Lysophosphatidic acid (LPA, 1-acyl-2-hydroxy-sn-glycero-3-phosphate) is the prototype member of a family of lipid mediators and second messengers. LPA and its naturally occurring analogues interact with G protein-coupled receptors on the cell surface and a nuclear hormone receptor within the cell. In addition, there are several enzymes that utilize LPA as a substrate or generate it as a product and are under its regulatory control. LPA is present in biological fluids, and attempts have been made to link changes in its concentration and molecular composition to specific disease conditions. Through their many targets, members of the LPA family regulate cell survival, apoptosis, motility, shape, differentiation, gene transcription, malignant transformation and more. The present review depicts arbitrary aspects of the physiological and pathophysiological actions of LPA and attempts to link them with select targets. Many of us are now convinced that therapies targeting LPA biosynthesis and signalling are feasible for the treatment of devastating human diseases such as cancer, fibrosis and degenerative conditions. However, successful targeting of the pathways associated with this pleiotropic lipid will depend on the future development of as yet undeveloped pharmacons.

Abbreviations
3CCPA, 18:1 oleoyl-3-carba cyclic phosphatidic acid; ATX, autotaxin; CHO, Chinese hamster ovary; CPA, cyclic phosphatidic acid (1-acyl-sn-glycerol-2,3-cyclic phosphate); CTGF, connective tissue growth factor; CYR61, cysteine-rich protein 61; DRG, dorsal root ganglion; EDG, endothelial gene; GPAT, glycerol-3-phosphate acyl transferase; GPCR, G protein-coupled receptor; HUVEC, human umbilical vein endothelial cells; iPLA2, Ca\(^{2+}\)-independent phospholipase A2; IUPHAR, International Union of Basic and Clinical Pharmacology; LDL, low-density lipoprotein; LPA, 1-acyl-2-hydroxy-sn-glycero-3-phosphate; LPC, lysophosphatidylcholine; LPS, lysophosphatidylserine; MEF, mouse embryonic fibroblast; MMP, matrix metalloproteinases; NPP, nucleotide pyrophosphatase/phosphodiesterase; OMPT, D-sn-1-O-oleyl-2-O-methyl-glycerol-3-phosphothionate; P2Y, purinoreceptor; PDZ domain, PSD-95/Disc-large/ZO-1 domain; PIP2, phosphatidylinositol 4,5-bisphosphate; PLD, phospholipase D; PPARY, peroxisome proliferator-activated receptor gamma; ROCK, Rho-associated kinase; S1P, sphingosine-1-phosphate; siRNA, small interfering RNA; TRIP6, thyroid receptor-interacting protein 6; TZD, thiazolidinedione; UDP, uridine diphosphate; UTP, uridine triphosphate; uPA, urokinase; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cells

The lysophosphatidic acid-like lipid mediator family
Lysophosphatidic acid (1-acyl-2-hydroxy-sn-glycerol-3-phosphate; LPA) represents the minimal glycerophospholipid based on the radyl-glycerol-phosphate scaffold. However, LPA is only one member of a family of endogenous lipid-like substances endowed with a host of biological actions that are often mediated through high-affinity...
interactions with specific cell surface and intracellular proteins (Figure 1). The specific recognition of LPA-like molecules by several receptors and enzymes provides a diversity of biological actions that have fascinated many investigators. What biomolecules constitute the LPA family? First, for the sake of brevity, we will ignore lysophospholipids built up on the sphingoid backbone. Although this creates an artificial oversimplification, it nevertheless allows for a single focus on a distinct subset of lipids, which in many instances interact with the same molecular targets. In most cases, sphingolipids, best represented by sphingosine-1-phosphate (S1P), have their own set of distinct targets that rarely overlap (but in some instances do; discussed below) with those of LPA.

Different molecular species of LPA, dominated by 16-, 18- and 20-carbon long acyl chains, have been detected in biological fluids as saturated, and mono- and poly-unsaturated variants of either the sn1 or the sn2 regioisomers. This natural variety within the LPA class leads to important biological and pharmacological consequences. First, most LPA G protein-coupled receptors (GPCR) show lower EC50 values (higher potency) for unsaturated species (Bandoh et al., 2000; Fujiwara et al., 2005; Williams et al., 2009). An extreme case of target selectivity is found for the peroxisome proliferator-activated receptor gamma (PPARγ), an intracellular target of LPA that is exclusively activated by unsaturated acyl species of LPA (Tsukahara et al., 2006). The LPA concentration in plasma is in the low nanomolar range, whereas in serum it increases to several micromoles (Baker et al., 2001; Watanabe et al., 2007b; Hosogaya et al., 2008). The poly-unsaturated 18:2 (38%) and 20:4 (39%) and the mono-unsaturated 18:1 (9%) LPA species constitute the overwhelming majority (86%) of LPA in serum (Sano et al., 2002). This enrichment in the unsaturated species increases the likelihood of the activation of those LPA targets that show preference for these fatty acyl species. In addition, sn1 and sn2 regioisomers of acyl LPA have been detected, and the LPA3, the purinoreceptor-5 (P2Y5) and GPR35 receptors have been suggested to show a slight preference for sn2 over sn1 LPA containing identical fatty acid chains (Bandoh et al., 2000; Yanagida et al., 2009; Oka et al., 2010). One unresolved concern is the stability of these regioisomers in vivo due to the relatively fast rate of acyl migration towards a 9:1 (sn1 : sn2) equilibrium ratio of the isomers (Wang et al., 1997; Croset et al., 2000).

There are two mouse phospholipase A1 enzymes that have been linked to production of unsaturated sn-2 LPA. A phosphatidic acid-specific PLA1α and β (Sonoda et al., 2002; Hiramatsu et al., 2003) from mice have been cloned, which are the equivalents of the human enzymes LIPH and LIPI respectively (Kazantseva et al., 2006; Aoki et al., 2007; Aoki et al., 2008; Pasternack et al., 2009; Shimomura et al., 2009a,b). Mutations in the LIPH/mPA-PLA1α gene have been identified as the cause of the hereditary disorder woolly hair, as it’s gene product supplies LPA to the P2Y5 receptor expressed in the hair bulb (Kazantseva et al., 2006; Pasternack et al., 2008;
2009; Shimomura et al., 2008; 2009a,b). Yet, another mouse PLA1 enzyme, designated PS-PLA1, secreted from rat platelets and specific to phosphatidylserine, has been identified by the Aoki group (Horigome et al., 1987; Sato et al., 1997). However, this enzyme is not found in human platelets and its role in human pathophysiology is unclear.

Alkyl-ether (Sugiura et al., 1999; Nakane et al., 2001) and alkenyl-ether analogues (Liliom et al., 1998) of LPA have been described. These lower-abundance mediators have been shown to be weaker agonists of LPA-specific GPCR (Bandoh et al., 2000; Fujiwara et al., 2005; Williams et al., 2009) with one exception. The LPAG/GPR92 receptor shows a preference for 1-O-alkyl glycerophosphate over the acyl analogues with the same chain length (Williams et al., 2009). Human platelets show preferential activation by 1-O-alkyl glycerophosphate over LPA; and the structure-activity relationship of the platelet response is similar, although not identical, to that of LPAG (Simon et al., 1982; Williams et al., 2009). Interestingly, 1-O-alkyl glycerophosphate shows a higher potency than LPA with respect to PPARγ activation (Zhang et al., 2009). CPA can be generated by autoxidation (ATX) under non-physiological conditions using a two-phase water/ether reaction system (Tsuda et al., 2006), but whether this enzyme contributes to the formation of CPA in blood remains an open question. Bacterial phospholipase D (PLD) can also yield CPA production (Friedman et al., 1996). In mammalian cells, CPA is generated intracellularly by PLD2 and acts as a second messenger which inhibits PPARγ (Tsukahara et al., 2010). CPA is a weak agonist of many LPA GPCR (Fujiwara et al., 2005; Williams et al., 2009). However, its synthetic hydrolysis-resistant 3-carba analogue (3CCPA 18:1) is a selective weak agonist of LPAG/GPR92 that fails to activate LPAG/12/23/4 and also inhibits ATX (Baker et al., 2006; Fujiwara, 2008). CPA and its synthetic 3CCPA analogues inhibit cancer metastasis through an as yet unidentified mechanism, which includes the inhibition of ATX (Baker et al., 2006; Uchiyama et al., 2007; Fujiwara, 2008).

In search of the minimal pharmacophore that activates LPA GPCR, we identified fatty alcohol phosphates that, depending on the length of the hydrocarbon chain and the headgroup (phosphate or thiophosphate), function as either antagonists or agonists (Durgam et al., 2005; Deng et al., 2007; Zhang et al., 2009a). Naturally occurring farnesyl phosphates are structurally similar to fatty alcohol phosphates. This similarity prompted us to confirm that farnesyl mono- and diphosphate activate various LPA GPCR targets (Liliom et al., 2006). Oh et al. (Oh da et al., 2008) reported that farnesyl monophosphate and arachidonoyl glycerol were more potent activators of LPAG than LPA. However, other groups have reported that LPA and 1-O-alkyl glycerophosphate are the most potent agonists of LPAG identified so far (Kotarsky et al., 2006; Lee et al., 2006; Williams et al., 2009). Despite this controversy, farnesyl mono- and diphosphate are important pharmacological tools that allow for the selective activation of LPAG (~50 nM EC50) without activation of LPAG,12,14 (in fact these reagents are weak inhibitors of LPAG with IC50 values in the mid-micromolar range) (Williams et al., 2009).

