiotriphosphate (GTPγS) binding data or supporting data from multiple expression systems may be used as empirical support. Second, the same authors observed an enhanced response to S1P in HEK293 cells under starvation. However, because HEK293 cells have endogenous responses to S1P and express several S1P receptors (6), enhanced responses to S1P and LPA even in nontransfected cells are expected under conditions of serum starvation, because S1P and LPA are well-known cell-proliferating constitu-

![Fig. 2. Phylogenetic tree of G protein-coupled receptors (GPCRs) for intercellular lipid mediators. The tree was constructed with amino acid sequences of human GPCRs for lipid mediators by using the MEGA II and TrecView programs.](Image URL)
ents of serum (72). Thus, an experimental approach using null cells (cells nonresponsive to S1P) would appear to be critically important. Third, the data in their report only addressed transiently transfected HEK293 cells; therefore, analysis with other cell lines as well as study of stably transfected cells are needed to bolster their claim.

The cited study by Uhlenbrock, Gassenhuber, and Kostenis (6) was supported in part by Ignatov et al. (7). Although the latter group studied only GPR12, functional study of its expression in two different systems made their data quite convincing (7). In CHO cells stably expressing promiscuous Gα16, transient transfection of GPR12 made the cells responsive to SPC (7). Furthermore, in oocytes coinjected with cRNAs that encode both GPR12 and GIRK (G protein gated inwardly rectifying K+ channel), SPC induced strong inward currents, thus partly supporting the earlier observation of Uhlenbrock, Gassenhuber, and Kostenis (6): activation of GPR12 by S1P in both assay systems and the involvement of Gαi/o proteins shown by the use of pertussis toxin (6, 7). However, Ignatov et al. (7) did not provide any binding data, and the experiments were successful only when they used signal peptide-tagged GPR12. Uhlenbrock, Gassenhuber, and Kostenis (6) found no activity of SPC on GPR12 in their HEK293 cells. On the other hand, Ignatov et al. (7) showed that SPC had higher affinity to GPR12 than did S1P and affected neuronal development; they suggested that the neuronal effect was mediated via GPR12, because there was no expression of any other known SPC receptors, such as OGR1 and GPR4, in the brain. Nevertheless, because there still exists a possibility of uncharacterized SPC receptors in the brain, it is somewhat premature to conclude that GPR12 mediates all SPC responses in the brain cells.

In 2003, Niedernberg et al. (8) suggested that GPR63 is a receptor for S1P and doPA. GPR63 and GPR45 are the human homologs of Xenopus PSP24, which was first reported in 1996 as a receptor for LPA (68–70). However, others have claimed that mammalian receptors, including GPR45 and GPR63, are not responsive to LPA in many assay systems (68–70). These authors also tested other lipid mediators, including S1P and phosphatidic acid, as candidate ligands for GPR45 or GPR63 in their assay systems, but they found no activity (68–70). Furthermore, LPA receptors in Xenopus, which are homologous to mammalian LPA receptors, were cloned as functional LPA receptors (68–70, 73). Niedernberg et al. (8) observed that S1P, dihydro-S1P, or doPA increased intracellular calcium concentration ([Ca2+]i) in CHO cells, cotransfected with GPR63 and Gaαq6q5myr, which is Gαq without six N-terminal amino acids and with four C-terminal amino acids of Gαq. A highly sensitive instrument that measures changes in intracellular Ca2+ concentration (i.e., the fluorometric imaging plate reader) and the enhancement of signals by coexpression of Gaαq6q5myr or equorin-green fluorescent protein (GFP) fusion protein made it possible to observe such changes, thereby leading the authors to match S1P and doPA with GPR63. However, the increase of basal responsiveness to S1P or doPA with expression of Gaαq6q5myr alone and the lack of binding data cast some uncertainty on these conclusion (8). Additional data, based on the pairing of GPR63 with S1P or doPA by an alternative assay method using other expression systems, are needed. The bioinformatic analysis of the phylogenetic tree of GPCRs suggests the possibility of peptide ligands for GPR45 and GPR63, because sequentially related neighbor GPCRs form a large class of peptide receptors (3).

The existence of additional LPA receptors has been suggested previously, because some responses to LPA could not be explained with the known LPA receptors (74, 75). Recently, Noguchi, Ishii, and Shimizu (18) reported GPR23, which is structurally distinct from the Edg family (Fig. 2), as another LPA receptor. They showed a specific binding of [3H]LPA to membranes of null-response cells, such as RH7777 and B103, thereby transiently expressing GPR23. Furthermore, reporter gene assay, Ca2+ mobilization, and adenyl cyclase activity confirmed the pairing of LPA with GPR23, and the GPR23-mediated signaling in several cell lines was further characterized after transient or stable transfection of GPR23 (18).

