Journal of Cellular Biochemistry 92:949–966 (2004)

PROSPECTS

G Protein Mediated Signaling Pathways in Lysophospholipid Induced Cell Proliferation and Survival

Julie Radeff-Huang, Tammy M. Seasholtz, Rosalia G. Matteo, and Joan Heller Brown*

Department of Pharmacology, University of California San Diego, La Jolla, California 92093

Abstract Agonist activation of a subset of G protein coupled receptors (GPCRs) stimulates cell proliferation, mimicking the better known effects of tyrosine kinase growth factors. Cell survival or apoptosis is also regulated via pathways initiated by stimulation of these same GPCRs. This review focuses on aspects of signaling by the lysophospholipid mediators, lysophosphatidic acid (LPA), and sphingosine 1 phosphate (S1P), which make these agonists uniquely capable of modulating cell growth and survival. The general features of GPCR coupling to specific G proteins, downstream effectors and signaling cascades are first reviewed. GPCR coupling to G_i and Ras/MAPK or to G_q and phospholipase generated second messengers are insufficient to regulate cell proliferation while $G_{12/13}$/Rho engagement provides additional complementary signals required for cell proliferation. Survival is best predicted by coupling to G_i pathways that regulate PI3K and Akt, but other signals generated through different G protein pathways are also implicated. The unique ability of LPA and S1P to concomitantly stimulate G_i, G_q, and $G_{12/13}$ pathways, given the proper complement of expressed LPA or S1P receptors, allows these receptors to support cell survival and proliferation. In pathophysiological situations, e.g., vascular disease, cancer, brain injury, and inflammation, components of the signaling cascade downstream of lysophospholipid receptors, in particular those involving Ras or Rho, may be altered. In addition, up or downregulation of LPA or S1P receptor subtypes, altering their ratio, and increased availability of the lysophospholipid ligands at sites of injury or inflammation, likely contribute to disease and may be important targets for therapeutic intervention. J. Cell. Biochem. 92: 949–966, 2004. © 2004 Wiley-Liss, Inc.

Key words: LPA; S1P; proliferation; survival; Rho; ERK; PI3K; Akt; G_i; G_q; $G_{12/13}$; vascular disease; astrogliosis; inflammation; cardiac hypertrophy

RECEPTOR-G PROTEIN COUPLING AND CELL GROWTH

Hormones, neurotransmitters, and other extracellular mediators that bind to cell surface receptors initiate complex intracellular biochemical signaling cascades. The largest family of hormone receptors, the G protein-coupled receptors (GPCRs), signal via interaction of the agonist-bound receptor with heterotrimeric G proteins. Hormone binding induces a conformational change in the GPCR that leads to receptor-G protein coupling. The alpha (α) subunit of the G protein releases GDP in exchange for GTP and is thereby "activated." Once the activated α subunit dissociates from the beta-gamma ($\beta\gamma$) subunits, either α or $\beta\gamma$ can interact with enzymes, scaffolds, or ion channels to induce biochemical changes that ultimately affect contraction, gene expression, or cell growth.

There are four major families of heterotrimeric G protein alpha subunits: $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$. $G\alpha_s$ activates adenylyl cyclase (AC) resulting in increases in cAMP, leading to activation of cAMP dependent protein kinase A (PKA) and subsequent phosphorylation of cellular proteins. Elevated cAMP stimulates cell growth in only a few cell systems (e.g., thyroid cells). In most, cAMP/PKA and the closely related cGMP/PKG pathway, inhibit cell growth and proliferation by modulating

Grant sponsor: NIH (to J.H.B.); Grant number: GM36927; Grant sponsor: American Heart Association Scientist Development (to T.M.S.); Grant sponsor: NIH training (to J.R.H.); Grant number: 2T82AG00216.

*Correspondence to: Joan Heller Brown, Department of Pharmacology, University of California San Diego, 9500 Gilman Drive-0636, La Jolla, CA 92093.
E-mail: jhbrown@ucsd.edu

Received 19 November 2003; Accepted 10 February 2004
DOI 10.1002/jcb.20094

© 2004 Wiley-Liss, Inc.

components in the signal transduction cascade. $G\alpha_i$ is also linked to AC, but in contrast to $G\alpha_s$, $G\alpha_i$ inhibits AC and thus lowers cellular cAMP. The bacterial toxin, pertussis toxin (PTX) ADP ribosylates the α subunits of the $G_{i/o}$ proteins. This prevents α subunit dissociation from $\beta\gamma$, and thereby inhibits the ability of $G_{i/o}$-coupled receptor agonists to affect downstream responses. Not only is the $G\alpha_i$-dependent inhibition of AC PTX-sensitive, but so is the regulation of effectors such as phospholipase C (PLC), AC, Src, and PI3 kinase by $\beta\gamma$ subunits released from G_i.

$G\alpha_q$ binds to and activates phospholipase C β, which in turn catalyzes the conversion of phosphatidylinostiol bisphosphate to diacylglycerol (DAG) and inositol trisphosphate (IP_3). DAG activates protein kinase (PKC), a response mimicked by phorbol esters, compounds originally identified as tumor promoters. The existence of various PKC isoforms in multiple subcellular locations throughout the cell contributes to the pleiotropic effects of PKC activation. $InsP_3$ binds to receptors on the endoplasmic reticulum, resulting in the mobilization of stored Ca^{++}. The subsequent increase in cytosolic Ca^{++} can activate a variety of Ca^{++}-dependent enzymes involved in signal transduction cascades. Some isoforms of AC and phosphodiesterase are regulated by changes in intracellular Ca^{++}, as are PLCδ, conventional PKCs, myosin light chain kinase, phospholipase A_2, and Ca^{++}/calmodulin-dependent kinase. In light of the myriad responses that can be elicited secondary to PKC activation and/or Ca^{++} elevation, it is not surprising that activation of some G_q-coupled receptors, or overexpression of GTPase-deficient, constitutively active $G\alpha_q$, can result in cell proliferation. Notably however, activation of PLC, Ca^{++}, or PKC signaling pathways are generally observed to be insufficient to promote cell proliferation.

The most recently identified class of heterotrimeric G proteins includes those of the $G\alpha_{12/13}$ family. $G\alpha_{13}$ was first identified as an oncogene, and overexpression of GTPase-deficient $G\alpha_{12}$ or $G\alpha_{13}$ has been demonstrated to alter cell shape, gene expression, and cell growth. It is now clear that these responses result, at least in part, from activation of the small G protein Rho. This conclusion has been deduced from investigations using dominant interfering forms of Rho, *Clostridium difficile* Toxin B, and C3 exoenzyme from *Clostridium botulinum* toxin, which ADP ribosylates and inactivates Rho. The Rho family of small G proteins includes more than 18 members, the best characterized of these being RhoA, Rac1, and Cdc42. A molecular link between the $G\alpha_{12/13}$ family and RhoA was recently identified through work demonstrating that a Rho guanine nucleotide exchange factor (GEF) p115RhoGEF binds $G\alpha_{12/13}$ proteins and is activated by $G\alpha_{13}$ [Hart et al., 1998]. Similar interactions have been shown for other GEFs, e.g., PDZRhoGEF and LARG, and $G\alpha_{13}$.

While Rho signaling was once considered most relevant to control of the actin cytoskeleton, it is now clear that RhoA also collaborates with the small G protein Ras to produce cell cycle progression. Ras activation can be elicited through a variety of GPCRs. Beta/gamma subunits released through activation of G_i-coupled receptors, as well as signals generated through activation of $G\alpha_q$ can increase Ras activity. Other mechanisms described for Ras/MAPK activation, include transactivation of receptor tyrosine kinases by release of paracrine or autocrine factors. In the case of the epidermal growth factor (EGF) receptor, stimulation of GPCRs leads to the shedding of an EGFR ligand. This activation of the EGF receptor can initiate a canonical sequence of events (i.e., Shc/Grb2/Sos) or recruit signaling molecules such as PI3K, which then lead to Ras and ERK activation. MAPK signaling cascades have also been shown to be elicited through scaffolding proteins. For example, GPCR-stimulation can promote complex formation between β-arrestin and ERK or JNK MAPK cascades, leading to their activation. Thus stimulation of GPCRs coupled to many different G proteins activate Ras through various pathways and lead to subsequent MAPK signaling. Notably, while Ras-dependent MAPK signaling has been described in many systems and in response to many GPCR ligands, not all GPCRs are able to promote proliferation. MAPK activation includes both a transient and a sustained activation phase. The sustained phase appears to reflect MAPK translocation to the nucleus where it can initiate gene transcription and cell cycle progression. This occurs only with a subset of GPCRs. Recent evidence indicates that $G_{12/13}$ signaling to Rho may be one factor that contributes to the nuclear translocation or maintenance of MAPK activity.

Our previous studies examining GPCR signaling pathways regulating gene expression

and proliferation included a comprehensive comparison of the effects of thrombin and muscarinic receptor activation. Activation of several signaling cascades was uniquely observed with thrombin. Since thrombin receptors are strongly coupled to $G_{12/13}$, we hypothesized that receptors which couple to $G_{12/13}$ family proteins and Rho pathways are most likely to generate growth-promoting signals. Simultaneous activation of either G_q or G_i may also be required, as there appears to be a synergy between activation (by G_q or G_i) of Ras/MAPK pathways and activation (by $G_{12/13}$) of Rho. There are several proposed mechanisms that can explain the positive effect of Rho on cell cycle progression. These include decreasing the expression of cell cycle inhibitors such as p21 and p27, increasing the expression of cyclin A, and facilitating the nuclear localization of ERK.

LYSOPHOSPHOLIPID RECEPTORS AND G-PROTEIN COUPLING

Lysophospholipids including lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) have emerged as important regulators of cell growth and survival. While it was once thought that high concentrations of these mediators were required to alter cellular responses, and did so via membrane perturbations, it is now clear that most responses to LPA and S1P are mediated via activation of GPCRs. LPA and S1P receptor subtypes have been identified, cloned, and expressed. The four LPA receptors identified to date are LPA_1, LPA_2, LPA_3 and the distantly related and newly identified LPA_4. For S1P, five receptors have been identified: $S1P_1$, $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$. Previous nomenclature classified all of the LPA and S1P receptors as EDG receptors, but the currently accepted nomenclature is LPA_{1-4} and $S1P_{1-5}$. While some GPCR receptor subtypes show selectivity in their interaction with particular G proteins, LPA and S1P receptor subtypes appear to couple to multiple heterotrimeric G proteins. Thus in heterologous expression studies, receptor subtype coupling to G proteins and downstream responses may only indicate what is possible rather than what occurs under physiological conditions. Accordingly, the precise LPA and S1P receptor subtypes that couple to specific G proteins have not, for the most part been satisfactorily delineated. However, studies using LPA receptor knockout mice and examining downstream signaling pathways suggest that LPA_1 couples efficiently to G_i-dependent regulation of AC, LPA_2 couples to G_{12}/Rho, and cytoskeletal responses and LPA_3 couples to G_q-mediated PLC activation [Ye et al., 2002]. For S1P receptor subtypes, G protein activation has been measured directly in both insect and mammalian heterologous expression systems using [^{35}S]GTPγS binding. These experiments reveal that $S1P_1$ is singularly coupled to G_i, while $S1P_2$ and $S1P_3$ receptors can all couple not only to G_i but also to G_q and G_{12} [Windh et al., 1999]. Studies using S1P receptor knockout mice suggest that coupling to PLC (via G_q) is mediated largely via $S1P_3$, while Rho activation (via $G_{12/13}$) occurs through $S1P_2$ and $S1P_3$ receptors. The receptor subtype mediated signaling mechanisms will be covered in greater detail below and are summarized in Figure 1.