SIP is currently classified as a specific ligand for five cognate receptors, S1P1,2,3,4,5, which are not activated by physiological concentrations of LPA. It is important to recognize that LPA1,2,3 receptors, at least when expressed heterologously, do show activation to SIP at concentrations in excess of 1 μM (Valentine et al., 2008a). Conversely, LPA binds to (Kd = 2.3 μM) and activates the S1P receptor as a low-affinity agonist (Lee et al., 1998). Because LPA concentrations can reach several micromoles in serum and tumour cell effusates (Baker et al., 2001; 2002; Surphen et al., 2004), promiscuous activation of S1P by LPA in pathophysiological conditions may occur. Murakami et al. (Murakami et al., 2008) recently reported that P2Y10 is a receptor for LPA that is also activated by SIP, making P2Y10, if confirmed, a dual-specificity receptor. To make things even more interesting, heterologously expressed P2Y10 is also activated by lysophosphatidyl serine, although it is much less potent than LPA on this target.

**LPA GPCRs**

The LPA GPCR are the most studied and best understood targets of LPA. In mammals, LPA GPCRs have been identified in two distinct gene families (Figure 2). Chronologically, the endothelial gene (EDG) family was the first shown to encode GPCR specific for SIP and LPA. Within the EDG family, three receptors – LPA1, LPA2 and LPA3 – are activated by LPA and its analogues. These three receptors share 45–56% overall amino acid identity. The overall homology of the transmembrane
domains of these three GPCRs is 86%, whereas only 6% of the amino acids are conserved in their C-terminal tails. In addition, five S1P-specific receptors, S1P1, S1P3, S1P4, S1P5, and S1P6, belong to this gene cluster. There are many excellent recent reviews on the biological function and signal transduction associated with the EDG family receptors; we refer the reader to these for a more in-depth description (Tigyi and Parrill, 2003; Choi et al., 2008; Mutoh and Chun, 2008; Rivera and Chun, 2008; Ishii et al., 2009; Noguchi et al., 2009; Peyruchaud, 2009).

A second cluster of LPA GPCR is found within the P2Y gene cluster (Ishii et al., 2009). The better-characterized LPA-sensitive members of this cluster are P2Y9/GPR23/LPA4 (this will be abbreviated as LPA4) (Noguchi et al., 2003; Yanagida et al., 2007) and GPR92/LPA5 (this will be abbreviated as LPA5) (Kotarsky et al., 2006; Lee et al., 2006; Williams et al., 2009; Yin et al., 2009). There are additional related GPCR that appear to be activated by LPA. In chronological order based on the first publication, these include the GPR87 (Tabata et al., 2007; Wetter et al., 2009), P2Y5 (Pasternack et al., 2008; Shimomura et al., 2008), P2Y10 (Murakami et al., 2008) and GPR35 (Oka et al., 2010) gene products. The LPA GPCR nomenclature was developed by the International Union of Basic and Clinical Pharmacology (IUPHAR) nomenclature committee (Chun et al., 2002), which has not met since 2002; but efforts to update this nomenclature are underway. Hence, the assignment of numbers to LPA GPCR beyond LPA5 has been arbitrary and confusing. According to the IUPHAR committee, numbering assignments should follow the chronological order of publication, yet some authors tend to ignore the report on GPR87 in 2007 (Tabata et al., 2007) that preceded publications on P2YS in 2008 (Pasternack et al., 2008; Shimomura et al., 2008). Now that GPR87 has been shown independently to be activated by LPA (Wetter et al., 2009), it might qualify for the designation LPA6; consequently, P2Y5 would be LPA7, P2Y10 and GPR35, if confirmed, would be LPA8 and LPA9, respectively. However, these designations will have to be officially assigned by the IUPHAR pending the publication of additional confirmatory evidence concerning the identity of their most potent natural ligand. A summary of the different established and putative LPA receptors and their ligands is shown in Table 1 and Figure 3.

LPA4 shows a wide tissue distribution with the highest density of transcripts in the ovary, uterus and placenta (Ishii et al., 2009). Interestingly, this receptor was not detectable by Northern blotting in the liver, spleen and testis. LPA4 has high transcript abundance at sites of implantation within the uterus and increased expression in the ovary during pregnancy. The ligand preference of LPA4 is LPA 18:1 > 18:0 > 16:0 > 14:0 > 1-O-alkyl glycerophosphate > 1-O-alkenyl glycerophosphate, and the Kd for LPA 18:1 binding to the human orthologue is 77 nM (Noguchi et al., 2003). The brain expresses relatively high levels of LPA4; and it has been shown, using heterologous expression in B103 neuroblastoma cells, to cause LPA-dependent growth cone collapse and neurite retraction through G12/13-RhoA-Rho-associated kinase (ROCK) activation (Lee et al., 2007). Although a practical issue, it is important to note that heterologous expression of the non-EDG family receptors is often difficult and requires special plasmids and/or receptor-G protein fusion constructs (Noguchi et al., 2003; Tabata et al., 2007; Murakami et al., 2008). Knockout mice for LPA4 do not display a noticeable phenotype, although mouse embryonic fibroblasts (MEF) isolated from the knockouts show increased migration in response to LPA, supporting the hypothesis that this receptor inhibits cell motility (Lee et al., 2008). Along with LPA5, LPA4 has been implicated in the inhibition of neurosphere formation and neuronal differentiation of human embryonic stem cells (Dottori et al., 2008). LPA4 shows high expression in platelets (Khandoga et al., 2008), and Smyth et al. proposed that it might mediate LPA-induced inhibition of platelet activation (Pamuklar et al., 2008). In patients whose platelets do not respond to LPA, LPA4

Figure 2
<table>
<thead>
<tr>
<th>Receptor</th>
<th>Location</th>
<th>Agonist preference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPA&lt;sub&gt;1&lt;/sub&gt;</td>
<td>9q31.3</td>
<td>18 : 2 &gt; 18 : 3 &gt; 20 : 4 &gt; 18 : 1 &gt; 16 : 0 &gt; 18 : 0</td>
</tr>
<tr>
<td>LPA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>19p12</td>
<td>18 : 3 &gt; 20 : 4 &gt; 18 : 2 = 18 : 1 &gt; 18 : 0 &gt; 16 : 0 &gt; 20 : 0</td>
</tr>
<tr>
<td>LPA&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1p22.3</td>
<td>18 : 3 &gt; 18 : 2 &gt; OMPT &gt; 18 : 1 &gt; 20 : 4 &gt;&gt; 18 : 0 &gt;&gt; 16 : 0</td>
</tr>
<tr>
<td>LPA&lt;sub&gt;α&lt;/sub&gt;/PR23/PRY9</td>
<td>Xq13-q21.1</td>
<td>18 : 1 &gt; 18 : 0 &gt; 16 : 0 &gt; 14 : 0 &gt; AGP18 : 0 &gt; AKG18 : 1</td>
</tr>
<tr>
<td>LPA&lt;sub&gt;α&lt;/sub&gt;/GPR92</td>
<td>12p13.31</td>
<td>AGP18 : 1 &gt; AGP18 : 0 &gt; 16 : 0 &gt; 18 : 3 &gt; 18 : 2 &gt; 18 : 1 = 16 : 0 = 20 : 4 &gt; FDP &gt; FMP &gt; AGP &gt; 18 : 0 &gt; 18 : 0 &gt; CPA &gt; 18 : 1 &gt; CPA18 : 1 &gt; NAG</td>
</tr>
<tr>
<td>GPR87</td>
<td>3q25</td>
<td>18 : 1 &gt; CPA18 : 1 &gt; 2CPA18 : 1</td>
</tr>
<tr>
<td>P2Y5</td>
<td>13q14.2</td>
<td>18 : 2 &gt; OMPT &gt; sn2-18 : 1 &gt; sn1-18 : 1 &gt; 20 : 4 &gt; 18 : 0 &gt; 16 : 0 &gt; 14 : 0</td>
</tr>
<tr>
<td>P2Y10</td>
<td>Xq21.1</td>
<td>18 : 1 &gt; S1P &gt; LPS18 : 1</td>
</tr>
<tr>
<td>GPR35</td>
<td>2q37.3</td>
<td>sn2-18 : 2 &gt; sn2-18 : 1 &gt; sn1-20 : 4 &gt; sn1-18 : 1 &gt;&gt; Zaprinast</td>
</tr>
<tr>
<td>PPARγ</td>
<td>3p25</td>
<td>Unsaturated AGP &gt; Unsaturated LPA</td>
</tr>
</tbody>
</table>

*Numbers alone always refer to LPA, AKGP 18:1 denotes alkenyl glycerophosphate 18:1.
*Compounds in italics show at least a 10-fold selectivity for the receptor subtype.
LPA, lysophosphatidic acid; N/A, not available.
mRNA is increased sixfold over platelets from patients that are LPA-responsive. Thus, LPA4 might be a cardioprotective receptor as patients with LPA-non-responsive platelets are less likely to suffer from coronary heart disease.

