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REVIEW

Insights into the pharmacological relevance of lysophospholipid receptors

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The discovery of lysophospholipid (LP) 7-transmembrane, G protein-coupled receptors (GPCRs) that began in the 1990s, together with research into the functional roles of the major LPs known as lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P), have opened new research avenues into their biological processes and mechanisms. Major examples of LP signalling effects include embryogenesis, nervous system development, vascular development, uterine implantation, immune cell trafficking, and inflammatory reactions. LP signalling also influences the pathophysiology of many diseases including cancer, autoimmune and inflammatory diseases, which indicate that LP receptors may be attractive targets for pharmacological therapies. A key example of such a therapeutic agent is the S1P receptor modulator FTY720, which upon phosphorylation and continued drug exposure, acts as an S1P receptor functional antagonist. This compound (also known as fingolimod or Gilenya) has recently been approved by the FDA for the treatment of relapsing forms of multiple sclerosis. Continued basic and translational research on LP signalling should provide novel insights into both basic biological mechanisms, as well as novel therapeutic approaches to combat a range of human diseases.

Abbreviations

GPCR, G protein-coupled receptor; IUPHAR, International Union of Basic and Clinical Pharmacology; LPA, lysophosphatidic acid; LPs, lysophospholipids; S1P, sphingosine 1-phosphate; SMC, smooth muscle cells; TCM, central memory T cell; TEM, effector memory T cells

Introduction

Lysophosphospholipids are cell membrane lipid derivatives that also act as extracellular signals, joining other lipid mediators that include prostaglandins, leukotrienes, platelet-activating factors, and endocannabinoids. Lysophospholipids (LP) are small (around 400 Da), 3-carbon backbone phospholipids derived from glycerophospholipids or sphingolipids, containing a single carbon chain of varied length and saturation. Examples of LPs include LPA (lysophosphatidic acid), S1P (sphingosine 1-phosphate), LPC (lysophosphatidylcholine), SPC (sphingosylphosphorylcholine), LPS (lysophosphatidylserine) and LPE (lysophosphatidylethanolamine) (Figure 1). This review will focus on LPA and S1P, both of which have been analysed extensively with respect to both ligand and receptor. However, it is notable that other LPs likely have distinct physiologies that remain to be elucidated, such as LPC, that is present at high concentration in blood (several hundred μM) (Croset *et al.*, 2000), and has been reported to modulate cardiac potassium channels through several G proteins (Ding *et al.*, 2011), suggesting involvement of one or more unidentified receptors. LPA and S1P have well documented *in vivo* functions mediated by cognate G protein-coupled receptors

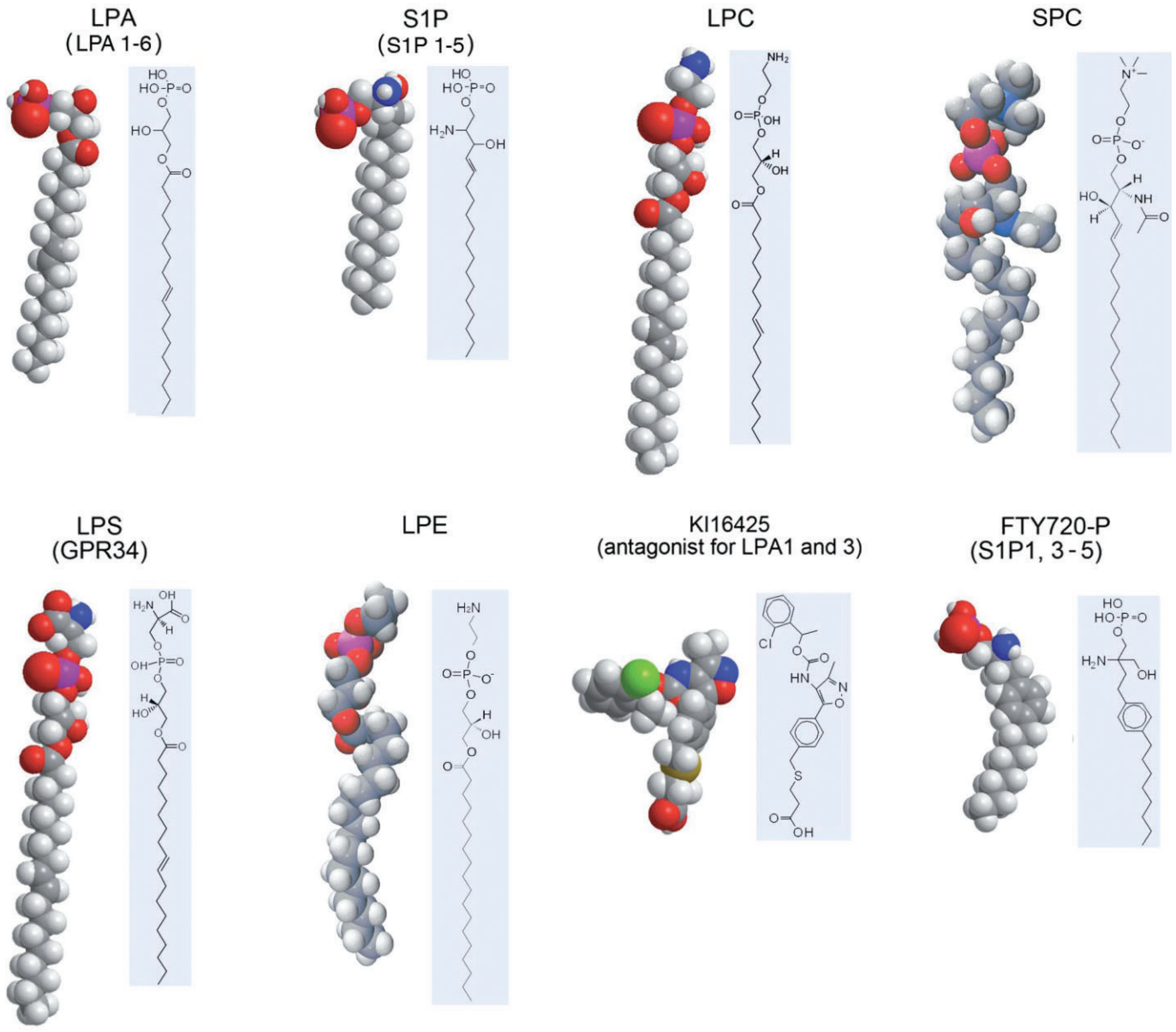

Figure 1

The molecular structure of major lysophospholipids. Space-filling molecular models and structural formulas of LPA, S1P, LPC, SPC, LPS and LPE and some major analogues are shown. High-affinity LP receptors are indicated in parentheses under the name of each ligand.