**PHYLOGENETIC ANALYSIS OF NEW GPCRS FOR S1P, LPA, SPC, AND DOPA**

Eleven GPCRs for S1P, LPA, and SPC/LPC, including five S1P receptors (S1P1–5), three LPA receptors (LPA1–3), and three SPC/LPC receptors (OGR1, GPR4, and G2A) have been identified. As described above, five additional GPCRs with these ligands have been reported: GPR3, GPR6, GPR12, GPR23, and GPR63. As shown in Fig. 2, GPR3, GPR6, and GPR12 are located near the Edg subfamily GPCRs for LPA and S1P and cannabinoid receptors on the phylogenetic tree. Their amino acid sequence identity is 57–61%, which is marginally higher than the identity among three LPA receptors. As Uhlenbrock, Gassenhuber, and Kostenis (6) suggested, S1P is an attractive candidate ligand, although the constitutive activation of G proteins without S1P addition and the lack of available binding data make their interpretation problematic (76). The suggestion that SPC is the ligand for GPR12 raises a question (76). Because the sequence of GPR12 is distinct from that of other putative SPC receptors, such as OGR1 and GPR4 (Fig. 2), it would represent an exception to the nearly invariant rule that “like ligands have like receptors” (76). The ligand assignments for GPR23 (LPA) and GPR63 (S1P) are also exceptional (Fig. 2). A phylogenetic analysis indicated very low homology between GPR23 and GPR63 and also with the known LPA/S1P receptors. The phylogenetic distances cast doubt on the veracity of these claims. However, there have been two similar exceptions: H2 histamine receptor is structurally separated from other histamine receptors (H1, H3, and H4), and the amino acid sequence of the recently identified prostaglandin D2 receptor (CRTH2/GPR44) is quite dissimilar from that of the previously identified prostaglandin D2 receptor (DP) (3, 77).

Computer modeling and mutation studies on LPA and S1P receptors suggested that the arginine-glutamine pair...
in LPAL and receptors and the arginine-glutamate pair in SIP; receptors are indispensable for the binding of LPA or SIP to the receptors (78, 79). These amino acids are not present in the newly proposed lysophospholipid receptors, suggesting differences in the binding mode between the known lysophospholipid GPCRs and proposed new members.

GPR40, GPR41, AND GPR43 RECEPTORS FOR FREE FATTY ACIDS

GPR40, GPR41, and GPR43 were shown to be activated by free fatty acids (9–13, 19). Three independent groups have reported that GPR40 is a receptor for medium- and long-chain fatty acids, which include saturated fatty acids of 12–16 carbon units and unsaturated fatty acids of 18–20 carbons (9, 10, 13). Two highly homologous receptors (GPR43 and GPR41) were found to be unresponsive to medium- and long-chain fatty acids, but they responded to shorter chain fatty acids (C1–C6) (11, 12, 19).

Among free fatty acids tested, eicosatetraenoic acid (C20:4, H2O2), docosahexaenoic acid (C20:6, well known as DHA), and arachidonic acid (C20:4) were found to be activators of GPR40 (9, 10), and the importance of the amino moieties of fatty acids for the activation was shown by comparing the responses to linoleic acid and methyl linolenic acid (9). The sensitivity of pancreatic comparing the responses to linoleic acid and cell is shown by activators of GPR40 (9, 10), and the importance of the amino moieties of fatty acids for the activation was shown by comparing the responses to linoleic acid and methyl linolenic acid (9). The sensitivity of pancreatic β cells to blood fatty acid levels makes interesting the expression of GPR40 in pancreatic β cells (9). Furthermore, Kotarsky et al. (13) observed that thiazolidinedione-type antidiabetic drugs such as rosiglitazone activated GPR40, suggesting the involvement of GPR40 in the action mechanism of the drug. Three research groups reported different expression profiles of GPR40 in other tissues besides the pancreatic β cells; however, they provided no binding data (Table 1) (9, 10). Only multiple assay systems in the hands of different research groups would strengthen the concept of pairing GPR40 with medium- and long-chain fatty acids. The EC50 values in the micromolar range might seem high, but they match the concentration of bloodborne free (i.e., unesterified, nonbound) long-chain fatty acids (9, 10, 13). Thus, although an endogenous lipid with a Kd value in the nanomolar range has not been identified, modulation of GPR40 by free fatty acids strongly suggests a sensing system for free fatty acids (9, 10, 13).

The closely related GPCRs GPR41, GPR42, and GPR43 were tested with free fatty acids also (11, 12, 19). Using a reporter gene assay, Brown et al. (12) found activation of GPR43 by acetate in yeast cells. In the yeast expression system, they narrowed the possible G protein partners to Gq, Gi, and G12 families by using Gsa chimeric proteins (12). This group also conducted a GTP binding assay in the plasma membrane prepared from HEK293T cells and measured Kd currents in GPR43 mRNA-injected Xenopus oocytes, thus strengthening their case (12). The short-chain fatty acids at GPR43 exhibited the following rank order of potency: acetate (C2) = propionate (C3) = butyrate (C4) > pentanoyl (C5) > hexanoyl (C6) = formate (C1) (11, 12). GPR41 was activated by the same fatty acids also, but its rank order potency was different: propionate = pentanoyl = butyrate > acetate > formate (12).