LPA5 is present in the heart, placenta, brain, dorsal root ganglia, small intestine and spleen. Intestinal CD8\(^+\) lymphocytes, as well as B cells, show abundant expression of LPA5 (Kotarsky et al. 2006), suggesting an immunoregulatory role. This receptor subtype is also highly expressed in human platelets, and pharmacological evidence of its preference for 1-O-alkyl glycerophosphate suggests that it mediates platelet activation (Williams et al. 2009). The ligand preference of LPA5 is 1-O-alkyl glycerophosphate 18:1 > LPA 18:0 > CPA 18:1 > CPA 16:0 > N-arachidonyl glycine (Williams et al., 2009), and the \(K_d\) for LPA 18:1 binding is 6.4 nM (Kotarsky et al., 2006). The physiological/pathophysiological classification of this receptor subtype is rudimentary at the present time. Analysis of LPA5 is currently limited to heterologous expression studies, and experiments using farnesyl phosphate as a selective, although not a specific, agonist. LPA5 knockout mice have deficits in the response to neuropathic pain (Sheardown et al., 2004; Kinloch and Cox, 2005). LPA5-lacZ reporter gene-expressing mice showed the strongest expression in cell bodies of dorsal root ganglion cells that extend C-fibres (sensory nerves conveying pain

Figure 3
Synthetic ligands of LPA GPCR (see Table 1 for details). GPCR, G protein-coupled receptor; LPA, lysophosphaticid acid.
signals) but not in those neurons with Aβ fibres (sensory nerves conveying non-pain signals). In these animals, LPA₅ staining was noted in the trigeminal ganglia but not the spinal cord or brain. Behavioural testing showed that the mutant mice had no abnormalities on a wide range of standard tests, including von Frey responses. There was, however, a significant delay in the tail flick test, indicating impaired nociceptive function. In vivo electrophysiological recordings (Kinloch and Cox, 2005) from the dorsal horn of mutant animals revealed significantly lower numbers of action potentials in response to noxious mechanical, thermal and cold stimuli applied to the hind foot than in wild-type mice. However, there was no difference in the number of action potentials recorded in response to non-noxious brush stimulation or to temperature in the non-noxious range. Knockout mice failed to develop allodynia using the Chung model of neuropathic pain for 13 days as measured by von Frey thresholds. In contrast wild-type mice developed allodynia in this model (Sheardown et al., 2004; Kinloch and Cox, 2005). In another study, LPA₃ protein was detected using immunohistochemistry in dorsal root ganglion cells of mice and humans (Oh da et al., 2008). In cultured small dorsal root ganglion (DRG) cell neurons, farnesyl diphosphate and N-arachidonoyl glycine both elicited Ca²⁺ responses in response to LPA (100 nM) with an EC₅₀ of 36 nM. This response is completely abolished with a GPR87 siRNA. These cells fail to respond to uridine diphosphate (UDP) and UDP-glucose; however, uridine triphosphate (UTP) treatment of the cells elicits a desensitization-like phenomenon of the LPA response. GPR87 transcripts were detected in the brain, skeletal muscle and the reproductive organs. The initial report on GPR87 also showed that the response to LPA (100 nM) was blocked by Ki16425 (1 μM) and diacylglycerol pyrophosphate (50 μM), which have been previously identified as antagonists of the LPA₁-₃ receptors (Tabata et al., 2007). Clearly, more information is necessary to fully characterize this receptor’s ligand selectivity and signal transduction properties, both of which should be derived from LPA-hyporesponsive cells. GPR87 is under the transcriptional regulation of p53; shows a very high expression level in squamous cell carcinomas, lung adenocarcinomas and transitional carcinomas of the urinary bladder and is involved in promoting cell survival and proliferation (Glatt et al., 2008; Gugger et al., 2008; Zhang et al., 2009b).

An unbiased genetic search for the gene causing familial Hypotrichosis simplex and autosomal recessive woolly hair has identified mutations in the P2Y5 receptor gene on chromosome 13q14.2–14.3 (Z = 17.97) (Pasternack et al., 2008; Shimomura et al., 2008) underlying the disease. Woolly hair is characterized by the presence of fine and tightly curled hair and is also linked to another locus on chromosome 3q27. This region contains the lipase H (LIPH) gene, which has been recently shown to underlie an autosomal-recessive form of hypotrichosis (Pasternack et al., 2009; Shimomura et al., 2009a,b). Lipase H is the human orthologue of the PA-PLA₁ enzyme that produces sn2-LPA from PA. The phenotypically indistinguishable autosomal-recessive woolly hair syndrome caused either by mutations in the P2Y5 or PA-PLA₁/LIPH genes points to the signalling axis that is colocalized to the Henle’s and Huxley’s layers of the inner root of the hair follicle. There are differences in LPA receptor expression between hair follicles in the eyebrow and those in the scalp. The former express LPA₅ in addition to P2Y5, whereas the latter express P2Y5 only; hence, hypotrichosis manifests only in the scalp (Pasternack et al., 2008). LIPH was originally identified by Kazantseva et al. (Kazantseva et al., 2006) as a gene linked to hair growth deficiency in Finno-Ugric families in current-day Russia. LIPH is homologous to mPA-PLA₁α, the enzyme that cleaves the sn1 fatty acid in phosphatidate to generate sn2-LPA (Sonoda et al., 2002). LIPH/mPA-PLA₁α is expressed in the...
skin concentrated near the hair bulb and follicle, lung, kidney and pancreas. Currently, the tissue expression of P2Y5 is not known outside the skin and the hair. P2Y5 shows a $K_d$ of 88.6 nM for sn1 LPA 18:1 binding and a rank order of activation of LPA 18:2 > LPA 18:1 > LPA 20:4 > LPA 18:0 > LPA 16:0 > LPA 14:0 (Yanagida et al., 2009). Yanagida et al. also compared the activation properties of P2Y5 by sn1 and sn2-LPA species and found that the sn2-substituted regioisomers were more potent and, in the case of arachidonoyl LPA, more efficacious than the corresponding sn1 regioisomer (Yanagida et al., 2009). D-sn1-1-O-oleyl-2-O-methyl-glyceryl-3-phosphothionate (OMPT), a previously proposed LPA3-selective agonist, was more potent at the P2Y5 receptor than LPA 18:1 (Yanagida et al., 2009). P2Y5 in heterologous expression systems has been found to couple to $G_{12/13}$ and $G_s$, whereas its ability to elicit Ca$^{2+}$ transients is disputed (Yanagida et al., 2009). D-sn1-1-O-oleyl-2-O-methyl-glyceryl-3-phosphothionate (OMPT), a previously proposed LPA3-selective agonist, was more potent at the P2Y5 receptor than LPA 18:1 (Yanagida et al., 2009). P2Y5 in heterologous expression systems has been found to couple to $G_{12/13}$ and $G_s$, whereas its ability to elicit Ca$^{2+}$ transients is disputed (Yanagida et al., 2009). Of note is the observation that transgenic mice overexpressing lipid phosphate phosphatase-1 (LPP1/PAP2a), an ectoenzyme thought to degrade LPA, show hair growth defects that is consistent with LPP1-mediated degradation of LPA in the skin (Yue et al., 2004). The physiological and pathophysiological functions of P2Y5 other than in hair growth will have to be addressed in future studies.