The goal of this article is to review and put into context the current literature regarding LPA and S1P receptor coupling to G proteins and their downstream effectors in order to understand how these pathways translate into LPA and S1P mediated changes in cell growth and survival. How these pathways relate to both physiological and pathophysiological conditions will be addressed.

LYSOPHOSPHOLIPID SIGNALING PATHWAYS

Rho Activation

LPA. It has been known for over a decade that LPA can produce Rho-dependent cytoskeletal responses such as stress fiber formation, cell rounding, and neurite retraction. More recently it has been demonstrated that LPA produces Rho-dependent gene expression and DNA synthesis. Rho was initially implicated in these pathways through experiments using activated or dominant-negative RhoA or by Rho inactivation with C3 exoenzyme. Only over the past few years has direct evidence for the ability of LPA to activate RhoA been obtained. Initially this evidence came from cell fractionation studies, which showed an increase in membrane-associated RhoA following treatment with LPA [Fleming et al., 1996]. In 1999, work from two separate laboratories used affinity precipitation of GTP-liganded, active RhoA, with a GST-fusion protein of the Rho binding domain of either Rho kinase or rhotekin, to show unequivocally that RhoA was

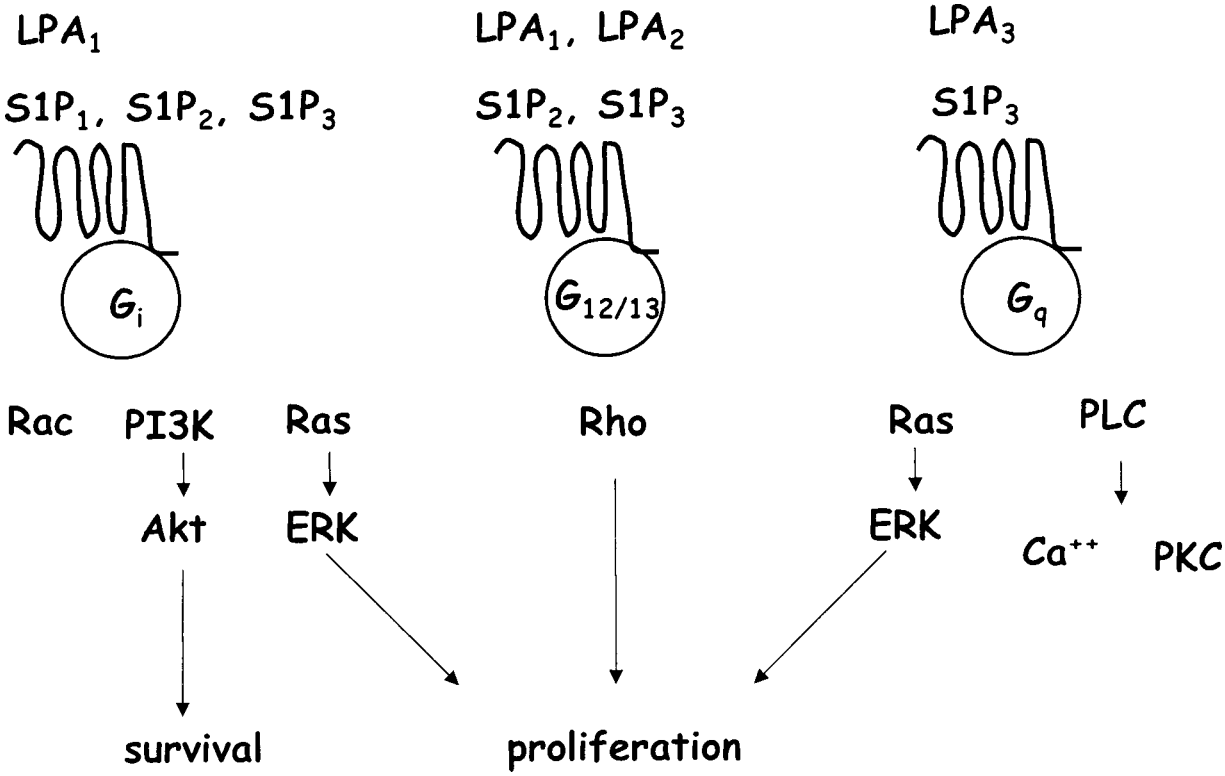

Fig. 1. Proposed model of lysophosphatidic acid (LPA) and sphingosine 1 phosphate (S1P) receptor-mediated regulation of cell proliferation and survival. LPA and S1P receptor subtypes can couple to G_i, G_q, and $G_{12/13}$ family heterotrimeric G proteins. Selectivity in receptor subtype coupling to specific G proteins is likely, but only partially resolved, as indicated here in the pairings shown. The coupling shown here is reflective of reports from endogenous or knockout systems rather than data from heterologous systems. Complex signaling cascades that ultimately regulate cell growth and protection from apoptosis are elicited through LPA and S1P receptor activation. Input from $G_{12/13}$-mediated Rho activation complement G_i and G_q-mediated Ras-ERK activation to coordinately regulate cell proliferation; G_i-mediated signaling through PI3K/Akt contributes to cell survival. Abberrant receptor subtype expression and altered ligand availability could contribute to a variety of pathophysiological disorders due to alteration of these tightly regulated signaling events.

activated by LPA [Kranenburg et al., 1999; Ren et al., 1999].

LPA activates RhoA through LPA receptor coupling to the $G\alpha_{12/13}$ family of proteins. Gohla et al. [1998] demonstrated that LPA could activate several G proteins ($G\alpha_{q/11}$, $G\alpha_{12}$, $G\alpha_{13}$, or $G\alpha_i$), in fibroblasts, as assessed by GTP azidoanilide photolabeling of the α subunit of these G proteins. However, RhoA activation in these cells was demonstrated to occur in response to overexpression of activated $G\alpha_{12}$ or $G\alpha_{13}$. Observations from other labs are consistent in demonstrating that $G\alpha_{12}$ and $G\alpha_{13}$ stimulate RhoA activation [Kranenburg et al., 1999; Sagi et al., 2001], whereas $G\alpha_i$ appears to be largely ineffective in this regard [Kranenburg et al., 1999]. Conflicting data exists regarding the ability of $G\alpha_q$ to activate RhoA [Sagi et al., 2001; Chikumi et al., 2002]. LPA appears to signal preferentially through $G\alpha_{13}$, rather than $G\alpha_{12}$, to produce Rho-dependent cytoskeletal changes (the opposite appears true for thrombin) in Swiss 3T3 fibroblasts, and the mechanism by which $G\alpha_{13}$ activates RhoA involves EGFR tyrosine kinase [Gohla et al., 1998]. Preferential coupling of LPA to $G\alpha_{13}$ has also been observed in HEK 293T cells using a novel assay based on active $G\alpha_{12}$ or $G\alpha_{13}$ binding to a GST-fused tetratricopeptide repeat domain of Ser/Thr phosphatase type 5 [Yamaguchi et al., 2003]. Seminal work from Sternweis and colleagues provides an important link between $G\alpha_{13}$ and RhoA activation, as mentioned above, by demonstrating that $G\alpha_{13}$ interacts with p115RhoGEF and enhances its ability to catalyze nucleotide exchange on RhoA [Hart et al., 1998].

While $G_{12/13}$ have been shown to link LPA receptors to activation of RhoA, recent evidence suggests that G_i couples LPA to activation of Rac1 [Van Leeuwen et al., 2003]. The balance

between Rho and Rac activity appears to be critical for changes in cell shape and resultant cell motility responses. Rac activation leads to increased focal contacts and cell spreading, as observed at the leading edge of migrating cells, whereas Rho produces cell rounding and contraction and contributes to the loss of adhesion observed at the trailing edge. These observations suggest that coordinated regulation of these two small G proteins is necessary for cell movement, with activation of Rac serving as a critical signal for cell migration (see below for $S1P_1$ receptor). The ratio of LPA receptor subtypes coupled to or signaling via G_i versus $G_{12/13}$ may determine the relative extent of activation of Rac and Rho and thus dictates the nature of the cytoskeletal response. Reciprocal modulation of Rho and Rac activation may be facilitated by the ability of Rho and Rac to negatively regulate the activity of one another. With regard to effects of Rho and Rac on cell proliferation, both have been reported to positively influence cell cycle progression although there is less mechanistic evidence for Rac than for Rho involvement (as discussed above). In the case of Rac, activation of its effector p21-activated kinase (PAK), superoxide production, and cyclin D1 accumulation have been suggested mechanisms.

S1P. There is a growing literature regarding the effect of S1P and specific S1P receptor subtypes on activation of Rho family proteins, in particular as associated with the ability of these receptors to stimulate or inhibit cell migration. Studies using endogenous S1P receptors or overexpression of S1P receptor subtypes, indicate that $S1P_1$ activates Rac1, presumably through G_i stimulation of a Rac GEF [Okamoto et al., 2000]. In contrast, $S1P_2$ inhibits Rac1 activation through stimulation of a Rac GAP [Okamoto et al., 2000]. This may reflect the ability of the $S1P_2$ receptor to couple to $G_{12/13}$, while $S1P_1$ couples exclusively to G_i. In line with this explanation, $S1P_2$ activates RhoA whereas $S1P_1$ is ineffective in this regard. $S1P_3$ [Sugimoto et al., 2003] and $S1P_4$ [Graler et al., 2003] activate both Rac1 and RhoA thus these receptors do not appear to confer the selectivity afforded by $S1P_1$ versus $S1P_2$ signaling. Data obtained using MEF cells from S1P receptor knockout mice also reveal that $S1P_2$ plays a major role in the activation of RhoA [Ishii et al., 2002], but combined deletion of $S1P_2$ and $S1P_3$ is necessary for complete loss of S1P receptor stimulated RhoA activation [Ishii et al., 2002]. The observation that the $S1P_1$ receptor remaining in $S1P_{2/3}$ null MEF cells fails to support RhoA activation is consistent with the primary coupling of $S1P_1$ to G_i and Rac rather than to $G_{12/13}$ and RhoA.

Phospholipase Activation

LPA. LPA mediated increases in PI hydrolysis occur primarily through what is now considered to be G_q regulated activation of phospholipase C (PLC). Early studies by van Corven et al. [1989] demonstrated that PI hydrolysis and subsequent increases in Ca^{++} and PKC were elicited by LPA and that these responses were not PTX-sensitive. Plevin et al. [1991] reported that LPA increased $InsP_3$ transiently, and led to parallel decreases in PIP_2 in fibroblasts. It is presently unclear whether only particular LPA receptor subtypes are involved in activating PLC. LPA_1, LPA_2, and LPA_3 receptors are all capable of increasing PLC activity in heterologous expression systems [Ishii et al., 2000]. In MEF cells from LPA_1 and LPA_2 receptor double knockout mice, LPA-stimulated PLC activation and Ca^{++} mobilization are markedly decreased, while loss of either single receptor alone confers a partial inhibition of these responses [Contos et al., 2002]. Thus, there appears to be redundancy between LPA_1 and LPA_2 receptor functions in PLC activation. As MEF cells have only low endogenous levels of the LPA_3 receptor, one cannot assess the relative role of the LPA_3 receptor from these studies. However, retroviral expression of LPA receptors in the $LPA_{1/2}$ double knockout MEFs restored PLC activation with LPA_3 being most efficacious [Contos et al., 2002]. That all three receptor subtypes can regulate PLC may be explained by the existence of several isoforms of PLC (α, β, γ, ε), regulated via different G-proteins or indirect pathways, as described in "Receptor-G protein coupling and cell growth."