The remaining member of the group, GPR42, has only six amino acids different from GPR41 and is expressed only in some human populations (80). Interestingly, GPR42 was not responsive to free fatty acids (12). Brown et al. (12) found that Arg174 in human GPR41 is conserved in other species but is a Trp residue in human GPR42. When Trp in GPR42 was changed to Arg, the GPR42 became responsive to propionate (12). GPR41 is highly expressed in adipose tissues, whereas GPR43 is found in monocytes and neutrophils (Table 1) (11, 12, 19). Nilsson et al. (11) independently conducted a similar experiment and reached the same conclusion, but they speculated further interference between short-chain fatty acids and GPR43 in the immune system, especially in the gut. However, the physiological relevance of GPR43 and fatty acids, including immune responses to enteric bacteria, needs to be investigated further (19).

---

**TABLE 1. New G protein-coupled receptors paired with lipid mediators**

| Receptor | Lipid | Major Expression | Assay | Range of Effective Concentrations |
|----------|-------|-----------------|-------|---------------------------------|
| GPR3, GPR6, GPR12 | SIP, DHS1P | Brain | [Ca²⁺]↑, cAMP↑, internalization | 30 nM–1 mM (6) |
| GPR12 | SPC >> SIP | Brain | [Ca²⁺]↑, K⁺ current | 100 nM–30 μM (7) |
| GPR5 | SIP, DHS1P, doPA | Brain | [Ca²⁺]↑ | 300 nM–30 μM (8) |
| GPR23 | LPA | Ovary | [Ca²⁺]↑, cAMP↑, reporter assay | 30 nM–50 μM (18) |
| GPR40 | Medium long FFAs | Pancreatic β cells | [Ca²⁺]↑, reporter assay | 1 μM–100 μM (9, 10, 13) |
| GPR41, GPR43 | Short FFAs | PBL, spleen, adipose (GPR41) | [Ca²⁺]↑, reporter assay K⁺ current, GTPyS binding | 30 nM–10 μM (11, 12, 19) |
| TG1019/R527 | 5-Oxo-ETE | Eosinophils, neutrophils, liver, kidney | [Ca²⁺]↑, cAMP↑, GTPyS binding | 1 μM–30 μM (14, 15) |
| BG37/TGR5 | Bile acids | Urothelial | cAMP↑, GTPyS binding, internalization | 100 nM–1 μM (16, 17) |

---

| [Ca²⁺]↑ | intracellular calcium concentration; | DHS1P, dihydro-sphingosine 1-phosphate; | doPA, dioleoylphosphatic acid; | GTPyS, guanosine 5’-y-[γ-35S]thiotriphosphate; | LPA, lysophosphatic acid; | 5-oxo-ETE, 5-oxo-6E,8E,11Z,14Z-eicosatetraenoic acid; | SIP, sphingosine 1-phosphate; | SPC, sphingosylphosphorylcholine; | PBL, peripheral blood leukocytes. | Upward arrows = increase of intracellular second messenger concentration (Ca²⁺ or cAMP); downward arrows = decrease. Potent long-chain free fatty acids are 5,8,11-eicosatetraenoic acid, α-4,7,10,13,16,19-docosahexaenoic acid, and γ-linolenic acid for GPR40. For GPR43 and GPR41, propionate and butyrate are potent short-chain free fatty acids. Potent bile acids are taurine-conjugated lithocholic acid and lithocholic acid. |
TG1019 (R527), A RECEPTOR FOR 5-OXO-6,8Z,11Z,14Z-EICOSATETRAENOIC ACID

TG1019, also named R527, has been cloned by two independent groups and shown to recognize eicosatetraenoic acids and polyunsaturated fatty acids, including 5-oxo-6Z,8Z,11Z,14Z-eicosatetraenoic acid (5-oxo-ETE), 5(S)-hydroxyperoxy-eicosatetraenoic acid [5(S)-HPETE], 5-hydroxy-eicosatetraenoic acid (5-HETE), and arachidonic acid (14, 15). These are eicosanoids, which are oxidized arachidonic acid derivatives with 20 carbons and four double bonds, where the oxidation is at C-5, C-12, or C-15. The four double bonds at positions 6, 8, 11, and 14 in the eicosanoids are found in arachidonic acid (C20:4) (14, 15). On oxidation by three different lipoxygenases (i.e., 5-lipoxygenase, 12-lipoxygenase, and 15-lipoxygenase), three different oxidized eicosatetraenoic acids (ETEs) are formed: 5-oxo-ETE, 12-oxo-ETE, and 15-oxo-ETE. Leukotrienes are the other well-known lipid mediators synthesized via the 5-lipoxygenase (5-LO) cascade.