P2Y10 was de-orphaned as an LPA-activated receptor by the Fujita group, using the same in silico process that led to their discovery of GPR87’s responsiveness to LPA (Murakami et al., 2008). The P2Y10 gene is localized to region 21.1 of the X chromosome near the gene for the LPA$_t$ receptor. LPA$_t$, LPA$_s$ and GPR87/LPA$_s$ show high $56\%$, $52\%$ and $45\%$ homology to P2Y10, which is similar to the 45% amino acid sequence identity among LPA$_t$, LPA$_s$ and LPA$_t$. Transcripts for this putative LPA receptor were detected in the uterus, prostate, heart, lung, placenta and skeletal muscle. P2Y10 is transcriptionally regulated by PU.1 and Spi-B of the Ets transcription factor family in B cells (Rao et al., 1999) and also expressed in platelets (Amisten et al., 2008). P2Y10 have been expressed functionally in Flp-In™ CHO cells either alone or as a G16 fusion construct. In the stable transfectants, LPA and S1P evoke Ca$^{2+}$ transients with EC$_{50}$ values of 53 nM and 130 nM respectively. Empty vector-transfected or G16-transfected CHO cells do not show Ca$^{2+}$ transients in response to S1P, and their endogenous LPA response is augmented in its size with a dose–response shifted to the left. These authors found no activation by dihydro-S1P or sphingosylphosphorylcholine or other lysophospholipids. Kitamura et al. (Kitamura et al., 2009) expressed P2Y10 in NIH3T3 and HEK293 cells and found that transfectants show morphological changes and TNFα secretion in response to lyso-phosphatidyl serine (LPS); the latter was inhibited by Y-27632, an inhibitor of the RhoA-ROCK signaling axis. These cells were highly responsive to LPA and S1P, and these lipids elicited similar responses in P2Y– and mock-transfected cells. In our hands, P2Y10-CHO cells respond to LPA > S1P > LPS (Figure 4), which is consistent with the findings of the Fujita and Aoki groups. GPR34, another P2Y family GPCR, has been recently de-orphaned as a receptor for LPS (Sugo et al., 2006). As for GPR87, also for P2Y10: additional studies will be needed to establish whether LPA is their most potent endogenous ligand. During the redaction of this article, Oka et al. (2010) reported that GPR35 responds to LPA with a unique preference for sn2-substituted LPAs. The rank order of ligand efficacy at GPR35 was sn2-18:2 > sn2-18:1 > sn2-20:4 > sn1-18:1. GPR35 activates the Rho GTPase and the ERK1/2 mitogen activated kinase. Kynurenic acid at concentrations $\equiv 100\ \mu M$ activated GPR35.
**Intracellular actions of LPA**

Identification of its many GPCR shifted the interest to the mediator role of LPA while many early reports on its intracellular actions became ignored (Tsai et al., 1989; Sando and Cherthihin, 1996; Yang et al., 2000; Chemin et al., 2005). LPA is generated intracellularly through several pathways. Glycerol-3-phosphate acyl transferase (GPAT) links fatty acyl-CoA with glycerol-3-phosphate to yield LPA. The metabolic fate of fatty acids taken up into cells goes through this important lipid biosynthetic pathway. In cells with the ability to take up fatty acids, this LPA production pathway might have an important regulatory role through activation of the nuclear hormone receptor PPARγ. PPARγ plays an essential role in regulating lipid and glucose homeostasis (Evans, 2005), as well as cell proliferation (Mueller et al., 1998), apoptosis (Elstner et al., 1998), and inflammation (Ricote and Glass, 2007). These responses have a direct impact on human diseases, particularly diabetes (Lehmann et al., 1995), atherosclerosis (Li et al., 2000) and cancer (Sarraf et al., 1998). Synthetic agonists of PPARγ include the thiazolidinedione (TZD) class, widely used to treat type 2 diabetes. Physiological agonists of PPARγ include 15d-PGJ2 (Forman et al., 2001), modified fatty acids (Baker et al., 2005) (Nagy et al., 1998), oxidized phospholipids (Davies et al., 2001) and select forms of LPA (McIntyre et al., 2003). The structure-activity relationship of PPARγ’s activation by LPA differs from that of the GPCR (Tsukahara et al., 2006). Whereas LPA GPCR are activated by both saturated and unsaturated fatty acid-substituted LPAs, PPARγ is only activated by unsaturated LPA. LPA GPCR do not show stereoselective responses to the S- and R-isomers of LPA; in contrast, PPARγ is only activated by the S-stereoisomer. Furthermore, with the exception of LPAs, the alkyl analogues of LPA are less potent agonists of the remaining LPA GPCR; however, 1-O-alkyl glycerophosphate is considerably more potent than LPA at PPARγ (Zhang et al., 2004; Tsukahara et al., 2006). The apparent Kd of 1-O-octadecenyl glycerophosphate binding was 60 nM in the PPARγ ligand binding domain, which is similar to that of rosiglitazone at 40 nM (Tsukahara et al., 2006). Some of the residues required for activation of PPARγ by 1-O-octadecenyl glycerophosphate differ from those required by rosiglitazone. When mutated to alanine, histidines 323 and 449 within the ligand-binding domain of PPARγ failed to abolish binding to and activation by 1-O-octadecenyl glycerophosphate but completely abolished the binding of and activation induced by rosiglitazone. Alanine mutation of arginine 288 significantly reduced binding and activation induced by 1-O-octadecenyl glycerophosphate but not those induced by rosiglitazone. Mutation of tyrosine 273 to phenylalanine abolished binding to and activation by both agonists (Tsukahara et al., 2006).

What is the physiological role of PPARγ activation by LPA? LPA is a key intermediate of fatty acid metabolism. Fatty acids taken up by cells are activated to form acyl-CoAs. Acyl-CoAs can be targeted for β-oxidation by conversion to acyl-carnitine or converted to LPA by different isoforms of GPAT. The resulting LPA can feed either phospholipid synthesis or fuel triacylglycerol synthesis and lipid storage. The LPA formed by GPAT can up-regulate PPARγ target genes involved in lipogenesis, lipid storage and adipocytic differentiation. In a pathophysiological context, LPA might be a key factor in non-alcoholic fatty liver disease (Nagle et al., 2009; Wendel et al., 2009). Thus, LPA might be a molecular link between fatty acid uptake and lipid storage. This hypothesis, although plausible, awaits experimental proof.

Another mechanism regulated by LPA-mediated activation of PPARγ is arterial wall remodelling, which can lead to plaque formation. All major cell types of the arterial wall respond to LPA. In endothelial cells, LPA has been shown to regulate the expression of adhesion molecules and stimulate proliferation, apoptosis, permeability, motility and cell-to-cell contacts responsible for transendothelial permeability (Rizza et al., 1999; Lin et al., 2006). LPA induces vascular smooth muscle cell (VSMC) contraction, proliferation (Tokumura et al., 1994) and phenotypic transdifferentiation in vitro (Hayashi et al., 2001; Zhang et al., 2004). LPA and oxidized low-density lipoprotein (LDL) inhibit macrophage/dendritic cell egress across endothelial cell monolayers (Angeli et al., 2004; Llodra et al., 2004). LPA also stimulates the formation of platelet-monocyte aggregates, which are considered an early marker of acute myocardial infarction (Fueller et al., 2003; Siess, 2006).

Yoshida et al. were the first to test the effects of lumena lly applied LPA on non-injured arterial wall in vivo (Yoshida et al., 2003). These authors infused LPA through the external carotid artery of rats into a ligated section of the common/internal carotid artery that was previously rinsed free of blood and maintained near the mean arterial perfusion pressure. In this model, which involves no mechanical injury or removal of endothelial cells within the common carotid artery, a 1-hour exposure to unsaturated but not to saturated species of LPA at low micromolar concentrations leads to neointima development. The structure-activity relationship of neointima induction does not match that of the
known LPA GPCR but matches that for PPARγ activation by LPA. PPARγ has long been implicated in atherogenesis (Diep and Schiffrin, 2001; Li and Glass, 2002). GW9662, a specific irreversible antagonist of PPARγ (Leesnitzer et al., 2002), completely abolished 1-O-octadecenyl glycerophosphate-, LPA- and rosiglitazone-induced neointima formation in the rat model (Zhang et al., 2004). In addition to this pharmacological evidence, studies conducted with LPA<sub>18:2</sub> and PPARγ conditional knockout mice confirmed the requirement of PPARγ and not LPA<sub>18:2</sub> GPCR for arterial wall remodelling in the non-injury model (Cheng et al., 2009). Several investigators have reported that systemic and chronic administration of rosiglitazone attenuates neointima in models with mechanical injury of the arterial wall, which differs from findings reported using the non-injury model (Lim et al., 2006; Lee et al., 2009). When 1-O-octadecenyl glycerophosphate was applied to an injured carotid artery, neointima formation was augmented, whereas rosiglitazone attenuated it. These observations underscore the differences between the non-injury and injury-induced models. It is important to note that circulating LPA bound to carrier proteins such as albumin is unable to activate PPARγ due to limited internalization of the lipid-protein complex (Zhang et al., 2004). Thus, the source of LPA is ether intracellular or although the uptake of lipid particles such as oxidized LDL (Siess et al., 1999; Zhang et al., 2004). The role of LPA in regulating vascular wall physiology is a very promising area for future research.

The LPA-PPARγ axis is also involved in mast cell and dendritic cell differentiation (Bagga et al., 2004; Leslie et al., 2008). Bagga et al. have shown that LPA caused mast cell hyperplasia by inducing the proliferation and maturation of these cells. These cellular responses are under dual control of LPA<sub>18:2</sub> GPCR and PPARγ as they were partially inhibited by GW9662, pertussis toxin or VPC-32179. Dendritic cells play an important role in antigen presentation. Serum lipids promote the expression of CD1d antigen-presenting molecules and enhance the activation of immune responses mediated by CD1d-restricted T cells. LPA and cardiolipin were identified as the two active ingredients in serum (Leslie et al., 2008). CD1 expression in immature dendritic cells was under the transcriptional control of PPARγ. This finding raises the hypothesis that LPA can regulate antigen presentation by dendritic cells through the expression of CD1 molecules. Although not mediated by PPARγ, another important immunoregulatory role of LPA has been described by Kanda et al. (Kanda et al., 2008), who found high expression of ATX in the high endothelial venules of lymph nodes. LPA induces chemokinesis in T lymphocytes and could regulate their homing into the lymph node. These observations combined with the role of S1P in lymphocyte egress underscore the importance of lysophospholipids in lymphocyte trafficking and assign LPA and S1P to distinct steps, homing and egress respectively.

