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Review

Lysophosphatidylinositol signalling: New wine from an old bottle

Roberto Piñeiro, Marco Falasca *

Queen Mary University of London, Barts and The London School of Medicine and Dentistry, Blizard Institute, Centre for Diabetes, Inositide Signalling Group, London E1 2AT, UK

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ABSTRACT

Lysophosphatidylinositol (LPI) is a bioactive lipid generated by phospholipase A2 which is believed to play an important role in several diseases. Indeed LPI can affect various functions such as cell growth, differentiation and motility, in a number of cell-types, including cancer cells, endothelial cells and nervous cells. Despite the fact that LPI-induced cellular functions had been known for more than twenty years, the recent discovery that in several cell-types the orphan G protein-coupled receptor GPR55 acts as the specific receptor for LPI has fuelled novel interest in this lysolipid. Different research groups, including our own, have recently suggested that LPI may be the specific and functional ligand for GPR55, triggering signalling cascades that are relevant to cell proliferation, migration, survival and tumourigenesis. Recently published data suggest that the LPI/GPR55 axis plays an important role in different physiological and pathological contexts. Here we review the available data supporting the role of LPI in cell signalling and the pharmacology of its putative receptor GPR55.

1. Introduction

Lysophospholipids (LPLs) are glycerophospholipids or sphingolipid products of the enzyme phospholipase A (PLA) (Fig. 1). This enzyme belongs to a family of esterases that hydrolyze fatty acid esters on sn-1 or sn-2 of membrane phospholipids (PLs), generating free fatty acids and LPLs. Like the PLs, LPLs are also components of the plasma membrane but present in smaller amounts [1]. Initially it was believed that LPLs were only capable to modulate the function of membrane proteins such as membrane ion channels, by changing the mechanical properties of these bilayers. Indeed, based on their structure and therefore on the shape, the accumulation of LPLs can alter the spontaneous curvature of the membrane (monolayer) and therefore affect the different conformational status of membrane proteins such as ion channels [2,3]. Subsequently, LPLs were considered as intermediate metabolites in phospholipid homeostasis and later as second messengers capable to modulate intracellular signalling events. Interest on LPLs increased since their role as signalling molecules was discovered as well as the existence of specific receptors on the cell surface able to regulate a wide range of physiological processes such as inflammation, reproduction, angiogenesis, tumourigenesis, atherosclerosis and nervous system regulation. The majority of studies on LPLs are mainly focused on lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P). These two LPLs are generated by enzymatic cleavage of stores of glycerophospholipids and sphingomyelin respectively and are associated with a wide range of cellular actions. LPA for instance has been shown to mediate cell proliferation and migration, platelet aggregation, smooth muscle contraction, actin stress fibres formation, cytokine and chemokine secretion and to prevent apoptosis [4], whereas S1P regulates cell movement, differentiation, survival, inflammation, angiogenesis, calcium homeostasis and immunity [5]. Nevertheless, there is an increasing evidence that other LPLs such as lysophosphatidylinositol (LPI) and lysophosphatidylcholine (LPC), may also play a crucial role as biologically active lipid mediators and are capable of initiating a variety of cellular responses in diverse cell types.

The intent of this review is to discuss current knowledge about the role of LPI in multiple aspects of intracellular signalling and the impact of these pathways on different diseases.

2. First discoveries in the lysophospholipids field

The interest on LPLs started in the late 80s, when a seminal paper was published reporting a biological action for LPA. In this study Moolenaar and his co-workers revealed that LPA derived from phosphatidic acid (PA) was able to act as mitogenic stimulus inducing cell proliferation [6]. The mitogenic activity of PA was previously reported by these authors and others in quiescent normal fibroblasts [7–9], but this study showed that among different PA-derived lipids, only LPA exerts a mitogenic effect for quiescent normal cells [6]. It is also noteworthy that the mitogenic effect of LPA was observed in a wide range of cells, including normal and transformed cells. This paper

* Corresponding author at: Inositide Signalling Group, Centre for Diabetes, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, 4 Newark Street, London E1 2AT, UK. Tel.: +44 2078828243; fax: +44 2078822186.
E-mail addresses: ropici@hotmail.com (R. Piñeiro), m.falasca@qmul.ac.uk (M. Falasca).