It is now well-established, that receptor mediated phosphoinositide hydrolysis can be elicited not only through $G\alpha_q$ effects on $PLC\beta_{1-3}$, but also via stimulation of $PLC\beta_2$ by released $\beta\gamma$ subunits (generally released from G_i). In addition, PI hydrolysis can occur via indirect activation of tyrosine kinase receptors that in turn phosphorylate and stimulate $PLC\gamma$. Most recently, PLC epsilon ($PLC\varepsilon$) has emerged as a novel PLC isoform that can be activated

through βγ, Ras, and via $G_{12/13}$-Rho signaling [Wing et al., 2003]. Activation by growth stimulatory signals such as $G_{12/13}$, Ras, and Rho suggests that PLCε may provide a novel pathway contributing to the regulation of cell growth.

LPA has also been demonstrated to activate phospholipase D (PLD). PLD hydrolyzes phosphatidylcholine (PC), generating phosphatidic acid (PA), and choline. Since PA can be further converted to DAG by phosphatidic acid phosphohydrolase, PKC can also be activated by this pathway, often in a more prolonged fashion. PLD activity can also be modulated through other second messengers including PKC, Ca^{++}, and small G proteins such as RhoA and ADP Ribosylation Factor (ARF), thus there are a variety of mechanisms for feedback or crosstalk to PLD signaling. LPA can stimulate PLD activity in a PKC-sensitive manner in fibroblasts [van der Bend et al., 1992]. In addition, LPA mediated NFκB and p70(S6K) activation have been shown to depend upon PLD signaling, as well as on PLC, PKC, and Ca^{++} [Shahrestanifar et al., 1999].

Interestingly, in several cultured cell systems LPA fails to activate PLC, as assessed by $InsP_3$ formation. Despite this, LPA induces intracellular Ca^{++} mobilization. This behavior is observed in both SH-SY5Y cells [Young et al., 1999] and in 1321N1 astrocytoma cells [Seasholtz et al., 2004]. A proposed explanation for this is that $InsP_3$-independent Ca^{++} mobilization occurs through a sphingosine kinase mediated generation of intracellular S1P which then affects Ca^{++} homeostasis as demonstrated for responses to muscarinic agonists [Meyer zu et al., 1998; Young et al., 2000; van Koppen et al., 2001] or EGF [Meyer zu et al., 1999]. This is discussed in more detail below.

S1P. S1P receptor coupling to G_q-mediated PLC activation, $InsP_3$ formation, and Ca^{++} mobilization, has also been demonstrated in a number of cell systems. Heterologous expression studies reveal that the $S1P_1$, $S1P_2$, and $S1P_3$ receptor subtypes are all capable of activating PLC and mobilizing Ca^{++} [An et al., 1999]. However, S1P receptor knockout studies demonstrated that in MEF cells there is preferential coupling of the $S1P_3$ receptor to G_q-mediated PLC and Ca^{++} signaling, as the response is abolished in $S1P_3$ receptor knockout cells [Ishii et al., 2001, 2002]. In these studies, the observed loss of S1P stimulated PLC activity could also be restored by retroviral introduction of the $S1P_3$ receptor. In contrast, in $S1P_2$ null mice, PLC and Ca^{++} signaling in MEF cells were not significantly diminished [Ishii et al., 2002]. $S1P_1$ receptors can also mediate PLC and Ca^{++} but this is achieved by signaling through a PTX-sensitive mechanism that involves the βγ subunits of G_i [Okamoto et al., 1998].

As cited above, an unusual property of S1P-mediated Ca^{++} signaling is that S1P appears capable of regulating Ca^{++} not only through action at its cell surface GPCR, but also via its action as an intracellular second messenger. This signaling mechanism can occur in response to various stimuli, including S1P and other GPCR agonists (e.g., muscarinic receptor agonists as stated above). While the precise intracellular targets of S1P are not fully understood, there is evidence that S1P can mobilize intracellular Ca^{++} by direct effects on the ER [Meyer zu et al., 1998] and can affect the store operated calcium channel (SOCE) that replenishes Ca^{++} stores following intracellular Ca^{++} depletion [Itagaki and Hauser, 2003].

S1P has also been shown to activate PLD signaling [Natarajan et al., 1994]. As described above, multiple pathways converge on or are required for PLD activation. A G_i mediated pathway has been implicated in S1P-stimulated PLD along with activation of PKC, Ca^{++}, Rho, and Rho kinase [Orlati et al., 2000; Wang et al., 2002; Meacci et al., 2003].

EFFECTS OF LYSOPHOSPHOLIPIDS ON ERK ACTIVATION AND CELL PROLIFERATION

LPA. LPA stimulates proliferation in many cell types. A seminal paper from Moolenar's laboratory published in 1989 [van Corven et al., 1989] demonstrated pronounced increases in ^{3}H-thymidine incorporation into Rat1 and human foreskin (HF) fibroblasts and characterized the signaling pathways underlying this response. Notably, and as now confirmed in numerous systems, the mitogenic response was sensitive to PTX pretreatment, implicating G-proteins of the $G_{i/o}$ family in the signaling pathway from LPA to cell proliferation. While LPA also increased $InsP_3$ formation and mobilized intracellular Ca^{++} in these cells, the phosphoinositide/PKC pathway was determined to be neither necessary nor sufficient to elicit cell proliferation.

The PTX sensitive pathway that contributes to the proliferative response is likely that mediated through ERK activation. Biphasic increases in ERK activation, and the requirement for a sustained late ERK response to elicit cell cycle re-entry, were originally proposed by studies from Pouyssegurs laboratory in which responses to thrombin were investigated [Vouret-Craviari et al., 1993]. Similar observations were made for LPA which was shown to elicit both an early PKC and Ca^{++} dependent initial phase, and a sustained second phase of ERK activation in Rat-1 cells. The sustained response was PTX sensitive and required for mitogenesis [Cook and McCormick, 1996]. There are several pathways by which $G_{i/o}$ protein activation might contribute to ERK activation. Activation of Ras is likely involved as an initiating step upstream of ERK and has been shown to be PTX sensitive in response to LPA [van Corven et al., 1993]. Multiple possible mechanisms for $G_{i/o}$ affecting Ras activation are described earlier. Most involve the effects of released $\beta\gamma$ subunits (as opposed to effects of the $G\alpha_1$ subunit, which inhibits AC) and include transactivation of EGF receptors, activation of tyrosine kinases, activation of PI3K and recruitment of cell scaffolds composed of β-arrestin and other signaling molecules [Hall and Lefkowitz, 2002].

LPA acts as a mitogen in fibroblasts, cell lines of embryonic or transformed origin, HeLa and PC12 cells, vascular smooth muscle cells, astrocytes and astrocytoma cell lines, neuronal cells and others. In cardiomyocytes, which are terminally differentiated, LPA induces hypertrophic cell growth, mediated through G_i and Rho dependent pathways [Goetzl et al., 2000; Hilal-Dandan et al., 2003]. On the other hand in some cell lines, particularly those of lymphoid origin, LPA is inhibitory to cell growth [Tigyi et al., 1994]. Inhibition of cell growth could result from LPA mediated increases in cAMP. Increases in cAMP have been observed in response to LPA, but LPA_{1-3} receptors are not coupled to G_s (but rather to G_i). Increases in cAMP would likely occur only when a particular ratio of LPA receptor subtypes and AC isoforms prevails. LPA_4 has been shown to increase cAMP, but this receptor is only distantly related to the other LPA receptor subtypes. It has not yet been proven that this increase in cAMP occurs directly via G_s versus indirectly through a cyclase or phosphodiesterase (PDE), for example. An inhibitory effect of LPA on cell proliferation appears to be the exception to the usual pronounced proliferative response to this mitogen.

Which receptor subtypes are most intimately involved in LPA mediated proliferation is not known. The LPA_1 receptor appears to be tightly coupled to G_i signaling pathways and mediates PTX-sensitive AC inhibition [Ishii et al., 2000; Contos et al., 2002]. Heterologous expression of the LPA_1 receptor induces DNA synthesis [Fukushima et al., 1998]. However, similar observations have been made for other receptor subtypes. Cell proliferation is regulated through more than a single receptor subtype, which is suggested by studies using MEF cells from LPA_1 and LPA_2 knockout mice. The ability of LPA to increase BrdU incorporation was only modestly diminished with loss of either receptor, but abolished when both LPA_1 and LPA_2 receptors were absent [Contos et al., 2002].

S1P. S1P was demonstrated to stimulate DNA synthesis in Swiss 3T3 cells in seminal studies published by the Spiegel laboratory in 1991 [Zhang et al., 1991]. Several lines of evidence suggested that this did not occur through PKC dependent pathways but rather through a PTX-sensitive mechanism, paralleling observations made regarding LPA receptor coupling to cell proliferation via G_i. Activation of Ras, MAP kinase and increases in the transcription factor AP-1 accompanied the proliferative response and were also largely PTX-sensitive. Astrocytes also respond to S1P with PKC-independent proliferative responses regulated through PTX-sensitive pathways, probably via PI3K activation [Pebay et al., 2001]. A wide range of cell types, including fibroblasts, astrocytes, endothelial cells, vascular smooth muscle cells, hepatoma cells and glioma and breast cancer cell lines have now been shown to proliferate in response to S1P. Cardiomyocytes respond to S1P with hypertrophy, as indexed by increased cell size and protein synthesis; the $S1P_1$ receptor has been implicated in mediating this response [Robert et al., 2001]. However, as seen with LPA, S1P can also lead to inhibition of proliferation in some systems. This occurs, for example, in keratinocytes and is partially PTX-sensitive [Vogler et al., 2003].

As indicated above and in Figure 1, the SIP_1 receptor is the subtype most exclusively linked to a G_i/PTX sensitive signaling [Windh et al., 1999]. However, $S1P_2$ and $S1P_3$ receptors, can

also activate G_i, but additionally couple to G_q and G_{12} family proteins [Windh et al., 1999]. The $S1P_2$ and $S1P_3$ receptors, by engaging Rho in addition to G_i-mediated signaling pathways, should have the greatest capacity to elicit cell proliferation or perhaps by virtue of Rho signaling, enhance cell survival. In support of this, hepatoma cells stably expressing either $S1P_2$ or $S1P_3$ receptors responded to S1P with enhanced cell proliferation, and altered gene expression mediated through both PTX and Rho sensitive pathways [An et al., 2000].

EFFECTS OF LYSOPHOSPHOLIPIDS ON CELL SURVIVAL AND APOPTOSIS

LPA. LPA has been shown to protect against apoptosis in a number of cell types including ovarian cancer cells, intestinal epithelial cells, fibroblasts, osteoblasts, hepatocytes, Schwann cells, macrophages, T-cells, and renal proximal tubular cells. The primary signaling mechanisms that appear to be involved in this protection include G_i, PI3K, Akt, and eNOS. In addition, ERK pathways may serve protective functions. A role for Rho in cell survival has also been suggested in studies with ovarian cancer cells [Baudhuin et al., 2002]. Specific LPA receptor subtypes have been implicated in activation of pathways that protect from apoptosis in some cell types. For example, overexpression of the LPA_1 receptor in Schwann cells [Weiner and Chun, 1999] or hepatocytes [Sautin et al., 2001] leads to PI3K-mediated decreases in apoptosis. Further support for a role of the LPA_1 receptor in protection from apoptosis is that there is an 80% increase in apoptosis in sciatic nerve Schwann cells from LPA_1 receptor null mice [Contos et al., 2000].