Lysophosphatidic acid is also produced in a spatially regulated fashion by the Ca<sup>2+</sup>-independent phospholipase A2 (iPLA2) at the leading edge of the migrating monocyte (Carnevale and Cathcart, 2001; Mishra et al., 2008). Cathcart et al. have shown that whereas cPLA2α generates arachidonic acid in the cytoplasm and localizes to the trailing edge in monocytes migrating towards MCP-1 chemokine, iPLA2 produces LPA and is localized to the pseudopods where actin polymerization is actively regulated. LPA GPCR have long been known to signal through the Rho-Rac-Cdc42 small GTPase-coupled pathways regulating the actin cytoskeleton (Ridley and Hall, 1992; 1994; Ridley et al., 1992; Ridley et al., 2004). However, there is compelling evidence for the interaction of LPA with actin-binding proteins, including gelsolin, formin, adseverin (Meerschaert et al., 1998; Goetzl et al., 2000b; Mintzer et al., 2006) and villin, the later of which is restricted to epithelial cells (Khurana et al., 2008; Tomar et al., 2009). LPA generation via iPLA2 in migrating cells could regulate actin capping, severing and polymerization through direct high-affinity interactions with these actin-binding proteins. The high-affinity interaction of actin binding proteins with phosphatidylinositol 4,5-bisphosphate (PIP2) has long been known among cell biologists. In this context, findings of the Khurana group are of importance (Khurana et al., 2008; Tomar et al., 2009). These authors showed higher affinity binding of LPA to villin (K<sub>d</sub> 22 μM) than that of PIP2 (K<sub>d</sub> 39.5 μM) and demonstrated the LPA displaced villin-bound PIP2 in vivo. They also provided evidence that LPA can regulate actin nucleation, depolymerization and capping, the latter of which is not affected by PIP2. LPA has been found to enhance Src-mediated phosphorylation of villin (Khurana et al., 2008; Tomar et al., 2009) and gelsolin (Meerschaert et al., 1998). This polarized production coupled with the direct interaction of LPA with members of the gelsolin-villin family proteins implicates it in the cellular compass mechanism underlying directional cell migration. Extracellularly applied LPA induces cell polarization, directional motility and metabolic burst in human neutrophils (Chettibi et al., 1994). It is intriguing to speculate how iPLA2 could be redistributed to the leading edge of the cell exposed to a chemokinetic gradient and...
whether extracellular LPA signals originating from LPA GPCR through an inside-out mechanism could feed back into the regulation of iPLA2 and the cellular compass (Figure 5).

Lysophosphatidic acid can also be produced intracellularly by PLD from lysophosphatidyl choline and potentially be a source of ligand for intracellular and/or cell surface LPA receptors (Xie et al., 2002).

Autotaxin – how to target it in vivo?

In extracellular biological fluids, LPA production is linked to ATX, which is a lysophospholipase D that cleaves the headgroup from lysophospholipids to generate LPA. ATX is a member of the nucleotide pyrophosphatase/phosphodiesterase (NPP) family and is also known as NPP2. ATX was originally identified as a secreted phosphatase in melanoma culture supernatant that promoted the motility of cancer cells (Stracke et al., 1992). Ten years after its original discovery, ATX was identified as a lysophospholipase D responsible for the production of LPA in serum (Umez u-Goto et al., 2002; Tokumura et al., 2002a). Knocking out ATX leads to embryonic lethality due to a defect in blood vessel formation (van Meeteren et al., 2006; Tanaka et al., 2006) and a lack of large lysosomes in the yolk sac visceral endoderm cells (Koike et al., 2009). This latter defect is due to a lack of LPA-mediated constitutive activation of the Rho-ROCK and LIM kinase pathways. Conditional knockout of ATX in the nervous system causes a neural tube defect that can be corrected in explants by the addition of LPA in the culture medium (Fotopoulou et al., 2010). Neural tube closure involves LPA-mediated activation of the hypoxia-inducible factor HIF-1α. In the developing nervous system, ATX also regulates the differentiation of oligodendrocytes (Dennis et al., 2005; 2008; Yuelling and Fuss, 2008; Nogaroli et al., 2009). In adult mice, the level of ATX affects haemostasis and thrombosis (Pamuklar et al., 2009).

Many cancers secrete ATX, which contributes to their invasive properties. Gene copy numbers increase in ovarian cancer in chromosomal region 8q24, which also contains the genes encoding the Myc oncogene (Dimova et al., 2006). This raises the possibility that the ATX gene might be amplified in ovarian cancer. Ectopic expression of ATX in mice has recently been shown to lead to the development of chronic mastitis, hyperplasia, mammary intraepithelial neoplasia, and invasive and metastatic tumours (Liu et al., 2009). Ovarian cancer cells produce high levels of LPA in tumour ascites (Baker et al., 2002; Sutphen et al., 2004). ATX inhibits paclitaxel-induced apoptosis in breast cancer cells.
(Samadi et al., 2009), and LPA renders ovarian cancer cells chemoresistant to cisplatin and adriamycin (E et al., 2009). ATX is overexpressed in patients with recurrent disease after prior treatment with chemotherapy (Jazaeri et al., 2005). An siRNA screen has identified ATX as a candidate drug-resistance gene in ovarian cancer (Vidot et al., 2010).

Autotxin shows product feedback inhibition, meaning that LPA and S1P inhibit its activity (Durgam et al., 2005; van Meeteren et al., 2005). This feedback inhibition of ATX by LPA has been proposed to be a factor in maintaining plasma levels of LPA in the low nanomolar range (van Meeteren et al., 2005). However, the rate of ATX production seems to be an overriding factor because ATX knock-out heterogeneous mice have half the normal plasma level of LPA (van Meeteren et al., 2006; Tanaka et al., 2006). Nevertheless, the search for ATX inhibitors is intensifying due to the potential therapeutic applicability of such compounds in cancer therapy (Clair et al., 2005; Baker et al., 2006; Ferry et al., 2008; van Meeteren et al., 2008; Parrill and Baker, 2008; Parrill et al., 2008; North et al., 2009; Zhang et al., 2009a). As proof of this concept, we and others have shown that CCPA analogues inhibit ATX-mediated melanoma invasion in vitro and metastasis in vivo (Baker et al., 2006; Uchiyama et al., 2007). However, inhibition of ATX does not lead to an anti-tumour effect, as cancer cells continue to proliferate although their metastasis is reduced. Zhang et al. (Zhang et al., 2009a) have developed alpha-bromophosphonate analogues of LPA that inhibit ATX activity and also are antagonists of LPA1,2,3,4,5 GPCR and showed that these dual-action compounds not only reduce breast cancer metastasis but also inhibit tumour growth. Hence, the challenge now is to combine receptor antagonism with the inhibition of ATX to obtain anti-tumour and anti-metastatic drug candidates. Pure ATX inhibitors also face another challenge; intravenously injected recombinant ATX is very rapidly >10-min cleared within the liver sinusoids (Jansen et al., 2009). This rapid clearance might also apply to endogenous ATX. Another new direction is to explore agonists of LPA4 and LPA5 that also inhibit ATX. LPA4 inhibits invasion and migration elicited by the EDG-family LPA receptors LPA1 and LPA2 (Lee et al., 2008). Hence, selective agonists of this receptor subtype that inhibit ATX might prove to be useful in limiting cancer invasion and metastasis (Baker et al., 2006; Williams et al., 2009). LPA receptors of the EDG family are dysregulated in ovarian cancer. In a study with 30 ovarian cancer patients, LPA1 expression was decreased, whereas LPA2 and LPA3 were increased (Murph et al., 2008) in the ovarian tissue. siRNA-mediated knock-down of LPA2 and LPA3 in SKOV and OVCAR-3 ovarian cancer cell lines reduced aggressiveness and increased survival after xenografting (Yu et al., 2008).