(GPCRs), despite their comparatively low concentrations (low μM range) (Okajima, 2002; Aoki, 2004). LPs are usually bound to lipoproteins *in vivo*. Serum LPA is bound by albumin, gelsolin and other proteins (Moolenaar *et al.*, 2004). S1P is bound by HDL and albumin (Okajima, 2002; Levkau *et al.*, 2004; Nofer *et al.*, 2004; Theilmeier *et al.*, 2006; Sato *et al.*, 2007). These lipoproteins stabilize LPs in the hydrophilic environment and possibly protect them from overly rapid degradation. LPs in isolation are unstable and are rapidly degraded within a few hours or less *in vivo* (Pages *et al.*, 2001). Dephosphorylation by lipid phosphate phosphatases (LPPs) inactivates LPA and S1P by conversion to monoacylglycerol (MAG) and sphingosine respectively (Morris *et al.*, 2009). S1P can be permanently degraded to hexadecanal and phosphoethanolamine by S1P lyase (Brindley and Pilquil, 2009). LPA can also be converted to phosphatidic acid by an LPA acyltransferase, and the long chain fatty acyl group can be hydrolysed by lysophospholipases (Lin *et al.*, 2010). Ligand availability, concentration and half-life may influence cellular responses mediated by LP receptors. The stabilization effect of lipoproteins is currently being studied (Moumtzi *et al.*, 2007).

Initially, LPs were shown to be precursors and metabolites in the *de novo* biosynthesis of phospholipids. It was previously thought that the effect of LPs and their mechanisms of action were largely non-specific. However, other bioactive properties were subsequently discovered. For instance, LPA was shown to function as an anti-hypertensive agent (Sen

et al., 1968; Tokumura *et al.*, 1978). LPA also acts as a cell growth and motility factor present in serum, and the signalling cascades mediated by LPA were shown to involve G proteins (van Corven *et al.*, 1989), suggesting that LPA's effects were mediated through GPCRs, although other GPCR-independent mechanisms were also possible considering the high concentrations often employed combined with a lack of identified receptors.

The first LP receptor was cloned from mouse brain cDNA by degenerate PCR with primers designed against GPCRs (Hecht *et al.*, 1996). This receptor, originally designated VZG-1, and now called LPA_1, was the first LP receptor discovered. Within several years of this initial report, several members of an orphan GPCR receptor family, called 'endothelial differentiation genes (Edg)', as well as other names were identified as GPCRs for both LPA and S1P (An *et al.*, 1997; Lee *et al.*, 1998; Im *et al.*, 2000a; Van Brocklyn *et al.*, 2000; Ishii *et al.*, 2004). For the LPA receptors, another group of less similar GPCR genes have also been identified (Noguchi *et al.*, 2003; Kotarsky *et al.*, 2006; Lee *et al.*, 2006). This latter group is more closely related to the family of P2Y purinergic receptor genes, indicating that LPA receptors have evolved via two distinct lineages in the rhodopsin GPCR family. To date, 11 *bona fide* LP receptors have been reported, 6 for LPA ($LPA_{1–6}$) and 5 for S1P ($S1P_{1–5}$), with additional putative LP G protein-coupled receptors (GPCRs) existing in the literature (Anliker and Chun, 2004b; Ishii *et al.*, 2004; Lee *et al.*, 2006; Rivera and Chun, 2008). All of these LP receptors are GPCRs capable of interacting with a number of heterotrimeric G proteins. The current nomenclature reflects the receptor's cognate ligand and chronological order of the relevant receptor's identification (Chun *et al.*, 2010) (Table 1). Protein receptor names are $LPA_{1–6}$ and $S1P_{1–5}$. Gene names for humans are *LPAR1-6* and *S1PR1-5*, or, for mice, *Lpar1-6* and *S1pr1-5* (Chun *et al.*, 2010). This nomenclature will be used throughout this review.

The synthesis of lipid mediators and subsequent receptor activation is tightly regulated under normal physiological conditions, and enzyme and/or receptor dysfunction can lead to a variety of disease conditions. LP signalling itself can also influence other signalling pathways, such as signalling crosstalk with S1P, and other pro-angiogenic growth factors, such as VEGF, EGF, IGF, PDGF, bFGF and IL-8 have been reported (Schwartz *et al.*, 2001; Spiegel and Milstien, 2003; Pyne *et al.*, 2007). Thus, the manipulation of lipid mediator signalling through enzyme inhibitors or receptor antagonists and agonists has great potential as a therapeutic approach to disease. This review summarizes our current knowledge of LP lipid mediators and the function of their cognate receptors. It also discusses the effects of genetic ablation or pharmacological inhibition of enzyme or receptor function on various pathophysiological processes. We will also discuss the normal physiological functions of LPA and S1P mediated by their cognate receptors and discuss diseases associated with these bioactive LP molecules and their pharmaceutical application or potential.

LP Receptors and GPCRs

LP receptor genes are distributed throughout the genome. The coding regions for the LPA receptors in the genomes of humans and mice are found in multiple exons ($LPA_{1–3,6}$) or single exons (LPA_4 and LPA_5), while the coding region of each of the 5 S1P receptors is contained within a single exon, with only non-coding exon(s) upstream (Contos and Chun, 2001; Contos *et al.*, 2000b; 2002). Several structural characteristics are shared between LPA and S1P receptors, including an extracellular N-terminus, seven *a*-helical transmembrane helices, and an intracellular C-terminus (Pierce *et al.*, 2002). No formal structural data have been reported for this family of GPCRs, although non-G protein binding, fusion-protein structures should be available for some receptors in the near future.