Eicosanoids have been implicated as lipid mediators acting on GPCRs. Hosoi et al. (14) found that TG1019 is a receptor for eicosanoids, with the most potent eicosanoid being 5-oxo-ETE. There was no significant activation by other 5-LO products, including leukotriene B4 and leukotriene D4, although some eicosanoids, including 5(S)-HPETE, 5-HETE, docosahexaenoic acid, and EPA, antagonized the agonistic activity of 5-oxo-ETE on TG1019 (14). In CHO cells expressing TG1019, Hosoi et al. (14) found the involvement of Gs proteins in the receptor signaling. By comparing the agonist lipid structures, they suggested that a length of carbon chain (20 carbons) and an olefin between C-8 and C-9 are the minimum requirements for the activators and that the functional group at C-5 is critical for potency (14).

5-Oxo-ETE, the most potent agonist, has been known as a potent chemotactic factor for eosinophils and neutrophils (14, 15). Jones et al. (15) cloned the same receptor and named it R527, although their receptor was truncated at the N terminus relative to TG1019; R527 starts from the second methionine residue at the 40 position of TG1019. Nevertheless, Jones et al. (15) found the results with R527 to be identical to those reported with TG1019.

BG37 (TGR5), A RECEPTOR FOR BILE ACIDS

BG37, also named TGR5, was first identified as a receptor for bile acids during the search of a ligand library (16, 17). Maruyama et al. (16) established BG37 expressing HEK293 cells and monitored intracellular cAMP and Ca2+ levels in response to various compounds. Bile acids increased intracellular cAMP levels (16), and the rank order potency of bile acids was as follows: taurine-conjugated lithocholic acid > lithocholic acid > deoxycholic acid > chenodeoxycholic acid > cholic acid (16, 17). Kawamata et al. (17) named the same receptor TGR5 and reached the same conclusion as Maruyama et al. (16), but with a different assay system. They further confirmed that the bile acids are ligands for TGR5 by demonstrating internalization of GFP-tagged TGR5, GTPyS binding in membrane fractions, and cAMP production in TGR5-expressing CHO cells (17). Although bile acids have nuclear receptors [farnesoid X receptor (nuclear bile acid receptor) (FXR)], the TGR5-mediated responses of bile acids are distinguishable from the nuclear receptor-mediated responses in three aspects: GPCR-mediated responses are much faster than responses through nuclear receptors, expression profiles for receptors in the nucleus and plasma membrane are different, and the rank order potencies of ligands are different (16, 17). Maruyama et al. (16) extended the significance of BG37 to gastrointestinal (GI) physiology (16), because they found the expression of BG37 in the GI tract, especially in enterocinocytic cell lines, but not in epithelial cells, implying that the interaction of bile acids with BG37 could induce the secretion of glucagon-like peptide-1 or cholecystokinin. Kawamata et al. (17) observed that bile acids suppressed LPS-stimulated cytokine production in a human monocyte cell line (THP-1) in a BG37-mediated manner. From these observations, it is obvious that the discovery of BG37 ligand opened a new avenue in the physiology of the lipids and also linked the actions of bile acids in the immune system and GI tissues to the GPCR (16, 17).

CONSIDERATIONS ON THE NEW GPCRS FOR LIPID MEDIATORS

In GPCR research on lipid mediators, the pharmacological criteria for ligand identification, especially specific binding and the reversibility of binding, are sometimes very difficult to satisfy, because the lipid mediators are easily absorbed, resulting in high nonspecific binding. Therefore, it is not always possible to demonstrate an interaction of lipids with candidate receptor proteins that is supported by specific, reversible, and high-affinity binding.

Among the 14 papers cited in this review, only one publication provided data on ligand binding (18). On the other hand, GTPyS binding experiments, which involve the functional analysis of GPCR activation in the plasma membrane preparation, were conducted in four studies in lieu of ligand binding (12, 14, 19, 81). The remaining nine publications did not provide data on ligand binding, which is a reflection of the difficulty of performing binding experiments in GPCR research for lipid mediators. To overcome this shortcoming, different assays in several expression systems are highly recommended to confirm conclusions and more importantly to avoid misinterpretations. Some of the reports mentioned above used only one or two functional assays. Therefore, their results would satisfy only one pharmacological criterion: the activation of GPCR by the candidate ligand. Without direct binding data, many alternative interpretations of the results of GPCR activation in intact cells are possible. For example, the candidate compound activates other receptors, which in turn triggers the secretion of endogenous...
ligands for the GPCR tested. Such an example was found in the case of the BLT1 leukotriene B4 receptor. This receptor was originally reported as an ATP receptor; however, it was later shown to be a leukotriene B4 receptor (82, 83). However, the original observation was partly correct, because ATP provokes the secretion of leukotriene B4 in the assay system. Enzymatic modification of the candidate lipid is an alternative possibility. Furthermore, GPCRs can be activated indirectly by candidate lipids, and such a possibility should be excluded.