LPA in malignant transformation, cancer metastasis, and radiation- and chemo-resistance

Lyosphosphatic acid is a mitogen, motogen and anti-apoptotic agent, all of which combined provide survival advantages to cells that generate LPA and utilize it in an autocrine or paracrine fashion. In the last decade, many reports have confirmed that several types of cancer cells secrete ATX, which in turn generates LPA in the immediate vicinity of the cell, so long as lyosphosphatidylcholine is available. Biological fluids are rich in lyosphosphatidylcholine. Perhaps the best-established example for the role of LPA in cancer pathobiology is ovarian cancer. Ovarian cancer ascites has highly elevated levels of LPA (Xu et al., 1995; Baker et al., 2002; Sutphen et al., 2004) due to increased ATX expression (Dimova et al., 2006). Ovarian cancers predominantly express LPA4 receptors, whereas this receptor is barely detected in ovarian epithelial cells (Goetzl et al., 1999a). In addition to LPA2, LPA4 is highly expressed in normal ovarian tissues (Noguchi et al., 2003). LPA4 might play an important role in the aggressive behaviour of ovarian cancer in at least two ways. First, LPA4 promotes the production of vascular endothelial growth factor (VEGF), urokinase (uPA) and matrix metalloproteinases (MMP) (Zebrowski et al., 1999; Hu et al., 2001; Huang et al., 2004; So et al., 2004; 2005). LPA increases VEGF production, and VEGF in turn up-regulates ATX production, which increases LPA levels (Ptaszynska et al., 2008) – a potential feed-forward loop. LPA-induced uPA production promotes invasion by several ovarian cancer cell lines (Pustilnik et al., 1999; Gil et al., 2008). uPA levels carry a prognostic value in ovarian cancer and correlate with poor prognosis (Schmalfeldt et al., 2001; Murthi et al., 2004). LPA conveys resistance to chemo- and radiation-therapy through its anti-apoptotic action (Fang et al., 2000; Deng et al., 2007; E et al., 2009). The LPA2 receptor plays a unique role in chemoresistance, mediated through its C-terminal interaction with the thyroid receptor-interacting protein 6 (TRIP6), the pro-apoptotic transcription factor Siva-1 and the PSD-95/Disc-large/ZO-1 domain (PDZ domain)-binding proteins NHERF2 and MAGI-3 (Yamada et al., 2005; Lin et al., 2007; Zhang et al., 2007a,b; E et al., 2009). LPA2 binds Siva-1 through a-C, C11,XXC motif and the complex becomes poly-ubiquitinated and degraded by the proteasome, which leads to the attenuation of the DNA-damage-
induced apoptosis. Siva-1 has also been shown to bind and sequester the anti-apoptotic BCL-XL protein and promote the progression of apoptosis via the mitochondrial pathway. LPA-induced molecular complex between LPA2 and Siva-1 plays a major role in the anti-apoptotic effect of LPA. LPA2 can form a ternary complex with TRIP6 and NHERF2 in such a way that TRIP6 and NHERF2 also attach to each other. This ternary complex is formed via the −347DSTL PDZ motif of LPA2 and the PDZ2 domain of NHERF2. NHERF2 homodimerizes with another NHERF2 via its PDZ1 binding domain, leaving the second PDZ2 domain available to bind the −TTDC PDZ motif of TRIP6. TRIP6 also binds to the −CXXC motif in LPA2 via its LIM3 domain completing the ternary complex (Figure 6). The ternary complex is required for the full activation of ERK1/2 and AKT kinases and through them essential for LPA-induced protection against radiation- and DNA-damage-induced apoptosis. LPA2-associated macromolecular signalosomes have been detected in ovarian cancer cells and for this reason also represent novel targets for prevention of therapeutic resistance of cancer (Figure 6). MMPs represent yet another group of important effectors of LPA in cancer cells (Fishman et al., 2001; Meng et al., 2004; So et al., 2004; 2005; Hope et al., 2009). LPA up-regulates MMP production, which in turn promotes the proliferation of cancer cells and invasion. Increased MMP production correlates with poor prognosis of ovarian cancer (Davidson et al., 1999a,b; Schmalfeldt et al., 2001). In view of the mitogenic, motogenic, pro-invasive and anti-apoptotic effects of LPA, receptor antagonists and ATX inhibitors offer potential for anti-cancer therapeutics.

**LPA in the nervous system**

The LPA1 receptor was originally identified from neuronal progenitor cells in the ventricular zone of the developing brain (Hecht et al., 1996). LPA is important in myelination and Schwann cell survival (Weiner et al., 1998; Moller et al., 1999; Li et al., 2003; Nogaroli et al., 2009). Perhaps the most profound morphological effect of LPA is on the development of cortical folds that lead to the development of gyri in the cerebral cortex (Kingsbury et al., 2003; Estivill-Torrus et al., 2008; Matas-Rico et al., 2008). There are several reviews dedicated to the topic of LPA in the brain, and the reader is referred to those for a more in-depth coverage of this exciting aspect of LPA developmental neurobiology (Birgbauer and Chun, 2006; Herr and Chun, 2007; Choi et al., 2008; Rivera and Chun, 2008; Noguchi et al., 2009).

One significant therapeutic effect of LPA appears to be its mediator role in neuropathic pain.
Intrathecally injected lysophosphatidylcholine (LPC) has been shown to elicit neuropathic pain and cause demyelination (Wallace et al., 2003; Inoue et al., 2008b). These effects of LPC require ATX; and the bioactive compound is LPA, the product of ATX-mediated LPC hydrolysis (Inoue et al., 2008b; Ahn et al., 2009). LPA initiates neuropathic pain and regulates the production of pain-related molecules through the LPA1 receptor (Inoue et al., 2004; 2008a,b; Ueda, 2006; Fujita et al., 2007; Xie et al., 2008). Recently, Ueda et al. proposed a feed-forward loop that involves LPA3 receptor-mediated production of lysophosphatidylcholine, which supplies the substrate to ATX, rich in the cerebrospinal fluid. These authors showed that LPA produced by ATX in turn can lead to LPA1-mediated neuropathic pain (Ma et al., 2009).

LPA1 up-regulates the voltage-gated Ca$^{2+}$ channel in the dorsal root ganglion cell, through a Rho-Rho kinase-coupled mechanism, causes demyelination of the fibres originating from the ganglion cells and leads to the reorganization of Aβ-fibres (Inoue et al., 2004; Fujita et al., 2007; Xie et al., 2008). In addition to the LPA1 receptor, LPA3 has also been implicated in neuropathic pain, although the mechanism responsible remains to be elucidated (Sheardown et al., 2004; Kinloch and Cox, 2005). Antagonists targeting LPA1 and LPA3 receptors might provide new ways to treat this currently difficult-to-manage neurological condition. Perhaps more than a curiosity is the fact that B lymphocytes from patients with bipolar disorders are hyper-responsive to LPA (Wasserman et al., 2004; Perova et al., 2008; 2009); and in patients with schizophrenia, LPA1 is among 18 genes that show close association with the disease (Bowden et al., 2006).

**LPA in bone metabolism**

LPA1 knockout mice show decreased bone density, shorter bones (J.P. Salles – personal communication) and a craniofacial dysmorphic phenotype due to abnormal development of the frontal bones of the skull (Contos et al., 2000). This abnormality might represent only one manifestation of defects in osteoclast and osteoblast function regulated by the LPA1 receptor. LPA has been identified as a mitogen for osteoblasts (Caverzasio et al., 2000; Grey et al., 2001; Lyons and Karin, 2001); it prevents osteoblast apoptosis (Grey et al., 2002) and stimulates alkaline phosphatase expression (Dziak et al., 2003) and cell migration. Osteocytes develop a mechanosensory network within the bone matrix and communicate through gap junctions established at their dendritic termini. LPA is a potent stimulator of dendrite outgrowth, which is blocked by the LPA1/LPA3 antagonist Ki16425 and pertussis toxin (Karagiosis and Karin, 2007). Resting zone chondrocytes generate LPA, which in turn promotes osteoblast differentiation, proliferation and survival (Hurst-Kennedy et al., 2009). Osteolytic metastasis of breast cancers also utilizes LPA as a mediator. LPA1 regulates the secretion of IL-6 and IL-8, which are potent bone resorption stimulators. Silencing expression or utilizing pharmacological inhibition of LPA1 in cancer cells reduces bone metastasis progression. Future pharmacological manipulation of LPA receptors might provide opportunities to prevent bone loss and reduce the bone metastasis of cancers.