LP receptors each have a heterogeneous spatiotemporal gene expression pattern and the same cell may express mul-

Table 1

Nomenclature of lysophospholipid receptors

IUPHAR nomenclature	Chromosomal location (Human)	Natural agonist ligand	Human gene name	Mouse gene name
LPA_1	9q32	LPA	*LPAR1*	*Lpar1*
LPA_2	19p12	LPA	*LPAR2*	*Lpar2*
LPA_3	1p22.3–p31.1	LPA	*LPAR3*	*Lpar3*
LPA_4	Xq13–q21.1	LPA	*LPAR4*	*Lpar4*
LPA_5	12p 13.31	LPA	*LPAR5*	*Lpar5*
LPA_6	13q14	LPA	*LPAR6*	*Lpar6*
$S1P_1$	1p21	S1P > SPC	*S1PR1*	*S1pr1*
$S1P_2$	19p13.2	S1P > SPC	*S1PR2*	*S1pr2*
$S1P_3$	9q22.1–q22.2	S1P > SPC	*S1PR3*	*S1pr3*
$S1P_4$	19p13.3	S1P > SPC	*S1PR4*	*S1pr4*
$S1P_5$	19p13.2	S1P > SPC	*S1PR5*	*S1pr5*

tiple receptors. These data were derived by examining mRNA for the presence of specific receptor transcripts and combined with functional assays. Notably, no antibodies or antisera have been clearly proven for use in immunohistochemical studies of native proteins, although many can identify over-expressed proteins in cell lines. Some reports have suggested a nuclear localizing protein fraction of LPA_1, but more physiological studies are still needed to elucidate the biological relevance of this fraction (Gobeil *et al.*, 2006).

LPA and S1P receptors couple to heterotrimeric G proteins, which consist of a Gα and associated Gβγ subunits. The heterotrimeric G proteins are thought to bind to the inner surface of the cell membrane. One receptor may couple to several different types of Gα protein subunits to form a complex signalling network (Figure 2). $LPA_{1,2,4,5,6}$ and $S1P_{2-5}$ all signal through $G\alpha_{12/13}$ to activate RhoA, a member of the family of Rho GTPases. LPA_{1-5} and $S1P_{2,3}$ couple to $G\alpha_{q/11}$ to activate phospholipase C (PLC). $LPA_{1-4,6}$ and $S1P_{1-5}$ also couple with $G\alpha_i$ to activate PLC, Ras, phosphoinositide-3 Kinase (PI3K), and to inhibit adenylyl cyclase (AC), but LPA_4 can also couple to Gαs to activate AC (Etienne-Manneville and Hall, 2002; Neves *et al.*, 2002; Chun *et al.*, 2010). When a ligand binds to a receptor, it exchanges GDP for GTP on the Gα subunit, and then Gα-GTP and Gβγ can activate the effector molecule complex for each signalling cascade. The desensitization of receptors is probably mediated by known mechanisms in other systems including phosphorylation of GPCRs by kinases and/or an uncoupling from G proteins by arrestins, followed by receptor internalization, recycling and/or degradation (Lefkowitz and Shenoy, 2005).

Endogenous regulation of LPA and S1P in vivo

It was previously thought that the main source of lysophospholipids was from blood. Reported concentrations of LPA and S1P vary in the literature, however most publications report around 1000 nM (200–5000 nM) in blood, and 0.2–100 $nmol \cdot g^{-1}$ in tissues under basal conditions (Das and Hajra, 1989; Eichholtz *et al.*, 1993; Olivera *et al.*, 1994; Yatomi *et al.*, 1997; Murata *et al.*, 2000; Min *et al.*, 2002; Okajima, 2002; Aoki, 2004; Berdyshev *et al.*, 2005; Bielawski *et al.*, 2006). Platelets contain large amounts of LPA and S1P, both of which can be released following platelet activation (Benton *et al.*, 1982; Yatomi *et al.*, 1997). For this reason, it was believed that platelets were the major source of S1P. However, it has been shown that erythrocytes can synthesize S1P by enzymatic pathways (Stoffel *et al.*, 1970), and it is now clear that sphingosine kinases (SPHKs) present in erythrocytes are responsible for S1P in blood (Pappu *et al.*, 2007). In addition, it has been shown that erythrocytes are able to import and store S1P, which can then be actively released upon stimulation (Hanel *et al.*, 2007). It was also believed that LPA in blood is mainly derived from activated platelets;

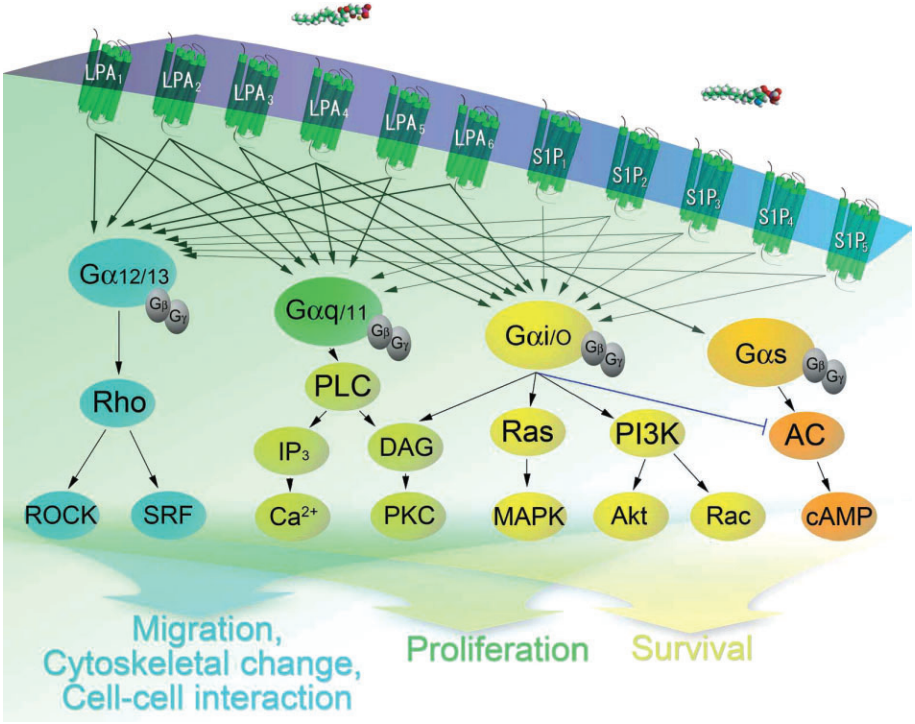

Figure 2

The network of LPA and S1P signalling through G protein-coupled receptors. Each LPA and S1P receptor couples to specific classes of G proteins. Ligand-binding activates or inhibits downstream second messenger molecules, and the most prominent cellular effects are illustrated. *Rock*, Rho-associated kinase; *SRF*, serum response factor; *IP3*, inositol 1,4,5-trisphosphate; PLC, phospholipase C; DAG, diacylglycerol; *PKC*, protein kinase C; *MAPK*, mitogen-activated protein kinase; *PI3K*, phosphoinositol 3-kinase; *DAG*, diacylglycerol.

however, it was reported that an LPA producing enzyme, lysophospholipase D (lysoPLD) in plasma, may also contribute to the total amount of LPA found in blood (Aoki, 2004). The plasma lysoPLD activity was directly measured, and half of this activity is attributed to autotaxin (ATX), one of the major lysoPLDs (Tanaka *et al.*, 2006). In addition to the aforementioned pathway, there might be local LP synthesis in specific tissues. For example, the highest expression of ATX is found in the floor plate of the developing embryo and in the choroid plexus and osteoblasts throughout development (Bachner *et al.*, 1999). Neurons are also a potential source of LPA in the developing brain, as nanomolar concentrations of LPA are found in conditioned medium from embryonic brain primary cultures as estimated in a biological activity assay (Fukushima *et al.*, 2000).