LPA induced protection from apoptosis has also been demonstrated to occur in vivo. In irradiated mice orally administered LPA, there was a decrease in apoptotic bodies in intestinal crypts [Deng et al., 2002]. Another study used a renal ischemia reperfusion injury model to show that LPA dose dependently inhibited apoptosis of tubular epithelial cells, as well as renal expression of TNF-α and influx of neutrophils, both markers of inflammation [de Vries et al., 2003].

While the data cited above indicates that LPA is generally protective, there are several reports that indicate that LPA can actually stimulate apoptosis. In TF-1 cells, Rho and Rho kinase appear to mediate LPA-induced apoptosis via loss of cell adhesion [Lai et al., 2003]. In hippocampal neurons, LPA also stimulates apoptosis and this response appears to be mediated via oxidative stress and increased NO production [Holtsberg et al., 1998]. Airway smooth muscle cells respond to low concentrations of LPA with mitogenesis, while higher concentrations of LPA stimulate apoptosis [Ediger and Toews, 2001].

S1P. Like LPA, S1P also acts as a survival signal for many cell types including melanocytes, hepatoma cells, neutrophils, macrophages, acute leukemia cells, PC-12 cells, HUVECs, keratinocytes, and hepatic myofibroblasts. As described above with regard to intracellular effects of S1P on Ca^{++} mobilization, S1P can act within the cell as well as via its GPCR and there is ample evidence for both receptor-mediated and intracellular effects of S1P on cell survival and protection from apoptosis.

S1P-mediated protection from apoptosis, like that of LPA, also appears to be mediated primarily through G_i-mediated PI3K/Akt/eNOS signaling. ERK and p38MAPK have also been implicated. A few examples are described below. S1P protects melanocytes [Kim et al., 2003] and hepatic myofibroblasts [Davaille et al., 2002] from apoptosis in an ERK and Akt dependent manner. In ovarian cancer cells, S1P elicits its protective effects through ERK, Akt, and p38MAPK [Baudhuin et al., 2002]. In neutrophils, the protective effects of S1P occur via G_i and p38, while ERK, Akt, and JNK are not required [Chihab et al., 2003]. In vascular endothelial cells, S1P has been shown to stimulate eNOS phosphorylation through a PTX-sensitive, Akt-mediated pathway [Igarashi et al., 2001]. Morales-Ruiz et al. [2001] have demonstrated that the $S1P_1$ receptor is responsible for G_i and Akt-dependent eNOS phosphorylation in endothelial cells. Consistent with this, S1P appears to activate NOS through a PTX-sensitive pathway and thereby mediate cytoprotection in HUVECs [Kwon et al., 2001]. This cytoprotection is mediated via $S1P_1$ or $S1P_3$ receptors since antisense to these receptors inhibited S1P stimulated increases in NO [Kwon et al., 2001]. $S1P_3$ receptor mediated signaling to PI3K and Akt has also been demonstrated in CHO cells [Banno et al., 2003]. Overall, the evidence indicates that $S1P_1$ and $S1P_3$ receptors signal via Gi to the

PI3K/Akt pathway to mediate S1P cytoprotection from apoptosis, and Akt-dependent phosphorylation and activation of eNOS is a primary contributor.

Several lines of evidence indicate that intracellularly generated S1P can also serve as a survival signal via its actions within the cell. Olivera et al. [1999] demonstrated that overexpression of sphingosine kinase in NIH3T3 fibroblasts and HEK293 cells increased intracellular S1P levels, without detectable S1P released into the media. They concluded that this was sufficient to increase cell growth in low serum media and protect against apoptosis. Intracellular S1P also protects cells from ceramide-induced apoptosis [Cuvillier et al., 1996] and blocks cytochrome C release and caspase activation [Cuvillier and Levade, 2001]. Microinjection of S1P into fibroblasts has been shown to increase DNA synthesis and to suppress apoptosis in HL-60 and PC-12 cells [Van Brocklyn et al., 1998]. Likewise, in PC-12 cells, overexpression of sphingosine kinase, which leads to an increase in intracellular S1P, suppressed apoptosis, independent of Akt, ERK, and G_i, suggesting non-receptor mediated mechansims [Edsall et al., 2001]. In HUVECs, TNF-α increased sphingosine kinase activity with a subsequent increase in S1P that protected against TNF-α stimulated apoptosis, further supporting a role for intracellular S1P in protection [Xia et al., 1999a]. Also, in rat heart fibroblasts, the ganglioside GM-1 increased intracellular S1P and protected these cells from apoptosis [Cavallini et al., 1999]. Most recently, it has been shown that overexpression of sphingosine kinase promotes cell survival and proliferation in MEF cells from $S1P_{2/3}$ knockout mice even in the presence of PTX, conditions under which there should be no signaling from any of these S1P receptors [Olivera et al., 2003]. Thus, at least in terms of cell protection and survival S1P signaling through its receptors is not required.

In some cell models intracellular S1P and GPCR-mediated S1P can combine to protect cells from apoptosis. For example in neuroblastoma $\times$ glioma hybrid cells, inhibition of sphingosine kinase increased thapsigargin-induced apoptosis and exogenous S1P protected cells from FCCP-induced cell death [Chin et al., 2002]. In hepatic myofibroblasts, which express $S1P_{1,2}$ and $_3$ receptors, receptor-dependent effects of S1P led to cell survival via G_i signaling through ERK and Akt pathways [Davaille et al., 2002]. Interestingly, intracellular S1P was shown to increase apoptosis, thus in these cells S1P effects on extra- and intracellular signaling pathways have opposing effects on cell survival [Davaille et al., 2002]. There is also evidence that S1P can function via "inside out" signaling, where intracellularly generated S1P is released and then acts upon its cell surface GPCR; this mechanism contributes to stress fiber formation and cell migration, rather than proliferation and survival [Olivera et al., 2003].

ROLE OF LYSOPHOSPHOLIPIDS IN PATHOPHYSIOLOGY

Lysophospholipids in Oncogenesis

As detailed above, GPCR stimulation can lead to cell proliferation, a response dependent on G_i and/or G_q activation of Ras MAPK pathways, which may also depend upon $G_{12/13}$ activation of Rho. Under normal physiological conditions, activation of the $G_{12/13}$-Rho pathway and hence cell proliferation, may be appropriately limited, because agonists for the $G_{12/13}$-coupled receptors (e.g, LPA, S1P, thrombin, and thromboxane A_2) are largely formed and released during stress, injury, and inflammation. However, upregulation of growth signals that activate Ras- or Rho-dependent pathways at various points in the signaling cascades described above, are now known to result in aberrant cell growth. Concomitant alterations in migration and invasion would further contribute to pathophysiological progression of metastatic cancer. Indeed, numerous findings indicate alterations in these processes in transformed cells. For example, in pancreatic carcinoma cell lines, LPA stimulates cell migration via a G_i/ERK pathway that requires Ras, Rac1, and RhoA [Stahle et al., 2003]. Not only have well-known mutations in Ras been identified in human tumors but various mutant forms of Rho GEFs (e.g., lbc, larg, dbl) have also been isolated from leukemic and other cancer cells. Expression of these Rho GEFs, like expression of the $G_{12/13}$ proteins that activate them, results in enhanced cell proliferation [Toksoz and Williams, 1994; Kourlas et al., 2000a,b]. Overexpression of RhoC, a homolog of RhoA, has also been observed in pancreatic and inflammatory breast cancer, and microarray data demonstrated an association between increases in RhoC and

metastasis [van Golen et al., 1999, 2000; Clark et al., 2000; Kleer et al., 2002].

Another potential mechanism by which dysregulated growth responses might occur is through increased production of ligands for $G_{12/13}$-coupled receptors. This has been reported to occur in ovarian cancer, where elevated concentrations of LPA and S1P have been found in the ascitic fluid of patients with ovarian cancer [Hong et al., 1999; Tanyi et al., 2003]. In addition, sphingosine kinase, the lipid kinase responsible for formation of S1P from ceramide has been reported to act as an oncogene and stimulate cell proliferation [Xia et al., 2000]. Several studies report overexpression of sphingosine kinase conferring a growth advantage of tumor cells. Conversely, lipid phosphate phosphatase-3, which selectively hydrolyzes LPA in vivo, has been shown to decrease the growth, survival, and tumorigenesis of ovarian cancer cells [Tanyi et al., 2003]. This suggests that balance between formation and metabolism of either S1P or LPA can determine cell fate and thus tumorigenic potential.

Signaling by mediators such as LPA and S1P could also be dysregulated through altered ratios of expression of the various receptor subtypes. In human ovarian cancer cells obtained from early and advanced tumors, LPA_2 and LPA_3 receptor expression is reported to be increased [Fujita et al., 2003] and indeed LPA_2 receptor expression has been suggested to serve as a "distinctive functional marker" for this disorder [Huang et al., 2002]. A carboxy-terminally extended gain of function mutation of the LPA_2 receptor has also been reported in ovarian cancer [Huang et al., 2002]. The ratio of receptor subtypes, and thus the appropriate balance in the signaling pathways that they control, may be of particular importance in determining cell survival and migration. Since LPA_1 has been shown to increase apoptosis of ovarian cancer cells, an increased LPA_2/LPA_1 ratio could prevent normal programmed cell death in response to LPA. In the case of S1P, the $S1P_2$ receptor has been shown to inhibit B16 melanoma cell invasion. Thus, the loss of $S1P_2$ receptors or increases in activation of competing pathways could alter the balance between proliferative and apoptotic signals, or pro- versus inhibitory-migratory signals, and thus could contribute to conversion from normal to carcinogenic to invasive phenotype. As a general concept, it is likely that in pathophysiological situations, the lysophospholipid receptors, (or molecules critical to their downstream signaling) may become limited or upregulated, altering the nature of the response. Compartmentation of cell signaling components localized in membrane domains such as lipid rafts or caveolae, may also be altered in and contribute to pathophysiological responses.

Lysophospholipids in Vascular Pathology

As described in detail above, in many cell types including vascular smooth muscle cells, LPA and S1P induce cell cycle progression and proliferation. In the case of vascular smooth muscle cells, enhanced cell growth responses are observed in vascular diseases such as hypertension and diabetes, and are involved in the pathogenesis of atherosclerosis, peripheral vascular disease, and renal failure. Since LPA and S1P are released from platelets and are key components in serum responsible for cell proliferation, one would predict that they could have profound effects on angiogenesis and vascular function and dysfunction.

S1P receptor deletion studies have revealed a crucial role for $S1P_1$ in angiogenesis and vascular maturation. $S1P_1-/-$ mice undergo intrauterine death at age E12.5-E14.5 due to defects in vascular smooth muscle migration subsequent to an inability to activate the small G protein Rac [Liu et al., 2000]. Interestingly, $S1P_1$ receptor levels are lower in VSMC from adult (versus young) animals [Kluk and Hla, 2001], an observation that may explain the diminished ability of S1P to induce cell migration, or proliferation in adult VSMC.

Decreased responsivity of adult VSMC to lysophospholipid mediators would serve as an adaptive response which acts to inhibit the proliferation of smooth muscle cells under normal physiological conditions. During vascular injury or the initiation and progression of vascular disease, however, alterations in signal transduction pathways might shift the balance toward enhanced lysophopholipid proliferative signaling. For example, evidence is rapidly accumulating which suggests that upregulation of Rho expression, activity and/or signaling might play a role in at least a subset of vascular disorders. Rho kinase inhibitors [Sawada et al., 2000; Shibata et al., 2001], dominant negative Rho kinase [Morishige et al., 2001], and statins [Mulder et al., 2000], which inhibit

the geranylgeranylation and therefore membrane-targeting of Rho, have been shown to reduce neointimal formation and coronary constrictive remodeling [Mulder et al., 2000]. Likewise, enhanced vascular RhoA expression, RhoA activity and Rho-dependent DNA synthesis have been reported in vessels and smooth muscle cells from hypertensive rats [Seasholtz et al., 2001]. Similar increases in Rho activation have also been described for animal models of diabetes, and hyperinsulinemia has been shown to potentiate LPA-stimulated, Rho-dependent gene expression [Chappell et al., 2000].