**LPA in reproductive disorders**

The effects of LPA on cells from the female reproductive system have been known since the late 1980s. We previously identified and purified LPA as a serum factor based on its activation of oscillatory Ca$^{2+}$ currents in Xenopus oocytes (Tigyi et al., 1990; Tigyi and Miledi, 1992). LPA enhances embryonic development from the pronuclear stage to the blastocyst stage in mice (Kobayashi et al., 1994). In luteal cells, LPA stimulates type V adenyl cyclase, which is important for the maintenance of pregnancy (Budnik and Mukhopadhyay, 1997). This response is likely to be mediated through LPA4, which, in contrast to the EDG family receptors, elevates cAMP and is highly expressed in the ovary (Noguchi et al., 2003). LPA stimulates contraction in uterine smooth muscle cells (Tokumura et al., 1980) and increases embryo transport in the oviduct of mice (Kunikata et al., 1999; Tokumura et al., 1999). In the follicular fluid, LPA concentration is as high as 25 nmol·mL$^{-1}$, which is nearly double its serum concentration of c. 15 nmol·mL$^{-1}$ (Tokumura et al., 1999). The LPA3 receptor, which is up-regulated by progesterone and down-regulated by oestrogen, is required for embryo implantation and affects embryo spacing in the womb (Ye et al., 2005; Hama et al., 2007). In view of its effects on implantation, it is plausible that LPA is involved in placental pathologies, including non-receptive endometrium, decidualization, embryo crowding, placenta previa and placenta acreta, any of which could lead to sterility or loss of pregnancy (Ye, 2008). There are some selective LPA3 antagonists available that include diacylglycerol pyrophosphate (Fischer et al., 2001), VPC12449 (Heise et al., 2001) and Ki16425 (Ohta et al., 2003). However, these antagonists also inhibit LPA1. Recently, a specific non-lipid antagonist, NSC161613, has been identified showing an IC$_{50}$ of 24 nM (Fells et al., 2008).
LPA1/2/3 are highly expressed in the male reproductive system. Mice deficient in all three receptors show a testosterone-independent reduction of mating activity and sperm production, with an increased prevalence of azoospernia in aging animals. These triple knockouts show a significant increase in germ cell apoptosis, leading to a reduction in germ cell proliferation (Ye et al., 2008). Similar azoospernia and reduced germ cell numbers were found in transgenic animals that overexpress lipid phosphate phosphatase, an enzyme that degrades LPA (Yue et al., 2004). There is a wealth of evidence that links LPA, LPA3, and LPA4 to the regulation of prostate cancer cells (Qi et al., 1998; Prenzel et al., 1999; Im et al., 2000; Daaka, 2002; Guo et al., 2006; Hwang et al., 2006). In contrast, little is known about the physiological role of LPA in the normal prostate. Selective ligands of the LPA3 receptor would likely offer novel drug candidates to modulate both male and female reproduction.

LPA in vascular pathologies

In 1978, Tokumura et al. reported that soy lipid extract contains a vasopressor lipid that they identified as LPA (Tokumura et al., 1978a; Tokumura et al., 1978b). LPA had hypertensive effects in rats and guinea pigs and hypotensive effects in cats and rabbits. Schumacher et al. discovered that factors developed during ‘aging’ of heparinized plasma kept at 36°C for 18–24 h and that these factors activated platelet aggregation and caused constriction of pulmonary vessels (Schumacher et al., 1979). These authors identified LPA as being one of the active factors and noted that it caused desensitization of the platelet response, a hallmark of GPCR action. Simon et al. discovered that the alkyl-ether analogue of LPA is a potent activator of human platelets at concentrations as low as 10⁻¹⁰ M, making this analogue nearly as potent as platelet-activating factor (Simon et al., 1982; 1984). The barrier function of vascular endothelial cells is altered by LPA (Schulze et al., 1997; Alexander et al., 1998; English et al., 1999), and LPA induces the expression of VCAM-1 and E-selectin on the surface of cultured human endothelial cells (Rizza et al., 1999). LPA induces endothelin-1 production in vitro and in vivo (Chua et al., 1998; Yakubu and Leffler, 1999). LPA is a mitogen for VSMC (Tokumura et al., 1994) and also promotes their phenotypic dedifferentiation from a contractile phenotype to a secretory phenotype (Hayashi et al., 2001; Yoshida et al., 2003). Topical application of unsaturated LPA species into the non-injured carotid artery of rodents induces arterial wall remodelling, and this response requires PPARγ but not LPA1 or LPA2 (Hayashi et al., 2001; Yoshida et al., 2003; Zhang et al., 2004; Cheng et al., 2009).

Lysophosphatidic acid is required for normal vascular development, as indicated by the embryonic lethality of ATX knockout mice at a stage when vascular stabilization begins (van Meeteren et al., 2006; Tanaka et al., 2006). In adult mice, ATX overexpression leads to haemorrhages due to an inhibitory effect of LPA on platelets, whereas ATX ± heterozygotes, which have a reduced plasma LPA concentration, are more prone to thrombosis (Pamuklar et al., 2009). LPA and ATX have been implicated in wet-type macular degeneration by promoting angiogenesis in the retina.

Nanomolar concentrations of LPA and minimally oxidized LDL induce shape change of washed platelets through LPA receptor-linked signal transduction pathways that presumably involve the activation of the heterotrimeric G₁₂/G₁₃ protein, the small GTPase Rho and Rho kinase, the Rho kinase-mediated inhibition of myosin light-chain phosphatase, and stimulation of myosin light-chain phosphorylation (Bauer et al., 1999; Retzer and Essler, 2000; Retzer et al., 2000). It seems that there are two types of LPA receptors that regulate LPA responsiveness in human platelets. One subtype inhibits platelet responses by elevating cAMP levels, which in turn attenuates activation, as proposed by Pamuklar et al. (Pamuklar et al., 2008). These authors ascribed this effect to LPA4. Khandoga et al. could not detect elevation in cAMP in LPA-treated human platelets (Khandoga et al., 2008). However, these authors found that the structure-activity relationship, characterized by the preference for alkyl over acyl LPA analogues, is similar to that of LPA₅, which makes this receptor a candidate for the activating LPA receptor subtype in platelets (Williams et al., 2009). Williams et al. identified two non-lipid antagonists of LPA₅, H2L 5987411 and H2L 5765834, that also blocked platelet activation by LPA (Williams et al., 2009). The former compound but not the latter is also a weak antagonist of LPA₄ (Kₐ 741 nM), yet both inhibited platelet responses to LPA. LPA₄ and LPA₅ transcripts are both abundant in human platelets mixed from multiple donors (Amisten et al., 2008). Tokumura et al. described human donors whose platelets failed to respond to alkyl glycerophosphate while maintaining normal responsiveness to acyl analogues of LPA (Tokumura et al., 2002b). This observation lends support to the hypothesis that multiple receptors are involved in platelet responses to LPA.

Lysophosphatidic acid also activates haptotactic migration in monocytes/macrophages (Zhou et al., 1995), inhibits the egress of dendritic cells from the vessel wall (Llodra et al., 2004), and promotes the formation of monocyte/platelet aggregates that are
an early marker of acute myocardial infarction (Fueller et al., 2003). Acyl and alkyl LPA accumulate in human atherosclerotic plaques and likely activate platelets and initiate thrombus formation upon plaque rupture (Siess et al., 1999; Rother et al., 2003). LPA production and the LPA receptor subtypes expressed by different subsets of blood cells and cells of the vessel wall are undoubtedly important potential drug targets for manipulating pathological angiogenesis and thrombosis.

**LPA as a mediator of organ fibrosis**

Lysophosphatidic acid has long been known for its mitogenic effect on fibroblasts (van Corven et al., 1989; 1992). In addition to being a mitogen, LPA increases connective tissue growth factor production, which promotes tissue fibrosis (Hahn et al., 2000; Muehlich et al., 2004; Vial et al., 2008). LPA induces the expression and activation of the CIC-3 chloride channel that is required for myofibroblast dedifferentiation during wound healing (Wang et al., 2002; Yin and Watsky, 2005; Yin et al., 2008). In light of these findings, it is perhaps not surprising that LPA has been implicated in renal (Pradere et al., 2009), hepatic (Watanabe et al., 2007a,b) and pulmonary fibrosis (Tager et al., 2008; Xu et al., 2009). In several models, inhibition of the LPA₁ receptor inhibits the progression of fibrosis (Pradere et al., 2007; Tager et al., 2008), but LPA-induced αVβ6 integrin-mediated TGF-β activity is mediated via the LPA₂ receptor (Xu et al., 2009). Tager et al. showed that LPA levels increase in bronchoalveolar lavage fluid following lung injury in the bleomycin model of pulmonary fibrosis and that mice lacking LPA₁ are protected from fibrosis in this model. These investigators showed that patients with idiopathic pulmonary fibrosis, had increased LPA levels in bronchoalveolar lavage fluid and that inhibition of LPA₁ reduced fibroblast responses to the chemotactic activity of the lavage fluid. A recent study using plasma and serum samples from systemic sclerosis patients found elevated LPA and S1P levels. Furthermore, serum LPA:LPC ratios of the 18:2 and 20:4 molecular species, and also the ratio of all species combined, were significantly higher in systemic sclerosis patients versus controls (Tokumura et al., 2009). Currently, a diagnosis of organ fibrosis leaves the physician with very limited therapeutic options that often come with severe side effects. Exploration of the role of LPA- and LPA receptor antagonist-based therapies might offer an entirely new therapeutic avenue of treatment.

**LPA in infection and immunity**

Activation of the RhoA GTPase and phospholipase D-dependent acidification of phagolysosomes can have profound effects on viral and bacterial entry into cells and also on the intracellular killing of bacteria. The cysteine-rich protein 61 (CYR61) and connective tissue growth factor (CTGF) regulate adhesion, migration, extracellular matrix deposition and cell differentiation and play a role in wound healing. Bacterial lipid extracts derived from *Yersinia, E. coli, Pseudomonas aeruginosa, Enterococcus faecalis* or *Staphylococcus aureus* induce expression of CYR61 and CTGF in epithelial cells. Wiedmaier et al. have shown that Ki16425, a selective antagonist of LPA₁/3 receptors (Ohta et al., 2003), inhibits Cyr61 and CTGF expression (Wiedmaier et al., 2008). LPA-induced CYR61 and CTGF mRNA expression requires Rho GTPases because it is abolished by *Clostridium difficile* toxin B, which inhibits RhoA and Rac-1. These authors raised the hypothesis that LPA GPCR could be involved in sensing bacterial lipid products, thereby regulating the host’s response to infection via CYR61 and CTGF expression.