As noted above, there is a wide concentration range of LPA and S1P reported in both blood and tissues. Possible reasons for the differences in concentration may include differences in platelet activation during blood collection, the presence or absence of catabolic enzymes in different tissues and the potential conversion of LPC to LPA by ATX. The inherently sticky nature of LPA also makes it particularly difficult to quantify, especially when levels are already low. LPA is also difficult to extract and differences in the pH of the extraction buffer can produce dramatic differences in sample recovery. It is also possible that LPA and S1P may vary with age, gender or strains of mice used in experiments.

As signalling mediators *in vivo*, the production and degradation of lysophospholipids is tightly controlled. LPs are produced by multi-step enzymatic pathways that are initiated by the de-esterification of membrane phospholipids. The metabolism of LPA has been partially characterized and involves a number of convergent biosynthetic pathways and enzymes of varied specificity (Meyer zu Heringdorf and Jakobs, 2007). To date, lysoPLD, ATX, phospholipase A1 (PLA1), phospholipase A2 (PLA2) and acylglycerol kinase (AGK) are enzymes reported to be involved in LP synthesis (Chun and Rosen, 2006). These enzymes, through multiple pathways, are responsible for LPA production (Meyer zu Heringdorf and Jakobs, 2007).

S1P metabolism involves a number of specific and highly conserved enzymes (Saba and Hla, 2004). Two SPHK isoforms, SPHK1 and SPHK 2, produce S1P from sphingosine (Kohama *et al.*, 1998; Liu *et al.*, 2000). Recently, it was shown by specific genetic removal of SPHK1 and 2 in erythrocytes that these cells are the major source of S1P in blood (Pappu *et al.*, 2007). SPHK activity is not only present in blood, but also in most mouse tissues (with high activity in thymus and lung) (Fukuda *et al.*, 2003).

The duration and strength of LP signalling likely depends, at least locally, on the activity of synthetic and degradative enzymes and their localization relative to the LP receptors. For example, it has been reported that S1P lyase (SPL) has an important role in maintaining a steep gradient of S1P between blood and tissues, which in part controls lymphocyte localization within lymphoid organs, particularly within lymph nodes where a low-to-high gradient exists in moving from the parenchyma to the efferent lymphatics (Schwab *et al.*, 2005; Schwab and Cyster, 2007). The fine distribution and potential LP gradients in other tissues remain to be elucidated. Pharmacological and molecular manipulation of LP metabolic enzyme activity is also an intriguing approach for cancer therapy or other clinical treatments.

LP receptors and clinical relevance

LPA$_1$

In adult tissues, LPA_1 shows broad gene expression, with mRNA present in brain, lung, heart, uterus, and other organs of both human and mouse (Choi *et al.*, 2010). Expression of the mouse homologue, *Lpar1*, is more spatially restricted during embryonic development, and is enriched in the brain (Table 2). *Lpar1* was initially called 'ventricular zone gene-1 (Vzg-1)' because of its enrichment in the neural progenitor zone of the mouse embryonic cerebral cortex, the 'ventricular zone' (Hecht *et al.*, 1996). The effects of *Lpar1* gene deletion are seen in studies of the CNS (Contos *et al.*, 2000a,b; Kingsbury *et al.*, 2003; Estivill-Torrus *et al.*, 2008; Matas-Rico *et al.*, 2008; Santin *et al.*, 2009; Castilla-Ortega *et al.*, 2010; Dubin *et al.*, 2010). Furthermore, the deletion of *Lpar1* in mice causes a reduction in litter size with 50% perinatal death (Contos *et al.*, 2000a,b; 2002). Intrathecal injection of LPA, but not S1P, initiates behavioural, morphological and biochemical symptoms of neuropathic pain via an LPA_1-mediated Rho/Rho-kinase (Inoue *et al.*, 2004; 2006; 2008). LPC conversion to LPA by ATX is necessary for these processes *in vivo* (Nagai *et al.*, 2010). LPA signalling modulation may be relevant to some forms of neuropathic pain, an area of significant, unmet medical need (Dworkin *et al.*, 2007). LPA_1 has also been linked to multiple disease processes, including cancer (Hama *et al.*, 2004), fibrosis in kidney (Pradere *et al.*, 2007) and lung (Tager *et al.*, 2008), and male infertility (Ye *et al.*, 2008), in conjunction with loss of other LPA receptors. Recent data has identified LPA_1 signalling as a mediator of hypoxic damage in the fetal brain (Herr *et al.*, 2011) and in the initiation of fetal hydrocephalus (Yung *et al.*, 2011).

LPA$_2$

LPA_2 has relatively high gene expression in human testis and leukocytes and moderate expression in prostate, spleen, thymus, and pancreas. Compared to the expression pattern of LPA_1, LPA_2 has a more restricted spatiotemporal expression pattern (An *et al.*, 1998; Contos *et al.*, 2000b; Choi *et al.*, 2010). In mice, *Lpar2* is highly expressed in kidney, uterus and testis, and moderately expressed in lung while lower expression levels are found in stomach, spleen, thymus, brain and heart (Contos and Chun, 2000; Anliker and Chun, 2004a). Expression is also detected in embryonic brain but decreases within a week after birth (Contos *et al.*, 2000b). In several cancer cell types, aberrant expression of LPA_2 has been reported, suggesting a possible tumour-promoting role for LPA_2 (Goetzl *et al.*, 1999a; Contos *et al.*, 2000b; Schulte *et al.*, 2001; Kitayama *et al.*, 2004; Shida *et al.*, 2004; Yamashita *et al.*, 2006; Hope *et al.*, 2009). Constitutive loss of *Lpar2* in mice does not cause a grossly abnormal phenotype. However, in combination with deletion of other LPA receptors, reproductive defects resulting from a decrease in germ cell survival are apparent in male mice (Ye *et al.*, 2008). In *ex vivo* experiments, this receptor is