Two structurally related ligands have been shown to act on a GPCR in many cases. Some S1P receptors are activated to different degrees by SPC or sphingosine (46, 56, 84–86), and GPR4 and G2A are similar cases (52, 87). They are activated by SPC and LPIC with different affinities. Multiplicity of ligands could give diverse degrees of receptor activation and provide fine modulation of the target cells. In contrast to the above, however, activation of a GPCR by two structurally unrelated endogenous ligands is very rare. The activation of GPR63 by structurally different S1P and doPA, but not by structurally related LPA, would be the first case of such an activation.

CONCLUDING REMARKS

The identification of GPCRs for new lipid mediators has contributed greatly to advancements in pharmacology and pathophysiology. Gene ablation (“knock-out”) studies of each GPCR, such as knock-out mice of S1P1 and G2A, also can provide critical information on the newly identified GPCRs (88, 89). The expression of recombinant DNAs would make it possible to carry out high-throughput screening of agonists or antagonists for each receptor and would be much more sensitive than classic tissue or cell preparations. The development of LPA antagonists has been accelerated by the identification of LPA receptors (59–64), and studies of the changes in the expression level of GPCRs in many pathophysiological conditions, such as cancer, would help in developing new drugs. Finally, changes in the ligand concentration of many biological fluids, such as the LPA concentration in ascites, should be studied in the future (90).

The discovery of orphan GPCRs has been accelerated largely by the full availability of human genomic information and highly sensitive high-throughput screening systems (4, 22, 91). The chemical structures of lipids, which match with the GPCRs, are familiar to the fields of nutrition, physiology, and biochemistry, and they are fortunately included in a library pool of candidate compounds for GPCRs, screened by many research groups. Of course, the possibility of their corresponding receptors in the plasma membrane has been suggested and implied in earlier reports. Nevertheless, experimentally matching lipids with GPCRs will have a great impact on many medical research areas and will be a springboard for the development of therapeutic agents targeting these receptors. To date, the field has been very successful in matching novel lipid mediators with orphan GPCRs, and there is the promise of additional discoveries to come.

The author thanks Heui-Soo Kim (Pusan National University) for constructing a dendrogram of GPCRs for lipid mediators and Kevin R. Lynch (University of Virginia) for critical reading of the manuscript and English usage. This work was supported by a Pusan National University research grant.

REFERENCES

1. Vane, J. R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat. New Biol. 231: 232–235.
2. Xie, G. X., A. Miyajima, and A. Goldstein. 1992. Expression cloning of cDNA encoding a seven-helix receptor from human plasma with affinity for opioid ligands. Proc. Natl. Acad. Sci. USA. 89: 4124–4128.
3. Vassilatis, D. K., J. G. Hofmann, H. Zeng, F. Li, J. E. Ranchalis, M. T. Mortrud, A. Brown, S. S. Rodriguez, J. R. Weller, A. C. Wright, J. E. Bergmann, and G. A. Gaitanaris. 2003. The G protein-coupled receptor repertoires of human and mouse. Proc. Natl. Acad. Sci. USA. 100: 4905–4908.
4. Im, D. S. 2002. Orphan G protein-coupled receptors and beyond. Jpn. J. Pharmacol. 96: 101–106.
5. Davinport, A. P. 2005. Peptide and trace amine orphan receptors: prospects for new therapeutic targets. Curr. Opin. Pharmacol. 3: 127–134.
6. Uhlenbrock, K., H. Gassenhuber, and E. Kostenis. 2002. Sphingosine-1-phosphate is a ligand of the human gpr3, gpr6 and gpr12 family of constitutively active G protein-coupled receptors. Cell. Signal. 14: 941–953.
7. Ignatov, A., J. Lintzel, I. Hermans-Borgmeyer, H. J. Kreienkamp, P. Joost, S. Thomsen, A. Mehtner, and H. C. Schaller. 2003. Role of the G-protein-coupled receptor GPR12 as high-affinity receptor for sphingosylphosphorylcholine and its expression and function in brain development. J. Neurosci. 23: 907–914.
8. Niedernberg, A., S. Tunaru, A. Blaukat, A. Ardati, and E. Kostenis. 2003. Sphingosine-1-phosphate and dioleoylphosphatidic acid are low affinity agonists for the orphan receptor GPR63. Cell. Signal. 15: 435–446.
9. Itoh, Y., Y. Kawamata, M. Harada, M. Kobayashi, R. Fujii, S. Fukumizu, K. Ogi, M. Hosoya, Y. Tanaka, H. Uejima, H. Tanaka, M. Maruyama, R. Satoh, S. Okubo, H. Kizawa, H. Komatsu, F. Matsumura, Y. Noguchi, T. Shinohara, S. Hinuma, Y. Fujisawa, and M. Fujino. 2003. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. Nature. 422: 175–176.
10. Briscoe, C. P., M. Tadayyon, J. L. Andrews, W. G. Benson, J. K. Joost, S. Thomsen, A. Mehtner, and H. C. Schaller. 2003. Role of the G-protein-coupled receptor GPR12 as high-affinity receptor for sphingosylphosphorylcholine and its expression and function in brain development. J. Neurosci. 23: 907–914.
11. Nilsson, N. E., K. Kotarsky, C. Owman, and B. Olde. 2003. Identification of a free fatty acid receptor, FFA(2)R, expressed on leukocytes and activated by short chain fatty acids. Biochim. Biophys. Res. Commun. 305: 1047–1052.
12. Brown, A. J., S. M. Goldsworthy, A. A. Barnes, M. M. Eilert, I. Tcheang, D. Daniels, A. I. Muir, M. J. Wigglesworth, I. Kinghorn, N. J. Fraser, N. B. Pike, J. C. Strum, K. M. Steplewski, P. R. Murdock, J. C. Holder, F. H. Marshall, P. G. Szeckeres, S. Wilson, D. M. Ignar, S. M. Foord, A. Wise, and S. J. Dowell. 2003. The orphan G protein-coupled receptor GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J. Biol. Chem. 278: 11312–11319.
13. Kotarsky, K., N. E. Nilsson, E. Floedtgen, C. Owman, and B. Olde. 2003. A human cell surface receptor activated by free fatty acids and thiazolidinedione drugs. Biochim. Biophys. Res. Commun. 301: 406–410.
14. Hosoi, T., Y. Koguchii, E. Sugikawa, A. Chikada, K. Ogawa, N. Tsuda, N. Suto, S. Tsunoda, T. Taniguchi, and T. Ohnuki. 2002. Identification of a novel human eicosanoid receptor coupled to G(i/o). J. Biol. Chem. 277: 31459–31465.
15. Jones, C. E., S. Holden, L. Tenaillon, U. Bhatia, K. Seuwen, P. Tranter, J. Turner, R. Kettle, R. Boulahel, S. Charlton, N. R. Nirmala, G. Jarai, and P. Finan. 2003. Expression and characterization of a 5-oxo-6E,7Z,11Z,14Z-eicosatetraenoic acid receptor highly expressed on human eosinophils. Biochem. Biophys. Acta 1582: 121–131.