Tsurudome et al. reported that LPA promotes cell-cell fusion in parainfluenza virus-infected Vero cells (Tsurudome et al., 2008). This process was sensitive to the Rho kinase inhibitor Y-27632. Thus, LPA, at least theoretically, could promote the cytopathic effect of parainfluenza virus infection. This hypothesis awaits experimental testing. Inhibition of RhoA activation elicited by respiratory syncytial virus infection is implicated in the antiviral mechanism of the macrolide antibiotics bafilomycin and clarithromycin (Asada et al., 2009). RhoA activation is associated with entry and exocytosis of viruses, and LPA might synergize with these events and also attenuate the antiviral efficacy of macrolide antibiotics.

Recent reports assign anti-microbial activity to LPA in *Mycobacterium tuberculosis* infection (Garg et al., 2006; Greco et al., 2010). LPA and S1P exert a cytotoxic effect in *Mycobacterium tuberculosis*-infected type II alveolar cells and activate PLD, which in turn leads to phagolysosome maturation, leading to mycobacterial killing and inhibition of bacterial dissemination in vitro (Greco et al., 2010). Garg et al. have shown that LPA enhances antimycobacterial activity in vitro and *ex vivo* in cells derived from the bronchoalveolar lavage of patients with tuberculosis. LPA activated PLD-dependent acidification of the phagolysosomes in these cells that had been chronically infected with endogenous mycobacteria in the lungs of the patients. The molecular target(s) of LPA underlying its antimycobacterial effect remain to be identified. None-
Nevertheless, LPA is known to activate PLD (Qi et al., 1998; Zhao et al., 2005) through a Ca\textsuperscript{2+}-dependent mechanism. Furthermore, ATX is important for normal lysosomal morphology and function (Koike et al., 2009). These exciting studies on the role of LPA in infection are at a very early stage; hence, it is difficult to predict whether LPA or LPA-based pharmacons might have therapeutic value in infection control.

The role of LPA in immune cells cannot be underestimated, and research in this field will likely lead to therapeutically important new discoveries. T lymphocytes (Goetzl et al., 2000a), B cells (Rosskopf et al., 1998; Satoh et al., 2007; Perova et al., 2008), eosinophils (Idzko et al., 2004), neutrophils (Chettibi et al., 1994), macrophages (Hornuss et al., 2001), mast cells (Bagga et al., 2004) and dendritic cells (Panther et al., 2002; Llodra et al., 2004; Chan et al., 2007) express functional LPA receptors. Natural killer cells respond to LPA with increased IFN\gamma production and chemotaxis (Jin et al., 2003; Maghazachi, 2003; Jo et al., 2008), although it was also found to inhibit their cytotoxic response through a cAMP-PKA-dependent mechanism. Lipopolysaccharide treatment of T\textsubscript{H}1 lymphocytes increases ATX expression that can supply LPA upon contact of the T cell with other immune cells (Li and Zhang, 2009). These findings lend support to the role of LPA in innate as well as acquired immunity. In the preceding sections, we have already described some effects of LPA on mast cell and dendritic cell differentiation and the regulation of T cell homing regulated by ATX-LPA GPCR and PPAR\gamma (Bagga et al., 2004; Llodra et al., 2004; Kanda et al., 2008; Leslie et al., 2008; Nakasaki et al., 2008). LPA has been shown to stimulate proliferation, migration and survival of T cells and macrophages (Koh et al., 1998; Goetzl et al., 1999b; Zheng et al., 2000; 2001). LPA regulates chemokine and cytokine production by immune cells that include IL-1\beta, IL-2, IL-8, IL-13, TNF\alpha, MCP-1 and MIP-1\beta (Zheng et al., 2000; Lee et al., 2002; Panther et al., 2002; Gobeil et al., 2003; Lin and Boyce, 2005; Rubenfeld et al., 2006). Perhaps the most compelling evidence for the immunomodulatory role of LPA comes from Leslie et al. (Leslie et al., 2008), who showed that it differentially regulates the development of group 1 (CD1a, CD1b and CD1c positive) and group 2 (CD1d) dendritic cells. LPA, in a completely reversible PPAR\gamma-dependent mechanism, inhibited the transcription and cell surface expression of group 1-specific CD1 markers, and conversely increased CD1d, involved in lipid antigen presentation by dendritic cells to T cells. Group 1-reactive T cells display a cytotoxic phenotype and secrete T helper type 1 cytokines, suggesting their role in microbial infection (Brigl and Brenner, 2004). CD1 molecules are also involved in T cell recognition of self-lipids (Leslie et al., 2002; Vincent et al., 2002; 2003). Thus, CD1-restricted T cells mediate not only host defense mechanisms but also pro-inflammatory responses in human autoimmune diseases. Including systemic lupus erythematoses (Siegel et al., 2000), multiple sclerosis (De Libero et al., 2002) and autoimmune thyroiditis (Roura-Mir et al., 2005). Based on these reports, LPA through its effect on dendritic cell maturation might promote the expansion of T helper 2 type cells relative to T helper 1 cells (Panther et al., 2002; Leslie et al., 2008), which has far-reaching implications for the physiological regulation of autoimmunity and host defense.

**Conclusion**

In recent years, there has been an explosion in the number of reports describing the numerous actions of LPA, making it impossible to write a completely up-to-date and comprehensive review of this rapidly changing field. The objective of the present article was not to be comprehensive but to highlight only certain aspects of the LPA story. The reader is referred to the many excellent and more in-depth reviews on specific aspects of LPA biology. Our aim in this article was to generate additional interest for this exciting and novel field of lipid research.

Lysosphatidic acid, with its pleiotropic effects and simple structure, provides an enticing opportunity for therapeutic exploration. LPA engages targets both as a mediator and a second messenger in almost every cell type in the body. The many LPA receptors represent an unparalleled redundancy of cellular signalling. The multiplicity of cell surface and intracellular LPA targets/receptors, often co-expressed in the same cell type and coupling to overlapping signal transduction pathways, poses an unmet challenge to designing LPA-based therapeutics.

A fundamental problem often neglected is the endogenous expression of LPA receptor transcripts in every mammalian cell line. Although there are a few cell lines that do not respond to LPA with Ca\textsuperscript{2+} responses, these cells still express low levels of LPA GPCR transcripts and show endogenous signalling responses to LPA (Valentine et al., 2008b). Functional expression of LPAR of the purinergic subcluster often requires plasmids with strong promoters such as pCXN2.1 (Niwa et al., 1991) and/or co-expression of promiscuous G
protein α-subunits (Gα16) due to low coupling efficiency of some LPAR. However, overexpression of Gα16 can increase the coupling efficiency of the endogenous LPAR and introduce Ca^{2+} transients into RH7777 and B103 cells even in the absence of heterologous LPAR (Valentine and Tigyi, unpublished). This complicates the characterization of the novel LPAR and emphasizes the need for ‘cleaner’ assay platforms (Wetter et al., 2009).

The S1P field has surpassed the LPA field in therapeutic exploration because the S1P receptor agonist FTY720 is now near completion of phase III clinical trials for the treatment of multiple sclerosis, whereas no LPA-based therapeutic has advanced this far. What might be the reason for such disparity between the two lysophospholipid fields? Perhaps one key difference lies in the obligatory role of S1P; in lymphocyte egress. Thus far, no matching (patho-)physiologically essential cellular response has been linked to a single LPAR, although the role of LPA3 in embryo implantation offers some degree of similarity. Thus, the greater redundancy and overlapping signalling pathways that prevail among the LPAR families might be the reason that no LPA-based drug has emerged thus far. Perhaps, the redundancy of LPA signalling is an indicator of the fundamental necessity of this mediator/second messenger for normal cell function. Indeed the S1P receptor knockout mouse is the only embryonic lethal lysophospholipid receptor knockout, whereas none of the LPAR knockouts cause embryonic lethality. This contrasts with the ATX knockout, which also causes embryonic lethality and indicates the essential requirement for LPA during development. More thorough characterization of LPAR knockouts under different pathophysiological challenges will likely pinpoint distinct and unique functions associated with a single or a select few LPAR.

The field still lacks drug-like compounds with the desired specificity or ubiquity for LPA targets. Currently, identified lipid-like LPA analogues lack the necessary potency and selectivity, and they have suboptimal pharmacokinetic profiles. With the identification of the novel LPAR, the selectivity and specificity of the previously developed and presumed ‘selective’ pharmacons begs further characterization. From now on, any new compound will have to be tested on the nine established and putative LPAR and the five S1P receptors. While the opportunity to control LPA-mediated pathophysologies is apparent, harnessing the LPA system through pharmacological tools remains a stimulating task for the academic community and a lucrative opportunity for the pharmaceutical industry.

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Conflicts of interest

The author is a shareholder in RxBio Inc.

References


Novel LPA targets


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