Table 2

Distribution and key functions of LP receptor subtypes

Receptor	mRNA distribution	Key functions
LPA_1	Widely expressed, including in the CNS	Vasculogenesis/angiogenesis
		Neural cell migration/function
		Nerve conduction
LPA_2	Lymphocytes	Cell migration
	Testis	
LPA_3	Uterus	Embryo implantation
LPA_4	Ovary, thymus	Cell migration
LPA_5	Dorsal root ganglion,	Unknown
	Thymus, spleen	
LPA_6	Intestinal mucosa	Maintenance of human hair growth
	Scalp hair follicles	
	Skin	
$S1P_1$	Widely expressed (high expression on lymphocytes, neural cells, and vasculature)	Lymphocyte egress from secondary lymphoid organs
		Neural cell migration/function
		Embryonic development of cardiovascular and nervous systems
		Blood vessel formation
		Endothelial barrier function
$S1P_2$	Widely expressed	Vascular tone
		Endothelial barrier function
		Inner ear maintenance
		affecting hearing and balance
		Endothelial barrier function
		Nerve conduction
$S1P_3$	Widely expressed, including the CNS (neural cells/astrocytes) and the endothelium	Endothelial barrier function
		Neural cell migration/function
$S1P_4$	Lymphocytes	Unknown
$S1P_5$	Oligodendrocytes	Oligodendrocyte function
	NK cells	Natural killer cell migration

also partially responsible for neural progenitor cell intracellular calcium responses, LPA-induced cortical folding (Kingsbury *et al.*, 2003; Dubin *et al.*, 2010), and contributes to synaptic functions in slice cultures derived from the adult CNS (Trimbuch *et al.*, 2009). A reduced incidence of tumour formation in LPA_2 deficient mice in a mouse model of colitis-associated cancer demonstrates a link to this form of cancer (Lin *et al.*, 2009). In addition, reduced eosinophil infiltration and prostaglandin E2 levels in the lung fluid of *Lpar2* heterozygous antigen-challenged mice compared with that of control mice suggests that *Lpar2* plays a role in proinflammatory responses (Zhao *et al.*, 2009).

LPA_3

LPA_3 gene expression is broad in human tissues with transcripts present in heart, testis, prostate, pancreas, lung and ovary (Bandoh *et al.*, 1999; Im *et al.*, 2000b). In mice, *Lpar3* is most abundant in uterus, testis, kidney, lung, stomach, spleen, brain and thymus (Contos *et al.*, 2000b).

Interestingly, in the murine uterus, *Lpar3* mRNA is exclusively expressed in the luminal endometrial epithelium at a crucial time in the embryo implantation window (Ye *et al.*, 2005; Hama *et al.*, 2007; Ye, 2008; Ye and Chun, 2010). *Lpar3* expression appears to be positively and negatively regulated by progesterone and oestrogen and plays a critical role in blastocyst implantation and proper embryo spacing through COX-2, which generates prostaglandins (PGs) E_2 and I_2 (Shah and Catt, 2005; Ye *et al.*, 2005; Hama *et al.*, 2006; Hama *et al.*, 2007). It was recently reported that the expression of several matrix metalloproteases are reduced in the *Lpar*$_3$ deficient uterus, which may lead to abnormal collagen subtype turnover and improper extracel-

lular matrix remodelling which may also contribute to the observed implantation defect (Diao *et al.*, 2010).

LPA_4

LPA_4 mRNA is more ubiquitously expressed than other LPA receptors; however, it is highly abundant in both human and mouse ovary (Noguchi *et al.*, 2003). In mice, *Lpar4* is also expressed in heart, skin, thymus, developing brain and embryonic fibroblasts (Lee *et al.*, 2007a; 2008). Whole mount *in situ* hybridization confirmed expression in the developing brain, as well as in limb buds, somites and facial processes (Ohuchi *et al.*, 2008). Gene deletion studies in mice show that *Lpar4* can affect embryonic viability; however, this effect appears to be dependent on genetic background (Lee *et al.*, 2008; Sumida *et al.*, 2010). The identified partially penetrant effect on embryonic lethality is due to a failure of cellular recruitment to the developing blood and lymphatic vessels (Sumida *et al.*, 2010).

Mouse embryo fibroblast (MEF) cells isolated from *Lpar4* deficient mice are hypersensitive to LPA-induced cell motility (Lee *et al.*, 2008). This result indicates that *Lpar4* plays an inhibitory role in LPA-dependent migratory responses. This inhibitory effect was reported to inhibit LPA-induced migratory effects mediated through *Lpar1*. Interestingly, a human cancer cell line ectopically expressing *Lpar4* was prevented from invading a Matrigel Basement Membrane Matrix (BD Biosciences, San Jose, CA, USA) (Lee *et al.*, 2008).

LPA_5

In humans, gene expression for LPA_5 is observed in spleen, heart, small intestine, placenta, liver and colon. *Lpar5* is relatively broadly expressed in murine tissues, including embryonic brain, small intestine, skin, spleen, stomach, thymus, lung, heart, liver and embryonic stem cells (Kotarsky *et al.*, 2006; Lee *et al.*, 2006). In addition to LPA, farnesyl pyrophosphate (FPP) and N-arachidonylglycine (NAG) have been reported as LPA_5 ligands (Oh *et al.*, 2008). However, independent studies gave support to LPA_5 as being a legitimate LPA receptor (Williams *et al.*, 2009; Yin *et al.*, 2009). To date, constitutive *Lpar5* gene knockout mice have not been reported. However, expression in immune tissues and dorsal root ganglion suggests a role for *Lpar5* in these tissues (Kotarsky *et al.*, 2006; Lee *et al.*, 2006; Oh *et al.*, 2008).

LPA_6

The orphan receptor P2Y5 was recently supported as a legitimate sixth LPA receptor and designated as LPA_6 by the International Union of Basic and Clinical Pharmacology (IUPHAR) committee on the basis of the emerging literature (Lee *et al.*, 2007b; Pasternack *et al.*, 2008; Yanagida *et al.*, 2009; Chun *et al.*, 2010). LPA_6 is more closely related to LPA_4 and LPA_5, which are members of the purinoreceptor (P2Y) family of receptors. Its gene structure is that of a nested gene located within intron 17 of the retinoblastoma gene, and it was reported that 2-acyl-LPA rather than 1-acyl-LPA is the preferred ligand of LPA_6 (Yanagida *et al.*, 2009). It is noteworthy that homozygous inactivation of LPA_6 has been implicated in bladder cancer development through unclear mechanisms that may reduce the activity of the tumour suppressor gene, RB1 (Lee *et al.*, 2007b). Human genetic studies indicate that LPA_6 influences forms of human hair growth (Pasternack *et al.*, 2008; Shinkuma *et al.*, 2010). Gene targeting of *Lpar6* in mice has not been reported, but loss of the receptor may reflect the human hair-growth phenotype.