16. Maruyama, T., Y. Miyamoto, T. Nakamura, Y. Tamai, H. Okada, E. Sugiyama, H. Itadani, and K. Tanaka. 2002. Identification of membrane-type receptor for bile acids (M-BAR). Biochem. Biophys. Res. Commun. 298: 714–719.

17. Kawamura, Y., R. Fujii, M. Hosoya, M. Harada, Y. Yoshida, M. Miwa, S. Fukusumi, Y. Habata, T. Itoh, Y. Shintani, S. Hinuma, Y. Fujigawa, and M. Tonomura. 2003. A G protein-coupled receptor responsive to bile acids. J. Biol. Chem. 278: 9435–9440.

18. Noguchi, K., S. Ishii, and T. Shimizu. 2003. Identification of p2y9/GPR23 as a novel G protein-coupled receptor for lysosphatidic acid, structurally distant from the Edg family. J. Biol. Chem. 278: 25690–25695.

19. Le Poual, E., C. Loison, S. Struyf, J. Y. Springael, V. Lannoy, M. E. Jarai, and P. Finan. 2003. Expression and characterization of a G protein-coupled receptor for lysosphatidic acid as a ligand for the G protein-coupled receptor EDG-1. Science. 279: 1592–1595.

20. Bourne, H. R., and M. von Zastrow. 2001. Drug receptors and pharmacodynamics. In Basic and Clinical Pharmacology, 8th edition, B. G. Katzung, editor. McGraw-Hill, New York, 9–34.

21. Marchese, A. S., R. George, L. F. Kolakowski, Jr., K. R. Lynch, and B. F. O’Dowd. 1999. Novel GPCRs and their endogenous ligands: expanding the boundaries of physiology and pharmacology. Trends Pharmacol. Sci. 20: 370–375.

22. Kenakin, T. P. 1987. Drug-Receptor Theory. Pharmacological Analysis of Drug-Receptor Interaction. Raven Press, New York, 1–30.

23. Samuelsson, B. 1983. From studies of biochemical mechanism to novel biological mediators: prostaglandin endoperoxides, thromboxanes, and leukotrienes. Nobel Lecture, 8 December 1982. Biochi. Reg. 3: 791–813.

24. Matsuda, L. A., S. J. Lollatii, M. J. Brownstein, A. C. Young, and T. J. Bonner. 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature. 346: 561–564.

25. Devane, W. A., A. G. McAllister, S. D. Feighner, Q. Liu, R. P. Nargund, L. H. Van der Poel, and A. A. Patchett. 2001. Orphan G-protein-coupled receptors and natural ligand discovery. Trends Pharmacol. Sci. 22: 132–140.

26. Howland, A. D., G. McAllister, P. E. May, E. J. Goetzl, and O. G. Hallmark. 1998. Characterization of a novel G protein-coupled receptor for lysosphatidic acid. J. Biol. Chem. 273: 7906–7910.