In addition to the effects of lysophospholipids on vascular smooth muscle cells, LPA and S1P have both been shown to mediate endothelial cell activation, which ultimately affects vascular function. These changes occur during the initiation and progression of vascular disease. The alterations include endothelial cell stress fiber formation and contraction, increased expression of adhesion molecules and decreased production of nitric oxide, which normally exerts inhibitory effects on vascular contraction, proliferation, and migration. Upon upregulation of adhesion molecules, leukocytes such as neutrophils and monocytes adhere to the endothelial cell layer and migrate into the intimal layer where they accumulate lipid, transform into foam cells and release factors that stimulate vascular smooth muscle cell growth and inflammation. An expanding literature also suggests that LPA is an important mediator of oxidized LDL effects on the vasculature. For instance, oxidized LDL has been shown to activate platelets presumably via platelet LPA receptors and through production of LPA [Siess et al., 1999]. In addition, biologically active LPA is found to be accumulated in human atherosclerotic plaques [Siess et al., 1999]. S1P has also been implicated in oxLDL mediated vascular smooth muscle cell mitogenesis [Auge et al., 1999] and in TNF-α-mediated adhesion protein expression in vascular endothelial cells [Xia et al., 1999b].

Progression of vascular disease, vessel occlusion, and plaque rupture are primary contributors to myocardial infarction and stroke. Other contributing factors include vasospasm. Interestingly, several reports suggest that S1P is particularly potent at eliciting contraction of cerebral (versus peripheral) vessels, suggesting a possible role for S1P in cerebral vasospasm. The basis for this selectivity may be that the expression of $S1P_3$ and $S1P_2$ receptors is approximately 4-fold higher in cerebral versus peripheral vessels [Coussin et al., 2002]. Another study using antisense constructs against either $S1P_3$ or $S1P_2$ demonstrated that only inhibition of $S1P_3$ receptor expression blocked S1P-stimulated constriction of cerebral arteries [Salomone et al., 2003]. Since C3 [Salomone et al., 2003] and the Rho kinase inhibitor, Y-27632 [Tosaka et al., 2001], (but not PTX), were also able to block the response, S1P would appear to produce contraction of cerebral vessels through activation of $S1P_3$ (and possibly $S1P_2$) but not $S1P_1$ receptors, consistent with a Rho-dependent signaling pathway. While many G_q- and G_i-coupled GPCR's mediate vascular contraction through increases in intracellular Ca^{++} and activation of myosin light chain kinase, Rho has been shown to increase contractility in the absence of changes in intracellular Ca^{++}. Rho through activation of Rho kinase promotes phosphorylation of the myosin binding subunit of myosin phosphatase, thereby inactivating the enzyme resulting in an accumulation of phosphorylated myosin. In addition to actions on cerebral vessels, S1P also appears to produce contraction of coronary artery smooth muscle cells, possibly through $S1P_2$ [Ohmori et al., 2003].

Lysophospholipids in Brain Injury

The first lysophospholipid receptor identified, vzg1/edg2/LPA_1, was isolated and cloned on the basis of its appearance in the ventricular zone, a region of the cortex in which there is active neuronal proliferation during development [Hecht et al., 1996]. Interestingly, $S1P_1$ is also expressed in this brain region during development [McGiffert et al., 2002]. LPA and S1P receptors appear to be temporally as well as spatially regulated during brain development [Hecht et al., 1996; Fukushima et al., 2002; McGiffert et al., 2002]. While the specific roles of each LPA and S1P receptor subtype are still being elucidated, it is clear that these receptors and their ligands are important for both the developing brain as well as the adult brain.

When the brain is injured, neurons and glia undergo a myriad of cellular responses including cell migration, proliferation, cytokine release, and apoptosis. A pathophysiologic event such as brain injury stimulates astrogliosis, defined by increased proliferation and migration of astrocytes to the site of injury,

resulting in glial scarring [McGraw et al., 2001]. The characteristic astroglial cell proliferation and migration is arguably both critical for and detrimental to axon regeneration after injury. Another characteristic often associated with brain injury is breakdown of the blood–brain barrier. This event allows LPA and S1P to enter the central nervous system via the bloodstream, as described above. By virtue of their pleiotropic effects, these ligands are poised to trigger the cellular events (e.g., ERK, Rho, Akt activation) required for proliferation, migration, and survival. The in vivo role of lysophospholipid receptors and their downstream signaling pathways in response to brain injury is, however, just beginning to be explored.

In cell culture, LPA and S1P elicit astrocyte proliferation and migration through multiple signaling pathways. The nature of lysophospholipid-induced astrocyte responses appears to vary based upon the specific brain region, the animal species, and the developmental stage of the astrocytes [Steiner et al., 2002]. Recently, Sorensen et al. [2003] showed that in vivo injection of LPA or S1P into the striatum of mouse brains induced astrogliosis. Furthermore, activation of the $G_{i/o}$-MAPK as well as the Rho kinase pathways was shown to be involved in astrocyte proliferation [Sorensen et al., 2003]. Preliminary data from our laboratory indicates that in a stab brain injury model $S1P_1$ receptor expression is altered suggesting a role for $S1P_1$ receptors in regulation of astrogliosis following brain injury. These data are consistent with the theory that LPA and S1P are involved in the pathophysiology associated with astrogliosis and other responses to brain injury. As discussed earlier, these ligands are also potent effectors of cerebrovascular constriction, suggesting a role in the pathophysiology of stroke. The ganglioside GM-1, which as mentioned previously can generate intracellular S1P, has been used in human stroke studies which have shown an amelioration of the neurologic deficits observed following the onset of stroke [Argentino et al., 1989; Lenzi et al., 1994]. This provides further evidence of the protective effects of the S1P in pathological conditions. The development of subtype-specific S1P and LPA receptor agonists and antagonists, which are able to cross the blood–brain barrier should provide crucial insight into the potential role of LPA and S1P receptors in the brain and their feasibility as therapeutic targets.

Effects of Lysophospholipids on Myocardial Growth and Survival

Cardiomyocytes are terminally differentiated cells and thus do not re-enter the cell cycle and proliferate. In response to demands imposed by increased workload or stress (e.g., hypertension, infarction) myocytes enlarge by a process referred to as hypertrophic growth. Increased cell size, protein synthesis, sarcomeric organization, switches in myofilament protein isoform expression (e.g., α vs. β myosin heavy chain), and re-expression of embryonic genes (e.g., ANF, BNP) characterize this response. Neonatal rat ventricular myocytes (NRVMs) cultured in the absence of serum and at low density recapitulate many of the features of in vivo hypertrophy when they are stimulated by the addition of serum. The effects of serum are mimicked by addition of ligands for GPCRs, in particular those that couple to G_q. The agonists that are most effective in this regard are norepinephrine, phenylephrine (PE), endothelin (ET), and PGF2 α, and many papers report on these responses, further elucidating the signal transduction pathways that are involved. Expression of the α subunit of G_q also induces hypertrophy. Thus, in contrast to proliferative responses, where G_q signaling pathways are relatively inefficient, at least alone, cardiac hypertrophy is highly dependent on G_q mediated responses.

Several studies have examined the ability of LPA and S1P to effect hypertrophic growth of NRVMS. In one study [Robert et al., 2001] S1P was demonstrated to increase protein synthesis, BNP secretion, and stress fiber formation. These effects were suggested to be mediated via $S1P_1$ receptors based on studies with $S1P_1$ antibodies, which were also used to suggest that this was the predominant receptor isoform expressed in these cells. S1P mediated activation of MAP kinases, PI3K, and Rho were all implicated as mediators of this hypertrophic response, as was the PTX-sensitive G_i protein. Interestingly, another group failed to observe hypertrophy in response to S1P, although sphingosylphosphorylcholine did increase protein synthesis and activate MAP kinases [Sekiguchi et al., 1999]. LPA has also been reported to increase protein synthesis, one aspect of the hypertrophic program, when added to NRVMs in the presence of gelsolin [Goetzl et al., 2000]. Our studies demonstrate

that LPA induces similar increases in protein synthesis even in the absence of gelsolin and also increases cell size, expression of ANF, and activation of MAP kinases [Hilal-Dandan et al., 2003]. Inhibition by PTX and C3 toxins indicated that G_i and Rho pathways are important components of the hypertrophic response to LPA, as suggested for S1P.

As a general statement, however, while S1P and LPA are capable of activating many features of hypertrophy, these ligands appear to be far less efficacious than PE, ET, or PGF2α in this regard. We speculate that the notably weak coupling of these receptors to G_q regulated pathways (PLC, PKC) limits the magnitude of the response elicited by S1P and LPA. For example, we observe little PLC stimulation (based on InsP formation) in NRVMs treated with LPA [Hilal-Dandan et al., 2003] or S1P (unpublished observation), while PE, PGF2α, or ET elicited robust responses. Thus, hypertrophic growth mediated through G_i and Rho pathways in response to these ligands is observed, but other functions for these receptors are suggested.

What appears as hypertrophic growth in serum deprived NRVMs may be partially explained as increased cell survival. Indeed one might argue that the more important effects of S1P and LPA in cardiomyocytes are activation of survival pathways. We have determined that LPA and S1P are highly effective activators of Rho and of the PI3K/Akt survival pathway [Hilal-Dandan et al., 2003; Xiao et al., 2003], while more traditional hypertrophic agonists like PE are not. Both LPA and S1P have been reported to enhance survival of NRVMs during hypoxia [Karliner et al., 2001] and S1P protects mouse hearts from global ischemia [Jin et al., 2002]. There is also considerable evidence that sphingomyelinase is activated during cardiac ischemia, and that sphingosine is formed and potentially converted to S1P in the blood [Hernandez et al., 2000; Levade et al., 2001; Cavalli et al., 2002]. We recently examined the role of S1P receptors in injury induced by ischemia and reperfusion in mouse hearts in vivo, comparing the extent of injury-induced cell loss in wild type and S1P receptor knockout mice. Our findings demonstrated increased infarct size in hearts from $S1P_{2/3}$ receptor null mice, and further showed that Akt activation was not observed in the absence of these receptors [Xiao et al., 2004]. Thus, it seems likely that S1P serves as a mediator that is formed in the heart in response to stresses such as ischemia/reperfusion, and that it protects cardiomyocytes from necrosis or apoptosis. Apoptotic cell death is also considered to be a critical component in the development of congestive heart failure, a condition in which hypertrophic growth is no longer able to compensate for increased stress, and in which the contractile function of the heart is diminished. Agents that activate lysophospholipid receptors coupled to the Rho or P13K/Akt pathways may thus prove beneficial in providing survival signals that slow or diminish the development of cardiomyocyte cell death and associated functional decompensation.

Lysophospholipids and the Immune System

LPA regulates growth and survival of several types of immune cells, including T lymphocytes, B lymphocytes, and macrophages. Mitogenic effects of LPA on B-lymphocytes and on T cells have been demonstrated, with the LPA_1 receptor being implicated in T cell proliferation, and LPA_2 receptor implicated in T-cell migration [Huang et al., 2002]. In addition, LPA protects macrophages and T cells from apoptosis, apparently via LPA_1 and LPA_2 receptor mediated signaling [Huang et al., 2002].