$S1P_1$

$S1P_1$ gene expression is observed in many tissues, with abundance in spleen, brain, heart, lung, adipose tissues, liver, thymus, kidney and skeletal muscle (Zhang *et al.*, 1999; Ishii *et al.*, 2001). Mice engineered to express the beta-galactosidase gene knocked into the *S1pr1* locus show that *S1pr1* protein exists in brain, lung, spleen, heart, vasculature and kidney in adult mice (Chae *et al.*, 2004). In humans, lymphocytes/leukocytes also strongly express $S1P_1$ (Balthasar *et al.*, 2006).

Constitutive $S1P_1$-null mice have severe defects in vascular maturation, and die between E12.5 to E14.5 *in utero* because of haemorrhaging (Liu *et al.*, 2000). Endothelial cell-specific deletion of $S1P_1$ showed that this abnormality was due to a maturation defect in vascular endothelial cells (Allende *et al.*, 2003). $S1P_1$ expression is localized to myocytes and perivascular SMCs, and bradycardia and hypertension in primates, including humans, is driven by $S1P_1$ activation (Brinkmann *et al.*, 2010; Chun *et al.*, 2010).

T cell-specific deletion showed that $S1P_1$ is crucial for egress of mature T cells from the thymus to the periphery (Allende *et al.*, 2004a). $S1P_1$ expression on lymphocytes also plays a profound role in the control of lymphocyte recirculation. Elegant studies with $S1P_1$ conditional or SPHK knockout mice showed that $S1P_1$ and S1P are necessary for the egress of thymocytes from the thymus and for egress of T and B cells from lymphoid tissues (Brinkmann *et al.*, 2004; Matloubian *et al.*, 2004; Schwab and Cyster, 2007).

The proper S1P gradient between efferent lymphatics and lymph node, together with lymphocyte receptor expression, has been proposed to be important for lymphocyte migration (Schwab *et al.*, 2005). It has been demonstrated that S1P stimulates migration of naïve T-cells via $S1P_1$. Following activation, T-cells temporarily suppress receptor expression which results in the loss of the S1P mediated migration response and retention of T cells in lymphoid organs. Terminally differentiated effector T-cells then up-regulate $S1P_1$ to egress from lymph nodes (Graeler and Goetzl, 2002; Matloubian *et al.*, 2004; Schwab and Cyster, 2007).

FTY720, a precursor compound that upon *in vivo* phosphorylation acts acutely as an S1P receptor agonist, but chronically as a functional antagonist, has been shown to produce selective retention of T cells (and B-cells) in peripheral lymphoid organs. Several reports have shown that FTY720 is phosphorylated *in vivo* by SPHK2 but not SPHK1 to produce FTY720-P (Allende *et al.*, 2004b; Kharel *et al.*, 2005), and this phosphorylated form inhibits T and B-cell egress from lymph nodes by modulating S1P signalling (Zemann *et al.*, 2006; Schwab and Cyster, 2007). In a recent study involving patients with relapsing MS, FTY720 was found to prevent the egress of CCR7-positive naive T cells and central memory T cells (TCM) from the lymph nodes, but not CCR7-negative effector memory T cells (TEMs) (Mehling *et al.*, 2008; Brinkmann *et al.*, 2010). Because FTY720 does not generally impair lymphocyte proliferation and/or functions, it is distinct from classical immunosuppressive therapies that are

accompanied by opportunistic infections and cancers (Gardell *et al.*, 2006; Rivera and Chun, 2007). Interestingly, in experimental autoimmune encephalomyelitis (EAE), a mouse MS model, nervous system specific-deletion of $S1P_1$ – particularly from astrocytes – attenuates FTY720 efficacy suggesting the possibility of a non-immunological target for S1P-related MS therapies (Choi *et al.*, 2011).

S1P$_2$

S1pr2 is abundantly expressed in the lung and heart, but less so in the brain of adult mice and rats (Okazaki *et al.*, 1993; Zhang *et al.*, 1999). However, during embryonic development, *S1pr2* is prominently expressed in the brain (MacLennan *et al.*, 1994).

$S1P_2$-null mice show a progressive loss of hearing and balance due to degeneration of vestibular and cochlear hair cells (MacLennan *et al.*, 2006; Herr *et al.*, 2007; Kono *et al.*, 2007). This suggests that it may be possible to prevent the degeneration of hair cells with a selective S1P signalling modulator. These studies are ongoing, and may offer novel treatment modalities for the prevention of age-related and ototoxic hearing loss.

$S1P_2$-null mutants in the C57Bl/6J background have also been reported to show electrophysiological defects and develop seizures (MacLennan *et al.*, 2001). These defects may be due to impairment of calcium-regulated signal transduction, but the $S1P_2$ pathways involved in neuronal excitability have not yet been determined (An *et al.*, 1999).

In zebrafish, a single-point mutation in the $S1P_2$-related *mil* gene leads to abnormal heart development (Kupperman *et al.*, 2000), however, this defect is not recapitulated in $S1P_2$ knockout mice (Ishii *et al.*, 2002).

S1P levels are up-regulated in the airways of asthmatic patients following allergen exposure. Cross-linking of IgE receptors on mast cells activates SPHK1 and increases S1P levels. Activation of $S1P_2$, and to a lesser extent $S1P_1$, promotes degranulation and chemotaxis of mast cells (Jolly *et al.*, 2002; 2004). Also, airway SMC express $S1P_{1-4}$, and they could modulate the SMC contraction and proliferation through the $G_{12/13}$ and $G_{i/o}$ pathways (Jolly *et al.*, 2002).