27. Kabarowski, J. H., K. Zhu, L. Q. Le, O. N. Witte, and Y. Xu. 2001. Sphingosylphosphorylcholine as a ligand for the immunoregulatory receptor GPR4. J. Biol. Chem. 276: 14281–14286.

28. Im, D. S., C. E. Heise, M. A. Harding, S. R. George, B. F. O’Dowd, D. Theodorescu, and K. R. Lynch. 2000. Molecular cloning and characterization of a novel human G-protein-coupled receptor, EDG7, for lysosphatidic acid. J. Biol. Chem. 274: 27776–27785.

29. Xu, Y., K. Zhu, G. Hong, W. Wu, L. M. Baudhuin, X. Xiao, and D. S. Damron. 2000. Sphingosylphosphorylcholine is a ligand for ovarian cancer G-protein-coupled receptor 1. Nat. Cell Biol. 2: 261–267.

30. Zhu, K., L. M. Baudhuin, G. Hong, F.S. Williams, K. L. Cristina, J. H. Kabarowski, O. N. Witte, and Y. Xu. 2001. Sphingosylphosphorylcholine and lysosphatidylcholine are ligands for the G protein-coupled receptor GPR4. J. Biol. Chem. 276: 41325–41335.

31. Kon, J., K. Zhu, L. Q. Le, O. N. Witte, and Y. Xu. 2001. Sphingosylphosphorylcholine as a ligand for the immunoregulatory receptor GPR2. Science. 293: 705–707.

32. Kabarowski, J. H., K. Zhu, L. Q. Le, O. N. Witte, and Y. Xu. 2001. Sphingosylphosphorylcholine and lysosphatidylcholine are ligands for the G protein-coupled receptor GPR4. J. Biol. Chem. 276: 41325–41335.

33. Hla, T., V. Y. Li, C. W. Huang, C. A. Blackman, T. Yokomizo, T. Shimizu, and T. Hla. 1998. Phosphoinositide-l-phosphate as a ligand for the G protein-coupled receptor EDG-1. Science. 279: 1592–1595.

34. Lee, S. T., H. Bleu, O. G. Hallmark, and E. J. Goetzl. 1997. Identification of cDNAs encoding two G protein-coupled receptors for lysosphingolipids. FEBS Lett. 417: 279–282.

35. Le Poual, E., C. Loison, S. Struyf, J. Y. Springael, V. Lannoy, M. E. Jarai, and P. Finan. 2003. Expression and characterization of a G protein-coupled receptor for lysosphatidic acid as a ligand for the G protein-coupled receptor EDG-1. Science. 279: 1592–1595.
57. Bandoh, K., J. Aoki, A. Taira, M. Tsujimoto, H. Arai, and K. Inoue. 2000. Lysophosphatidic acid (LPA) receptors of the EDG family are differentially activated by LPA species. Structure-activity relationship of cloned LPA receptors. FEMS Lett. 478:159–165.

58. Brinkmann, V., and K. R. Lynch. 2002. FTI720: targeting G-protein-coupled receptors for sphingosine 1-phosphate in transplanted and autoimmune tissue. Curr. Opin. Immunol. 14:560–575.

59. Hasegawa, Y., J. R. Erickson, G. J. Goddard, S. Yu, S. Liu, K. W. Hasegawa, Y., J. R. Erickson, G. J. Goddard, S. Yu, S. Liu, K. W. Ohta, H., K. Sato, N. Murata, A. Damirin, E. Malchinkhuu, J. Kon, K. Heise, C. E., W. L. Santos, A. M. Schreihofer, B. H. Heasley, Y. V. Heise, C. E., W. L. Santos, A. M. Schreihofer, B. H. Heasley, Y. V. Ku, N. Suzuki, H. Murooka, T. Sakai, T. Nishitoba, D. S. Im, H. Nochi, K. Tanoto, H. Tomura, and F. Okajima. 2003. K16425, a subtype-selective antagonist for EDG-family lysophosphatic acid receptors, Mol. Pharmacol. 64:994–1005.

60. Fischer, D. J., N. Nusser, T. Virag, K. Liliom, V. M. Sardar, A. L. Parrill, and G. Tigyi. 2001. Short-chain phosphatidates are subtype-selective antagonists of lysophosphatidic acid receptors. Mol. Pharmacol. 60:776–784.

61. Virag, T., D. B. Elrod, K. Liliom, V. M. Sardar, A. L. Parrill, K. Yokoyama, G. Durgam, W. Deng, D. D. Miller, and G. Tigyi. 2003. Fatty alcohol phosphates are subtype-selective agonists and antagonists of lysophosphatidic acid receptors. Mol. Pharmacol. 63:1032–1042.

62. Heise, C. E., W. L. Santos, A. M. Schreihofer, B. H. Heasley, Y. V. Ku, N. Suzuki, H. Murooka, T. Sakai, T. Nishitoba, D. S. Im, H. Nochi, K. Tanoto, H. Tomura, and F. Okajima. 2003. K16425, a subtype-selective antagonist for EDG-family lysophosphatic acid receptors, Mol. Pharmacol. 64:994–1005.