The best-studied effects of S1P on the immune system involve changes in cell migration/chemotaxis/lymphocyte homing or altered expression of inflammatory cytokines/adhesion molecules. It has also been shown recently that S1P inhibits T cell proliferation and migration [Dorsam et al., 2003; Jin et al., 2003] via the $S1P_1$ receptor [Dorsam et al., 2003]. This is contrary to what has been described for other cell types, including endothelial cells.

Development of FTY-720, which is an agonist for all S1P receptor subtypes except $S1P_2$, has strengthened the concept that S1P serves important physiological roles in the immune system. In the past year, there have been numerous reports of the therapeutic potential of this compound in the context of autoimmune disorders and transplantation. FTY-720 appears to be a powerful immunosuppressant that, when used in combination with cyclosporine, abrogates xenograft rejection in several systems [Kimura et al., 2003; Koshiba et al., 2003; Maeda et al., 2003]. The protective effect is thought to be due to a decrease in lymphocyte infiltration into the xenograft site. FTY-720 has

also been demonstrated to prevent autoimmune diabetes development in a nonobese diabetic mouse model [Yang et al., 2003]. Furthermore, FTY-720 protects rats from EAE, an experimental model of multiple sclerosis [Brinkmann et al., 2002; Fujino et al., 2003]. While the precise signaling events that are responsible for the protective effects of S1P in the immune system have not been fully elucidated, it is likely that regulation of cell growth and apoptosis by S1P complements the effects of this ligand on cell migration, and contributes to the protective effects observed in the immune system.

CONCLUDING REMARKS

There has been a great deal of detail uncovered regarding the pathways by which LPA and S1P receptors signal to elicit regulation of proliferation and survival. Nonetheless, the existing literature leaves numerous gaps regarding receptor-G protein coupling under physiological circumstances and, in particular which receptor subtypes control specific downstream responses. This knowledge is key to the rational design of therapeutic interventions targeting these novel and pivotal pathways, which transduce messages on behalf of ligands made available through stress, injury, or inflammation. Whether these ligands, by enhancing cell proliferation, migration, and survival serve protective or maladaptive effects remains to be defined, but it is clear nonetheless that they are important players in disease.

REFERENCES

An S, Bleu T, Zheng Y. 1999. Transduction of intracellular calcium signals through G protein-mediated activation of phospholipase C by recombinant sphingosine 1-phosphate receptors. Mol Pharmacol 55:787–794.

An S, Zheng Y, Bleu T. 2000. Sphingosine 1-phosphate-induced cell proliferation, survival, and related signaling events mediated by G protein-coupled receptors Edg3 and Edg5. J Biol Chem 275:288–296.

Argentino C, Sacchetti ML, Toni D, Savoini G, D'Arcangelo E, Erminio F, Federico F, Milone FF, Gallai V, Gambi D, et al. 1989. GM1 ganglioside therapy in acute ischemic stroke. Italian Acute Stroke Study—Hemodilution + Drug. Stroke 20:1143–1149.

Auge N, Nikolova-Karakashian M, Carpentier S, Parthasarathy S, Negre-Salvayre A, Salvayre R, Merrill AH, Jr., Levade T. 1999. Role of sphingosine 1-phosphate in the mitogenesis induced by oxidized low density lipoprotein in smooth muscle cells via activation of sphingomyelinase, ceramidase, and sphingosine kinase. J Biol Chem 274:21533–21538.

Banno Y, Takuwa Y, Yamada M, Takuwa N, Ohguchi K, Hara A, Nozawa Y. 2003. Involvement of phospholipase D in insulin-like growth factor-I-induced activation of extracellular signal-regulated kinase, but not phosphoinositide 3-kinase or Akt, in Chinese hamster ovary cells. Biochem J 369:363–368.

Baudhuin LM, Cristina KL, Lu J, Xu Y. 2002. Akt activation induced by lysophosphatidic acid and sphingosine-1-phosphate requires both mitogen-activated protein kinase kinase and p38 mitogen-activated protein kinase and is cell-line specific. Mol Pharmacol 62:660–671.

Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumruker T, Hiestand P, Foster CA, Zollinger M, Lynch KR. 2002. The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem 277:21453–21457.

Cavalli AL, Ligutti JA, Gellings NM, Castro E, Page MT, Klepper RE, Palade PT, McNutt WT, Sabbadini RA. 2002. The role of TNFα and sphingolipid signaling in cardiac hypoxia: Evidence the cardiomyocytes release TNFα and sphingosine. Basic Appl Myol 12:167–175.

Cavallini L, Venerando R, Miotto G, Alexandre A. 1999. Ganglioside GM1 protection from apoptosis of rat heart fibroblasts. Arch Biochem Biophys 370:156–162.

Chappell J, Golovchenko I, Wall K, Stjernholm R, Leitner JW, Goalstone M, Draznin B. 2000. Potentiation of Rho-A-mediated lysophosphatidic acid activity by hyperinsulinemia. J Biol Chem 275:31792–31797.

Chihab R, Porn-Ares MI, Alvarado-Kristensson M, Andersson T. 2003. Sphingosine 1-phosphate antagonizes human neutrophil apoptosis via p38 mitogen-activated protein kinase. Cell Mol Life Sci 60:776–785.

Chikumi H, Vazquez-Prado J, Servitja JM, Miyazaki H, Gutkind JS. 2002. Potent activation of RhoA by Galpha q and Gq-coupled receptors. J Biol Chem 277:27130–27134.

Chin TY, Hwang HM, Chueh SH. 2002. Distinct effects of different calcium-mobilizing agents on cell death in NG108-15 neuroblastoma X glioma cells. Mol Pharmacol 61:486–494.

Clark EA, Golub TR, Lander ES, Hynes RO. 2000. Genomic analysis of metastasis reveals an essential role for RhoC. Nature 406:532–535.

Contos JJ, Fukushima N, Weiner JA, Kaushal D, Chun J. 2000. Requirement for the lpA1 lysophosphatidic acid receptor gene in normal suckling behavior. Proc Natl Acad Sci USA 97:13384–13389.

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

Cook SJ, McCormick F. 1996. Kinetic and biochemical correlation between sustained p44ERK1 (44 kDa extracellular signal-regulated kinase 1) activation and lysophosphatidic acid-stimulated DNA synthesis in Rat-1 cells. Biochem J 320:237–245.

Coussin F, Scott RH, Wise A, Nixon GF. 2002. Comparison of sphingosine 1-phosphate-induced intracellular signaling pathways in vascular smooth muscles: Differential role in vasoconstriction. Circ Res 91:151–157.

Cuvillier O, Levade T. 2001. Sphingosine 1-phosphate antagonizes apoptosis of human leukemia cells by

inhibiting release of cytochrome c and Smac/DIABLO from mitochondria. Blood 98:2828–2836.

Cuvillier O, Pirianov G, Kleuser B, Vanek PG, Coso OA, Gutkind S, Spiegel S. 1996. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. Nature 381:800–803.

Davaille J, Li L, Mallat A, Lotersztajn S. 2002. Sphingosine 1-phosphate triggers both apoptotic and survival signals for human hepatic myofibroblasts. J Biol Chem 277: 37323–37330.

de Vries B, Matthijsen RA, van Bijnen AA, Wolfs TG, Buurman WA. 2003. Lysophosphatidic acid prevents renal ischemia-reperfusion injury by inhibition of apoptosis and complement activation. Am J Pathol 163: 47–56.

Deng W, Balazs L, Wang DA, Van Middlesworth L, Tigyi G, Johnson LR. 2002. Lysophosphatidic acid protects and rescues intestinal epithelial cells from radiation- and chemotherapy-induced apoptosis. Gastroenterology 123: 206–216.

Dorsam G, Graeler MH, Seroogy C, Kong Y, Voice JK, Goetzl EJ. 2003. Transduction of multiple effects of sphingosine 1-phosphate (S1P) on T cell functions by the S1P1 G protein-coupled receptor. J Immunol 171:3500–3507.

Ediger TL, Toews ML. 2001. Dual effects of lysophosphatidic acid on human airway smooth muscle cell proliferation and survival. Biochim Biophys Acta 1531: 59–67.

Edsall LC, Cuvillier O, Twitty S, Spiegel S, Milstien S. 2001. Sphingosine kinase expression regulates apoptosis and caspase activation in PC12 cells. J Neurochem 76: 1573–1584.

Fleming IN, Elliot CM, Exton JH. 1996. Differential translocation of Rho family GTPases by lysophosphatidic acid, endothelin-1, and platelet-derived growth factor. J Biol Chem 271:33067–33073.

Fujino M, Funeshima N, Kitazawa Y, Kimura H, Amemiya H, Suzuki S, Li XK. 2003. Amelioration of experimental autoimmune encephalomyelitis in Lewis rats by FTY720 treatment. J Pharmacol Exp Ther 305:70–77.

Fujita T, Miyamoto S, Onoyama I, Sonoda K, Mekada E, Nakano H. 2003. Expression of lysophosphatidic acid receptors and vascular endothelial growth factor mediating lysophosphatidic acid in the development of human ovarian cancer. Cancer Lett 192:161–169.

Fukushima N, Kimura Y, Chun J. 1998. A single receptor encoded by vzg-1/lp_{A1}/edg-2 couples to G proteins and mediates multiple cellular responses to lysophosphatidic acid. Proc Natl Acad Sci USA 95:6151–6156.

Fukushima N, Weiner JA, Kaushal D, Contos JJ, Rehen SK, Kingsbury MA, Kim KY, Chun J. 2002. Lysophosphatidic acid influences the morphology and motility of young, postmitotic cortical neurons. Mol Cell Neurosci 20:271–282.

Goetzl EJ, Lee H, Azuma T, Stossel TP, Turck CW, Karliner JS. 2000. Gelsolin binding and cellular presentation of lysophosphatidic acid. J Biol Chem 275:14573–14578.

Gohla A, Harhammer R, Schultz G. 1998. The G-protein G_{13} but not G_{12} mediates signaling from lysophosphatidic acid receptor via epidermal growth factor receptor to Rho. J Biol Chem 273:4653–4659.

Graler MH, Grosse R, Kusch A, Kremmer E, Gudermann T, Lipp M. 2003. The sphingosine 1-phosphate receptor S1P4 regulates cell shape and motility via coupling to Gi and G12/13. J Cell Biochem 89:507–519.

Hall RA, Lefkowitz RJ. 2002. Regulation of G protein-coupled receptor signaling by scaffold proteins. Circ Res 91:672–680.

Hart MJ, Jiang X, Kozasa T, Roscoe W, Singer WD, Gilman AG, Sternweis PC, Bollag G. 1998. Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by $G\alpha_{13}$. Science 280:2112–2114.

Hecht JH, Weiner JA, Post SR, Chun J. 1996. Ventricular zone gene-1 (*vzg-1*) encodes a lysophosphatidic acid receptor expressed in neurogenic regions of the developing cerebral cortex. J Cell Biol 135:1071–1083.

Hernandez OM, Discher DJ, Bishopric NH, Webster K. 2000. Rapid activation of neutral sphingomyelinase by hypoxia-reoxigenation of cardiac myocytes. Circ Res 86: 198–204.

Hilal-Dandan R, Means CK, Gustafsson Ä B, Morissette MR, Adams J, Brunton LL, Brown JH. 2004. Lysophosphatidic acid induces hypertrophy of neonatal cardiac myocytes via activation of G_i and Rho. J Mol Cell Can (in press).