S1P$_3$

In mice, *S1pr3* is highly expressed in heart, lung, spleen, kidney, intestine, diaphragm and certain cartilagnous regions (Ishii *et al.*, 2001). LPA and S1P have vaso-regulatory functions, such as regulation of heart rate, blood pressure, platelet aggregation and smooth muscle contraction (Siess *et al.*, 2000; Karliner, 2004). HDL, which includes S1P as a component, induces vasodilation, in part, by activation of Akt through $S1P_3$ (Levkau *et al.*, 2004; Nofer *et al.*, 2004). Importantly, in an *in vivo* ischemia/reperfusion (I/R) mouse model of cardiac injury, HDL, and to a greater extent S1P, protected the heart from injury. HDL and S1P reduced infarction size by inhibiting inflammation caused by polymorphonuclear leukocyte recruitment and cardiomyocyte apoptosis in a NO-dependent manner (Theilmeier *et al.*, 2006). In another I/R mouse study, it was shown that infarct size is significantly increased in mice lacking both *S1pr2* and *S1pr3*, but not in mice deficient in only one of these receptors. Furthermore, S1P-induced cardiomyocyte Akt activation was only ablated when both receptors were deleted, suggesting a redundant protective role for *S1pr2* and *S1pr3* (Means *et al.*, 2007). These studies demonstrated that vasorelaxation, myocardial cell survival, and protection from ischemic damage are mediated by the $S1P_3$-Akt-eNOS/NO pathway (Levkau *et al.*, 2004; Nofer *et al.*, 2004; Theilmeier *et al.*, 2006; Means *et al.*, 2007). $S1P_3$ influences on cardiac fibrosis (Takuwa *et al.*, 2009) and myofibroblasts have also been reported (Keller *et al.*, 2007; Niessen *et al.*, 2008).

S1P$_4$

$S1P_4$ gene expression is enriched in lymphoid tissues, including immune system cells (Graler *et al.*, 1998; Graeler and Goetzl, 2002). It is also present in SMCs of the human lung (Jolly *et al.*, 2002). Despite wide expression of *S1pr4* throughout the cells of the immune system, no obvious immune cell deficiencies have been reported in *S1pr4* null mutant mice, and the only apparent immune cell abnormality is a perturbation in megarkaryocyte cell morphology. However, *in vitro* megakaryocytes from *S1pr4*-deficient mice have reduced proplatelet formation, and platelet recovery is delayed in an experimental thrombocytopenia model (Golfier *et al.*, 2010). These data suggest that *in vivo S1pr4* plays a role in platelet formation; however, functional redundancies with other S1P receptors likely exist. Crosses of *S1p4* mice with other S1P receptor knockout mice may reveal other roles for *S1pr4* in the immune system.

S1P$_5$

$S1P_5$ gene expression is enriched in the spleen and white matter tracts of the CNS, primarily on oligodendrocytes (Im *et al.*, 2000a; Malek *et al.*, 2001; Jaillard *et al.*, 2005). In a recent study using *S1pr5*-constitutive null-mutant mice, it was found that this receptor influences natural killer (NK) cell egress through a T-bet/Tbx21 transcription factor mechanism involving various immunological compartments (Jenne *et al.*, 2009). The functions of *S1pr5* in oligodendrocytes under normal and pathological conditions remain to be determined.

Possible relevance of LP signalling to human diseases

Both LPA and S1P have been shown to act as immunomodulators in the regulation of T cells, B cells and macrophages. Overlapping expression patterns suggest that immune cells are likely regulated by combinations of LP receptors. For example, LPA and S1P acting through $LPA_{1,2}$ and $S1P_{2,3}$, respectively, may serve as survival factors for T-cells by suppressing Bax (Goetzl *et al.*, 1999b). LPA induces migration and suppression of IL-2 production in unstimulated T cells via LPA_2, however, following T cell stimulation, LPA inhibits cell migration but activates IL-2 production via LPA_1 (Zheng *et al.*, 2000; 2001). LP receptor expression can also dynamically vary with changes in cell activation (Zheng *et al.*, 2000; Graler and Goetzl, 2002; Rosen *et al.*, 2003).

As mentioned previously, LP signalling has relevance to cancer. One of the better-characterized possible cancer links is to ovarian cancer. LPA elevation in the ascites of patients was reported to elicit growth factor-like activity (Mills *et al.*, 1988), although there is controversy over the reproducibility of this initial report. It has also been shown that LP receptors and the enzymes involved in LPA and S1P metabolism are highly expressed in multiple cancer types (e.g. ovarian cancer and glioblastoma cells have high levels LPA_2 and LPA_3) (Murph *et al.*, 2006). S1P has both positive and negative effects on cancer cell growth (Hong *et al.*, 1999). FTY720 has anti-tumour effects *in vitro* and *in vivo*, and this may be due to a direct effect on tumour cells themselves, and also to indirect effects, like the inhibition of angiogenesis (Azuma *et al.*, 2002; Ho *et al.*, 2005; LaMontagne *et al.*, 2006).

S1P can act to prevent intrinsic-, chemical- and irradiation-induced oocyte apoptosis. S1P injection into the ovarian bursa, ahead of ionizing irradiation, improves the rate of successful pregnancy in irradiated mice (Morita *et al.*, 2000; Tilly, 2001). These data complement effects of LPA signalling on implantation and spermatogenesis (Ye *et al.*, 2005; 2008) and support possible roles in the treatment of human infertility.

LPA and S1P in blood may enter the brain during CNS injury and cause pathological effects. Experimentally induced brain haemorrhage can provide an influx of 1–10 μM of LPA in the CSF (Tigyi *et al.*, 1995). In cerebral infarction, platelet aggregation can release micromolar concentrations of LPA and can also lead to increased LPA levels in CSF (Eichholtz *et al.*, 1993). Indeed, intracranial injection of LPA or S1P causes astrogliosis *in vivo* (Sorensen *et al.*, 2003). Reactive astrogliosis is a prominent component of CNS injury, including diseases like multiple sclerosis, and recent data support S1P signalling as a therapeutically tractable mechanism in reducing astrogliosis (Choi *et al.*, 2011). Ongoing studies of LP signalling should reveal novel aspects of this therapeutic approach.

LPA pharmacological tools

Over 1% of genes in the human genome are estimated to encode GPCRs (over 1000). Their therapeutic value is proven, but not yet optimally realized because drugs in the clinic target only around 30 of the approximately 400 orphan GPCRs in the genome (Klabunde and Hessler, 2002). Even so, GPCRs are the target of half of all the drugs currently on the market, and remain the focus of major pharmaceutical companies (Klabunde and Hessler, 2002; Gloriam *et al.*, 2009). Chemical tools have been developed towards providing experimental uses and proof-of-concept data. Several LPA receptor agonists or antagonists have been reported, although most show modest selectivity and a relative lack of *in vivo* validation, which must be considered in any experimental usage and particularly for *in vivo* studies.