63. Clemens, J. J., M. D. Davis, K. R. Lynch, and T. L. Macdonald. 2003. Synthesis of para-alkyl aryl amide analogues of sphingosine-1-phosphate: discovery of potent SIP receptor agonists. Bioorg. Med. Chem. Lett. 13:3401–3404.

64. Lin, P., and R. D. Ye. 2003. The lysophospholid receptor G2A activates a specific combination of G proteins and promotes apoptosis. J. Biol. Chem. 278:14379–14386.

65. Ludwig, M. G., M. Vanek, D. Guerini, J. A. Gasser, C. E. Jones, U. Junker, H. Hofstetter, R. M. Wolf, and K. Seuwen. 2003. Proton-sensing G-protein-coupled receptors. Nature. 425:93–98.

66. Bektaş, M., L. S. Barak, P. S. Jolly, H. Liu, K. R. Lynch, E. Acana, K. B. Suhr, S. Milbien, and S. Spiegel. 2003. The G protein-coupled receptor GPR4 suppresses ERK activation in a ligand-independent manner. Biochemistry. 42:12181–12191.

67. Guo, Z., K. Liliom, D. J. Fischer, I. C. Bathurst, L. D. Tomei, M. C. Kieler, and G. Tigyi. 1996. Molecular cloning of a high-affinity receptor for the growth factor-like lipid mediator lysophosphatidic acid from Xenopus laevis. Biochim. Biophys. Acta. 12181–12191.

68. Marchese, A., M. Sawdzargo, T. Nguyen, R. Cheng, H. H. Heng, T. Nowak, D. S. Im, K. R. Lynch, S. R. George, and B. F. O’Dowd. 1999. Discovery of three novel orphan G-protein-coupled receptors. J. Biol. Chem. 275:1979–1985.

69. Kawsawaya, Y., K. Kame, T. Izumi, and T. Shimizu. 2000. Mammalian LPA1 (Edg-2) and LPA3 (Edg-3) receptors are not responsive to lysophosphatidic acid in mammalian expression systems. Biochem. Biophys. Res. Commun. 276:957–964.

70. Eggerickx, D., J. F. Denef, O. Labbe, Y. Hayashi, S. Refetoff, G. Vas- tors in Xenopus oocytes and mammalian cells. J. Biol. Chem. 276:15309–15315.

71. Contos, J. J., I. Ishii, N. Fukushima, M. A. Kingsbury, X. Ye, S. Kawamura, J. H. Brown, and J. Chun. 2002. Characterization of lpa(2) (Edg4) and lpa(1)/lpa(2) (Edg2/Edg4) lysophosphatidic acid receptor knockout mice: signaling defects without obvious phenotypic abnormality attributable to lpa(2). Mol. Cell. Biol. 22:6921–6929.

72. Hooks, S. B., W. L. Santos, D. S. Im, C. E. Heise, T. L. Macdonald, and K. R. Lynch. 2001. Lysophosphatidic acid-induced mitogene-sis is regulated by lipid phosphate phosphatases and is Edg-receptor independent. J. Biol. Chem. 276:4611–4621.

73. Lynch, K. R. 1998. G protein-coupled receptor informatics and the orphan problem. In Identification and Expression of G Protein-Coupled Receptors. K. R. Lynch, editor. Wiley-Liss, New York. 54–72.

74. Hooks, S. B., W. L. Santos, D. S. Im, C. E. Heise, T. L. Macdonald, and K. R. Lynch. 2001. Lysophosphatidic acid-induced mitogenesis is regulated by lipid phosphate phosphatases and is Edg-receptor independent. J. Biol. Chem. 276:4611–4621.

75. Lynch, K. R. 1998. G protein-coupled receptor informatics and the orphan problem. In Identification and Expression of G Protein-Coupled Receptors. K. R. Lynch, editor. Wiley-Liss, New York. 54–72.

76. Hooks, S. B., W. L. Santos, D. S. Im, C. E. Heise, T. L. Macdonald, and K. R. Lynch. 2001. Lysophosphatidic acid-induced mitogenesis is regulated by lipid phosphate phosphatases and is Edg-receptor independent. J. Biol. Chem. 276:4611–4621.

77. Hooks, S. B., W. L. Santos, D. S. Im, C. E. Heise, T. L. Macdonald, and K. R. Lynch. 2001. Lysophosphatidic acid-induced mitogenesis is regulated by lipid phosphate phosphatases and is Edg-receptor independent. J. Biol. Chem. 276:4611–4621.

78. Hooks, S. B., W. L. Santos, D. S. Im, C. E. Heise, T. L. Macdonald, and K. R. Lynch. 2001. Lysophosphatidic acid-induced mitogenesis is regulated by lipid phosphate phosphatases and is Edg-receptor independent. J. Biol. Chem. 276:4611–4621.