Holtsberg FW, Steiner MR, Bruce-Keller AJ, Keller JN, Mattson MP, Moyers JC, Steiner SM. 1998. Lysophosphatidic acid and apoptosis of nerve growth factor-differentiated PC12 cells. J Neurosci Res 53:685–696.

Hong G, Baudhuin LM, Xu Y. 1999. Sphingosine-1-phosphate modulates growth and adhesion of ovarian cancer cells. FEBS Lett 460:513–518.

Huang MC, Graeler M, Shankar G, Spencer J, Goetzl EJ. 2002. Lysophospholipid mediators of immunity and neoplasia. Biochim Biophys Acta 1582:161–167.

Igarashi J, Bernier SG, Michel T. 2001. Sphingosine 1-phosphate and activation of endothelial nitric-oxide synthase. Differential regulation of Akt and MAP kinase pathways by EDG and bradykinin receptors in vascular endothelial cells. J Biol Chem 276:12420–12426.

Ishii I, Contos JJ, Fukushima N, Chun J. 2000. Functional comparisons of the lysophosphatidic acid receptors, LP(A1)/VZG-1/EDG-2, LP(A2)/EDG-4, and LP(A3)/EDG-7 in neuronal cell lines using a retrovirus expression system. Mol Pharmacol 58:895–902.

Ishii I, Friedman B, Ye X, Kawamura S, McGiffert C, Contos JJ, Kingsbury MA, Zhang G, Brown JH, Chun J. 2001. Selective loss of sphingosine 1-phosphate signaling with no obvious phenotypic abnormality in mice lacking its G protein-coupled receptor LP_{B3}/EDG-3. J Biol Chem 276:33697–33704.

Ishii I, Ye X, Fredman B, Kawamura S, Contos JJA, Kingsbury MA, Yang AH, Zhang G, Brown JH, Chun J. 2002. Marked perinatal lethality and cellular signaling deficits in mice null for the two sphingosine 1-phosphate receptors, $S1P_2$/LP_{B2}/EDG-5 and $S1P_3$/LP_{B3}/EDG-3. J Biol Chem 277:25152–25159.

Itagaki K, Hauser CJ. 2003. Sphingosine 1-phosphate, a diffusible calcium influx factor mediating store-operated calcium entry. J Biol Chem 278:27540–27547.

Jin ZQ, Zhou HZ, Zhu P, Honbo N, Mochly-Rosen D, Messing RO, Goetzl EJ, Karliner JS, Gray MO. 2002. Cardioprotection mediated by sphingosine-1-phosphate and ganglioside GM-1 in wild-type and PKC epsilon knockout mouse hearts. Am J Physiol Heart Circ Physiol 282:H1970–H1977.

Jin Y, Knudsen E, Wang L, Bryceson Y, Damaj B, Gessani S, Maghazachi AA. 2003. Sphingosine 1-phosphate is a novel inhibitor of T-cell proliferation. Blood 101:4909–4915.

Karliner JS, Honbo N, Summers K, Gray MO, Goetzl EJ. 2001. The lysophospholipids sphingosine-1-phosphate and lysophosphatidic acid enhance survival during hypoxia in neonatal rat cardiac myocytes. J Mol Cell Cardiol 33:1713–1717.

Kim DS, Hwang ES, Lee JE, Kim SY, Park KC. 2003. Sphingosine-1-phosphate promotes mouse melanocyte survival via ERK and Akt activation. Cell Signal 15: 919–926.

Kimura T, Hasegawa T, Nakai H, Azuma T, Usui N, Sasaki T, Okada A. 2003. FTY720 reduces T-cell recruitment into murine intestinal allograft and prevents activation of graft-infiltrating cells. Transplantation 75:1469–1474.

Kleer CG, van Golen KL, Zhang Y, Wu ZF, Rubin MA, Merajver SD. 2002. Characterization of RhoC expression in benign and malignant breast disease: A potential new marker for small breast carcinomas with metastatic ability. Am J Pathol 160:579–584.

Kluk MJ, Hla T. 2001. Role of the sphingosine 1-phosphate receptor EDG-1 in vascular smooth muscle cell proliferation and migration. Circ Res 89:496–502.

Koshiba T, Van Damme B, Rutgeerts O, Waer M, Pirenne J. 2003. FTY720, an immunosuppressant that alters lymphocyte trafficking, abrogates chronic rejection in combination with cyclosporine A. Transplantation 75:945–952.

Kourlas PJ, Strout MP, Becknell B, Veronese ML, Croce CM, Theil KS, Krahe R, Ruutu T, Knuutila S, Bloomfield CD, Caligiuri MA. 2000b. Identification of a gene at 11q23 encoding a guanine nucleotide exchange factor: Evidence for its fusion with MLL in acute myeloid leukemia. Proc Natl Acad Sci USA 97:2145–2150.

Kranenburg O, Poland M, van Horck FPG, Drechsel D, Hall A, Moolenaar WH. 1999. Activation of RhoA by lysophosphatidic acid and $G\alpha_{12/13}$ subunits in neuronal cells: Induction of neurite retraction. Mol Biol Cell 10:1851–1857.

Kwon YG, Min JK, Kim KM, Lee DJ, Billiar TR, Kim YM. 2001. Sphingosine 1-phosphate protects human umbilical vein endothelial cells from serum-deprived apoptosis by nitric oxide production. J Biol Chem 276:10627–10633.

Lai JM, Hsieh CL, Chang ZF. 2003. Caspase activation during phorbol ester-induced apoptosis requires ROCK-dependent myosin-mediated contraction. J Cell Sci 116: 3491–3501.

Lenzi GL, Grigoletto F, Gent M, Roberts RS, Walker MD, Easton JD, Carolei A, Dorsey FC, Rocca WA, Bruno R, et al. 1994. Early treatment of stroke with monosialoganglioside GM-1. Efficacy and safety results of the Early Stroke Trial. Stroke 25:1552–1558.

Levade T, Auge N, Veldman RJ, Cuvillier O, Negre-Salvayre A, Salvayre R. 2001. Sphingolipid mediators in cardiovascular cell biology and pathology. Circ Res 89: 957–968.

Liu Y, Wada R, Yamashita T, Mi Y, Deng CX, Hobson JP, Rosenfeldt HM, Nava VE, Chae SS, Lee MJ, Liu CH, Hla T, Spiegel S, Proia RL. 2000. Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate,is essential for vascular maturation. J Clin Invest 106:951–961.

Maeda A, Goto M, Zhang J, Bennet W, Groth CG, Korsgren O, Wennberg L. 2003. Immunosuppression with FTY720 and cyclosporine A inhibits rejection of adult porcine islet xenografts in rats. Transplantation 75:1409–1414.

McGiffert C, Contos JJ, Friedman B, Chun J. 2002. Embryonic brain expression analysis of lysophospholipid receptor genes suggests roles for s1p(1) in neurogenesis and s1p(1-3) in angiogenesis. FEBS Lett 531:103–108.

McGraw J, Hiebert GW, Steeves JD. 2001. Modulating astrogliosis after neurotrauma. J Neurosci Res 63:109–115.

Meacci E, Nuti F, Catarzi S, Vasta V, Donati C, Bourgoin S, Bruni P, Moss J, Vaughan M. 2003. Activation of phospholipase D by bradykinin and sphingosine 1-phosphate in A549 human lung adenocarcinoma cells via different GTP-binding proteins and protein kinase C delta signaling pathways. Biochemistry 42:284–292.

Meyer zu HD, Lass H, Alemany R, Laser KT, Neumann E, Zhang C, Schmidt M, Rauen U, Jakobs KH, van Koppen CJ. 1998. Sphingosine kinase-mediated Ca^{2+} signalling by G-protein-coupled receptors. EMBO J 17:2830–2837.

Meyer zu HD, Lass H, Kuchar I, Alemany R, Guo Y, Schmidt M, Jakobs KH. 1999. Role of sphingosine kinase in Ca(2+) signalling by epidermal growth factor receptor. FEBS Lett 461:217–222.

Morales-Ruiz M, Lee MJ, Zollner S, Gratton JP, Scotland R, Shiojima I, Walsh K, Hla T, Sessa WC. 2001. Sphingosine 1-phosphate activates Akt, nitric oxide production, and chemotaxis through a Gi protein/phosphoinositide 3-kinase pathway in endothelial cells. J Biol Chem 276: 19672–19677.

Morishige K, Shimokawa H, Eto Y, Hoshijima M, Kaibuchi K, Takeshita A. 2001. In vivo gene transfer of dominant-negative rho-kinase induces regression of coronary arteriosclerosis in pigs. Ann NY Acad Sci 947:407–411.

Mulder HJ, Bal ET, Jukema JW, Zwinderman AH, Schalij MJ, van Boven AJ, Bruschke AV. 2000. Pravastatin reduces restenosis two years after percutaneous transluminal coronary angioplasty (REGRESS trial). Am J Cardiol 86:742–746.

Natarajan V, Jayaram HN, Scribner WM, Garcia JG. 1994. Activation of endothelial cell phospholipase D by sphingosine and sphingosine-1-phosphate. Am J Respir Cell Mol Biol 11:221–229.

Ohmori T, Yatomi Y, Osada M, Kazama F, Takafuta T, Ikeda H, Ozaki Y. 2003. Sphingosine 1-phosphate induces contraction of coronary artery smooth muscle cells via S1P2. Cardiovasc Res 58:170–177.

Okamoto H, Takuwa N, Gonda K, Okazaki H, Chang K, Yatomi Y, Shigematsu H, Takuwa Y. 1998. EDG1 is a functional sphingosine-1-phosphate receptor that is linked via a Gi/o to multiple signaling pathways, including phospholipase C activation, Ca^{2+} mobilization, Ras-mitogen-activated protein kinase activation, and adenylate cyclase inhibition. J Biol Chem 273:27104–27110.

Okamoto H, Takuwa N, Yokomizo T, Sugimoto N, Sakurada S, Shigematsu H, Takuwa Y. 2000. Inhibitory regulation of Rac activation, membrane ruffling, and cell migration by the G protein-coupled sphingosine-1-phosphate receptor EDG5 but not EDG1 or EDG3. Mol Cell Biol 20:9247–9261.

Olivera A, Kohama T, Edsall L, Nava V, Cuvillier O, Poulton S, Spiegel S. 1999. Sphingosine kinase

expression increases intracellular sphingosine-1-phosphate and promotes cell growth and survival. J Cell Biol 147:545–548.

Olivera A, Rosenfeldt HM, Bektas M, Wang F, Ishii I, Chun J, Milstien S, Spiegel S. 2003. Sphingosine kinase type 1 induces G12/13-mediated stress fiber formation yet promotes growth and survival independent of G protein coupled receptors. J Biol Chem 278:46452–46460.

Orlati S, Porcelli AM, Hrelia S, Van Brocklyn JR, Spiegel S, Rugolo M. 2000. Sphingosine-1-phosphate activates phospholipase D in human airway epithelial cells via a G protein-coupled receptor. Arch Biochem Biophys 375: 69–77.

Pebay A, Toutant M, Premont J, Calvo CF, Venance L, Cordier J, Glowinski J, Tence M. 2001. Sphingosine-1-phosphate induces proliferation of astrocytes: Regulation by intracellular signalling cascades. Eur J Neurosci 13: 2067–2076.

Plevin R, MacNulty EE, Palmer S, Wakelam MJ. 1991. Differences in the regulation of endothelin-1- and lysophosphatidic-acid-stimulated Ins(1,4,5)P3 formation in rat-1 fibroblasts. Biochem J 280(Pt 3):609–615.

Ren XD, Kiosses WB, Schwartz MA. 1999. Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. EMBO J 18:578–585.