One LPA receptor agonist is N-acyl ethanolamide phosphate (NAEPA), an LPA analogue that has an ethanol amine backbone (Lynch *et al.*, 1997). To identify receptor selective compounds, a screen with 2-oleoyl LPA, a derivative that had a pyran ring to stabilize the head group, was performed, and one LPA_1-selective agonist, two LPA_3-selective LPA agonists and one LPA_3-selective antagonist were identified (Tamaruya *et al.*, 2004).

Other LPA receptor antagonists have also been reported. For example, antagonists to both LPA_1 and LPA_3 include VPC-12449, a compound that can protect against LPA_3-mediated renal ischemia-reperfusion injury in a mouse model (Okusa *et al.*, 2003), a natural lipid metabolite, diacylglycerol pyrophosphate (DGPP) (Fischer *et al.*, 2001), and Ki16425, an antagonist with little resemblance to LPA (Ohta *et al.*, 2003) that can inhibit breast cancer cell proliferation and metastasis in mice (Boucharaba *et al.*, 2006). A unique LPA analogue, diastereoisomaeric α-bromophosphonates (BrP-LPA) showed antagonistic activity to LPA_{1-4}, and notably inhibited lysophospholipase D activity of autotaxin at a nanomolar level. The anti-isomer of BrP-LPA showed superior efficacy to paclitaxel in reducing the size and blood vessel density of tumours in a mouse orthotopic breast cancer xenograft model (Zhang *et al.*, 2009). Recently, two oral LPA_1 selective antagonists, with chemical structures having little resemblance to LPA, were reportedly used for treatment of lung fibrosis in a rodent model. One compound, AM095, showed anti-fibrotic activity without affecting wound-healing processes (Swaney *et al.*, 2010, 2011).

S1P pharmacological tools

FTY720 (fingolimod) is the best-characterized S1P receptor tool and deserves special consideration (Brinkmann, 2009; Brinkmann *et al.*, 2010; Chun and Hartung, 2010). The US Food and Drug Administration recently approved FTY720 (fingolimod) as an orally active immunomodulatory drug for the treatment of relapsing forms of multiple sclerosis (Novartis). It binds 4 of the 5 identified S1P receptors ($S1P_1$ and $S1P_{3-5}$) with high affinity (0.3–3.1 $nmol \cdot L^{-1}$). A unique feature of FTY720 is that, following its *in vivo* phosphorylation, it first binds S1P receptors and transiently activates downstream pathways; however with continued exposure, it down-regulates the S1P receptor by irreversible internalization and proteasomal degradation (Graler and Goetzl, 2004; Oo *et al.*, 2007). Therefore, FTY720 produces S1P receptor functional antagonism. FTY720 was initially reported to originate from the fungal species known as *Cordyceps*, part of the larger division *Ascomycota* (*Ascomycetes*) in 1995 (Adachi *et al.*, 1995; Suzuki *et al.*, 1996).

Another $S1P_1$ agonist, AAL-(R), is a non-selective S1P receptor agonist with structural and functional similarities to FTY720 (Brinkmann *et al.*, 2002; Rosen *et al.*, 2003). An $S1P_1$ partially selective agonist, KRP-203, sequesters circulating lymphocytes into peripheral lymphoid organs (Shimizu *et al.*, 2005), and prevents allograft rejection, but does not affect $S1P_3$ signalling (Fujishiro *et al.*, 2006). KRP203 ($S1P_1$ > $S1P_3$) is currently in Phase II clinical trials for the treatment of subacute cutaneous lupus erythematosus (Novartis). An $S1P_1$-specific antagonist called W146 can induce loss of capillary integrity (Sanna *et al.*, 2006). SEW2871 and AUY954 are $S1P_1$ specific agonists and have been shown to functionally prevent lymphocyte egress and inhibit allograft rejection, respectively (Sanna *et al.*, 2004; Pan *et al.*, 2006). AUY954 inhibits receptor recycling following internaliza-

tion, similar to phosphorylated FTY720, thus acting as a functional antagonist for $S1P_1$ (Choi *et al.*, 2011). These data suggest that at least some FTY720-related agonists can also remove receptors from further agonism, thus producing functional antagonism, as described above.

Aside from mild bradycardia, FTY720 produces few undesirable side effects in humans. In mice, bradycardia is mediated through $S1P_3$; however, several unpublished reports in primates, including humans, using $S1P_1$-selective antagonists show that it is mediated through $S1P_1$ (Chun and Hartung, 2010). The mechanism through which FTY720 operates in multiple sclerosis remains unclear, but likely involves both immunological as well as CNS effects (Chun, 2007; Choi *et al.*, 2011), and this remains an active research area. FTY720 represents the first generation of LP receptor modulators that may have therapeutic value. Other data concerning the efficacy of FTY720 administration for the treatment of type I diabetes, uveoretinitis, thyroiditis, myocarditis, systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis in animal models have been reported (Suzuki *et al.*, 1998; Hozumi *et al.*, 1999; Kurose *et al.*, 2000; Matsuura *et al.*, 2000; Okazaki *et al.*, 2002; Fujino *et al.*, 2003; Webb *et al.*, 2004).

For the other S1P receptors, JTE-013 is an $S1P_2$ specific antagonist (Yokoo *et al.*, 2004). Recently, it was reported that JTE-013 ameliorates pancreatic Beta cell failure in a mouse diabetes model (Imasawa *et al.*, 2010). In addition, there are a number of agonists/antagonists that have been described with varying affinities for the different receptor subtypes (Im *et al.*, 2001; Clemens *et al.*, 2003; 2004; Davis *et al.*, 2005).

Several oral-S1P lyase inhibitors have been developed and clinical trials for the treatment of rheumatoid arthritis have begun. These compounds can increase tissue S1P levels predominantly in lymphoid tissue without other overt physiological effects in rodents. Human clinical trials for the S1P lyase inhibitor LX2931 are being pursued (Bagdanoff *et al.*, 2010).

Another way to inhibit S1P signalling may be through the use of S1P specific monoclonal antibodies. Anti-S1P mAbs have been reported to arrest tumour-associated angiogenesis in a mouse xenograft model (Visentin *et al.*, 2006). Validation of the mechanism and efficacy profile await further study.

Current and future efforts to modulate LP signalling should provide attractive and tractable tools and potentially drugs for the treatment of medically important diseases (Chun and Rosen, 2006; Delgado *et al.*, 2007; Herr and Chun, 2007).

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Conflicts of interest

Jerold Chun has been a consultant for Novartis and a Scientific Advisory Board member for Amira Pharmaceuticals. Tetsuji Mutoh and Richard Rivera have no conflict of interest.

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