Robert P, Tsui P, Laville MP, Livi GP, Sarau HM, Bril A, Berrebi-Bertrand I. 2001. Edg1 receptor stimulation leads to cardiac hypertrophy in rat neonatal myocytes. J Mol Cell Cardiol 33:1589–1606.

Sagi SA, Seasholtz TM, Kobiashvilla M, Wilson BA, Toksoz D, Brown JH. 2001. Physical and functional interactions of $G\alpha_q$ with Rho and its exchange factors. J Biol Chem 276:15445–15452.

Salomone S, Yoshimura S, Reuter U, Foley M, Thomas SS, Moskowitz MA, Waeber C. 2003. S1P(3) receptors mediate the potent constriction of cerebral arteries by sphingosine-1-phosphate. Eur J Pharmacol 469:125–134.

Sautin YY, Crawford JM, Svetlov SI. 2001. Enhancement of survival by LPA via Erk1/Erk2 and PI 3-kinase/Akt pathways in a murine hepatocyte cell line. Am J Physiol Cell Physiol 281:C2010–C2019.

Sawada N, Itoh H, Ueyama K, Yamashita J, Doi K, Chun T-H, Inoue M, Masatsugu K, Saito T, Fukunaga Y, Sakaguchi S, Arai H, Ohno N, Komeda M, Nakao K. 2000. Inhibition of Rho-associated kinase results in suppression of neointimal formation of balloon-injured arteries. Circ 101:2030–2033.

Seasholtz TM, Zhang T, Morissette MR, Howes AL, Yang AH, Brown JH. 2001. Increased expression and activity of RhoA is associated with increased DNA synthesis and reduced $p27^{Kip1}$ expression in the vasculature of hypertensive rats. Circ Res 89:488–495.

Seasholtz TM, Radeff-Huang J, Sagi SA, Matteo RG, Weems JM, Stoudt A, Buckmaster C, Feramisco J, Brown JH. 2004. Lysophosphatidic acid and thrombin differentially effect Rho-mediated cell rounding: Relationship to phosphatidylinositol bisphosphate hydrolysis and Rac1 inhibition. In press.

Sekiguchi K, Yokoyama T, Kurabayashi M, Okajima F, Nagai R. 1999. Sphingosylphosphorylcholine induces a hypertrophic growth response through the mitogen-activated protein kinase signaling cascade in rat neonatal cardiac myocytes. Circ Res 85:1000–1008.

Shahrestanifar M, Fan X, Manning DR. 1999. Lysophosphatidic acid activates NF-kappaB in fibroblasts. A requirement for multiple inputs. J Biol Chem 274:3828–3833.

Shibata R, Kai H, Seki Y, Kato S, Morimatsu M, Kaibuchi K, Imaizumi T. 2001. Role of Rho-associated kinase in neointima formation after vascular injury. Circ 103:284–289.

Siess W, Zangl KJ, Essler M, Bauer M, Brandl R, Corrinth C, Bittman R, Tigyi G, Aepfelbacher M. 1999. Lysophosphatidic acid mediates the rapid activation of platelets and endothelial cells by mildly oxidized low density lipoprotein and accumulates in human athlerosclerotic lesions. Proc Natl Acad Sci USA 96:6931–6936.

Sorensen SD, Nicole O, Peavy RD, Montoya LM, Lee CJ, Murphy TJ, Traynelis SF, Hepler JR. 2003. Common signaling pathways link activation of murine PAR-1, LPA, and S1P receptors to proliferation of astrocytes. Mol Pharmacol 64:1199–1209.

Stahle M, Veit C, Bachfischer U, Schierling K, Skripczynski B, Hall A, Gierschik P, Giehl K. 2003. Mechanisms in LPA-induced tumor cell migration: Critical role of phosphorylated ERK. J Cell Sci 116:3835–3846.

Steiner MR, Urso JR, Klein J, Steiner SM. 2002. Multiple astrocyte responses to lysophosphatidic acids. Biochim Biophys Acta 1582:154–160.

Sugimoto N, Takuwa N, Okamoto H, Sakurada S, Takuwa Y. 2003. Inhibitory and stimulatory regulation of Rac and cell motility by the G12/13-Rho and Gi pathways integrated downstream of a single G protein-coupled sphingosine-1-phosphate receptor isoform. Mol Cell Biol 23:1534–1545.

Tanyi JL, Morris AJ, Wolf JK, Fang X, Hasegawa Y, Lapushin R, Auersperg N, Sigal YJ, Newman RA, Felix EA, Atkinson EN, Mills GB. 2003. The human lipid phosphate phosphatase-3 decreases the growth, survival, and tumorigenesis of ovarian cancer cells: Validation of the lysophosphatidic acid signaling cascade as a target for therapy in ovarian cancer. Cancer Res 63:1073–1082.

Tigyi G, Dyer DL, Miledi R. 1994. Lysophosphatidic acid possesses dual action in cell proliferation. Proc Natl Acad Sci USA 91:1908–1912.

Toksoz D, Williams DA. 1994. Novel human oncogene *lbc* detected by transfection with distinct homology regions to signal transduction products. Oncogene 9:621–628.

Tosaka M, Okajima F, Hashiba Y, Saito N, Nagano T, Watanabe T, Kimura T, Sasaki T. 2001. Sphingosine 1-phosphate contracts canine basilar arteries in vitro and in vivo: Possible role in pathogenesis of cerebral vasospasm. Stroke 32:2913–2919.

Van Brocklyn JR, Lee MJ, Menzeleev R, Olivera A, Edsall L, Cuvillier O, Thomas DM, Coopman PJ, Thangada S, Liu CH, Hla T, Spiegel S. 1998. Dual actions of sphingosine-1-phosphate: extracellular through the Gi-coupled receptor Edg-1 and intracellular to regulate proliferation and survival. J Cell Biol 142:229–240.

van Corven EJ, Groenink A, Jalink K, Eichholtz T, Moolenaar WH. 1989. Lysophosphatidate-induced cell proliferation: Identification and dissection of signaling pathways mediated by G proteins. Cell 59:45–54.

van Corven EJ, Hordijk PL, Medema RH, Bos JL, Moolenaar WH. 1993. Pertussis toxin-sensitive activation of p21ras by G protein-coupled receptor agonists in fibroblasts. Proc Natl Acad Sci USA 90:1257–1261.

van der Bend RL, de Widt J, van Corven EJ, Moolenaar WH, van Blitterswijk WJ. 1992. The biologically active phospholipid, lysophosphatidic acid, induces phosphatidylcholine breakdown in fibroblasts via activation of phospholipase D. Comparison with the response to endothelin. Biochem J 285(Pt 1):235–240.

van Golen KL, Davies S, Wu ZF, Wang Y, Bucana CD, Root H, Chandrasekharappa S, Strawderman M, Ethier SP, Merajver SD. 1999. A novel putative low-affinity insulin-like growth factor-binding protein, LIBC (lost in inflammatory breast cancer), and RhoC GTPase correlate with the inflammatory breast cancer phenotype. Clin Cancer Res 5:2511–2519.

van Golen KL, Wu ZF, Qiao XT, Bao LW, Merajver SD. 2000. RhoC GTPase, a novel transforming oncogene for human mammary epithelial cells that partially recapitulates the inflammatory breast cancer phenotype. Cancer Res 60:5832–5838.

van Koppen CJ, Meyer zu HD, Alemany R, Jakobs KH. 2001. Sphingosine kinase-mediated calcium signaling by muscarinic acetylcholine receptors. Life Sci 68:2535–2540.

Van Leeuwen FN, Olivo C, Grivell S, Giepmans BN, Collard JG, Moolenaar WH. 2003. Rac activation by lysophosphatidic acid LPA1 receptors through the guanine nucleotide exchange factor Tiam1. J Biol Chem 278: 400–406.

Vogler R, Sauer B, Kim DS, Schafer-Korting M, Kleuser B. 2003. Sphingosine-1-phosphate and its potentially paradoxical effects on critical parameters of cutaneous wound healing. J Invest Dermatol 120:693–700.

Vouret-Craviari V, Van Obberghen-Schilling E, Scimeca JC, Van Obberghen E, Pouyssegur J. 1993. Differential activation of $p44^{mapk}$ (ERK1) by a-thrombin and thrombin-receptor peptide agonist. Biochem J 289:209–214.

Wang L, Cummings R, Usatyuk P, Morris A, Irani K, Natarajan V. 2002. Involvement of phospholipases D1 and D2 in sphingosine 1-phosphate-induced ERK (extracellular-signal-regulated kinase) activation and interleukin-8 secretion in human bronchial epithelial cells. Biochem J 367:751–760.

Weiner JA, Chun J. 1999. Schwann cell survival mediated by the signaling phospholipid lysophosphatidic acid. Proc Natl Acad Sci USA 96:5233–5238.

Windh RT, Lee MJ, Hla T, An S, Barr AJ, Manning DR. 1999. Differential coupling of the sphingosine 1-phosphate receptors Edg-1, Edg-3, and h218/Edg-5 to the G_i, G_q, and G_{12} families of heterotrimeric G proteins. J Biol Chem 274:27351–27358.

Wing MR, Snyder JT, Sondek J, Harden TK. 2003. Direct activation of phospholipase C-epsilon by Rho. J Biol Chem 278:41253–41258.

Xia P, Wang L, Gamble JR, Vadas MA. 1999a. Activation of sphingosine kinase by tumor necrosis factor-alpha inhibits apoptosis in human endothelial cells. J Biol Chem 274:34499–34505.

Xia P, Vadas MA, Rye KA, Barter PJ, Gamble JR. 1999b. High density lipoproteins (HDL) interrupt the sphingosine kinase signaling pathway. A possible mechanism for protection against atherosclerosis by HDL. J Biol Chem 274:33143–33147.

Xia P, Gamble JR, Wang L, Pitson SM, Moretti PA, Wattenberg BW, D'Andrea RJ, Vadas MA. 2000. An oncogenic role of sphingosine kinase. Curr Biol 10:1527–1530.

Xiao CY, Means CK, Howes AL, Kawamura S, Zhang T, Omens JH, Ishii I, Chun J, Brown JH. 2004. $S1P_2$ and $S1P_3$ receptors provide protection against myocardial ischemia-reperfusion injury through regulation of Rho and Akt. In press.

Yamaguchi Y, Katoh H, Negishi M. 2003. N-terminal short sequences of alpha subunits of the G12 family determine selective coupling to receptors. J Biol Chem 278:14936–14939.

Yang Z, Chen M, Fialkow LB, Ellett JD, Wu R, Brinkmann V, Nadler JL, Lynch KR. 2003. The immune modulator FYT720 prevents autoimmune diabetes in nonobese diabetic mice small star, filled. Clin Immunol 107:30–35.

Ye X, Ishii I, Kingsbury MA, Chun J. 2002. Lysophosphatidic acid as a novel cell survival/apoptotic factor. Biochim Biophys Acta 1585:108–113.

Young KW, Challiss RA, Nahorski SR, MacKrill JJ. 1999. Lysophosphatidic acid-mediated Ca^{2+} mobilization in human SH-SY5Y neuroblastoma cells is independent of phosphoinositide signalling, but dependent on sphingosine kinase activation. Biochem J 343(Pt 1):45–52.

Young KW, Channing DR, Nahorski SR. 2000. Effect of dimethylsphingosine on muscarinic M(3) receptor signalling in SH-SY5Y cells. Eur J Pharmacol 402:55–59.

Zhang H, Desai NN, Olivera A, Seki T, Brooker G, Spiegel S. 1991. Sphingosine-1-phosphate, a novel lipid, involved in cellular proliferation. J Cell Biol 114:155–167.