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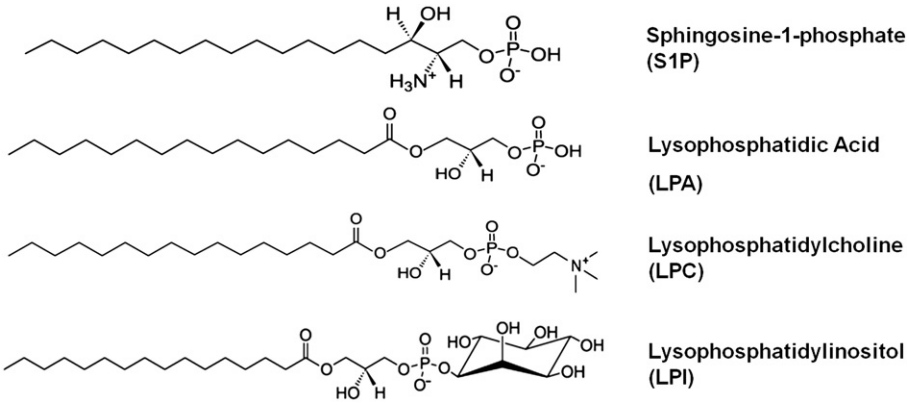

Fig. 1. Lysophospholipids structure.

represented one of the initial demonstrations that LPLs can act as intracellular messengers with biological functions rather than being just structural components of the plasma membrane playing a role in membrane plasticity; and represented the beginning of the research in a field that has hugely developed in the last two decades.

Very soon after the publication of this paper another report came out showing the effect on DNA synthesis and cell proliferation of the sphingolipid sphingosine (SP) on quiescent Swiss 3T3 fibroblasts via a mechanism independent on protein kinase C (PKC) [10]. This effect was shown to be accompanied with an increase in the concentration of the very potent mitogen PA in the cytoplasm of Swiss 3T3 fibroblasts [11]. Following this study data were reported suggesting that sphingosine (SP) is enzymatically converted inside the cell to sphingosine 1-phosphate (S1P) [12]. The authors demonstrated that in permeabilised DDT1MF-2 smooth muscle cells, sphingosine administration was able to mobilise Ca^{2+} from intracellular stores, and they suggested that this effect was mediated by S1P generated from sphingosine conversion. This study showed again evidences supporting the function of LPLs as intracellular second messenger. This was further confirmed by another report showing that the addition of mitogenic concentrations of SP to Swiss 3T3 cells stimulates PA synthesis, and increased the formation of S1P [13]. Moreover, addition of S1P was sufficient to stimulate the proliferation of quiescent Swiss 3T3 cells and to induce mobilisation of Ca^{+2} from intracellular stores. These early data indicated that these membrane lipid derivates can act co-ordinately on a common intracellular second messenger system or network regulating biological processes. For instance it was demonstrated that the addition of S1P to quiescent Swiss 3T3 fibroblast was accompanied by an increase in the concentration of PA which correlated with the effect on DNA synthesis. Therefore, S1P was presented as a mediator of the effect of SP on PA accumulation and as a candidate to regulate cellular proliferation by affecting multiple transmembrane signalling pathways [14].

An important development in LPLs research was achieved with the discovery of specific LPL receptors on the plasma membrane. The existence of a cell surface receptor activated by LPA and coupled to G proteins was suggested very early [6]. This was demonstrated by evidence showing that LPA induced its mitogenic stimulus through activation of certain G proteins. Results obtained using pertussis toxin suggested that G_i was crucial for the LPA mitogenic effect [6]. In this respect, evidence showed that S1P could also stimulate proliferation of Swiss 3T3 fibroblast through activation of mitogen-activated protein kinases (MAP kinases) in a mechanism involving G proteins, in particular G_i/G_o proteins [15]. Here the existence of a receptor at the plasma membrane able to bind S1P was suggested, and it was also proposed that such receptor must be different from the LPA-bound receptor, since the physiological responses mediated by these lipids were clearly different [15].

LPA and S1P are found circulating in blood plasma, indicating that they can be secreted by cells. Indeed, they have been both shown to be released from activated platelets [16,17]. This was the first identified source of these bioactive lipids in humans; currently it is known that LPA can also be released by other cell types such as adipocytes, peritoneal mesothelial cells, and fibroblasts; and S1P is secreted by epithelial cells, cerebellar granule cells, cerebellar astrocytes and thrombocytes [18]. The identification and characterisation of the different LPA and S1P receptor isoforms together with evidence of LPA and S1P synthesis and secretion by diverse cell types indicate that LPA and S1P can act as extracellular signalling molecules, as well as intracellular mediators, that regulate diverse cellular functions.

3. Lysophospholipid receptors: GPCRs

The first evidence of the existence of specific receptors for LPLs was reported in 1992, with the identification of a putative LPA membrane receptor in various LPA responsive cell lines [19]. It was suggested, that the membrane protein able to bind LPA was a member of the superfamily of seven-transmembrane-domain receptors, the G protein-coupled receptors (GPCRs) [19]. Similarly, it was reported that S1P activated a receptor triggering Rho-mediated cytoskeletal changes in the neuroblastoma cell line NIE-115 cells [20]. Interestingly, the conclusions of this work suggested that S1P and LPA may be part of a group of bioactive LPLs possibly acting through a family of GPCRs. The first LPA receptor was identified in 1996 as a GPCR, and it was named as ventricular zone gene-1 (vzg-1) [21]. This receptor is now named as LPA_1 (Edg2) and it is one of at least six GPCRs binding LPA. Subsequently, many other GPCRs have been described to be activated by LPLs (Table 1 lists GPCRs of the LPLs discussed in this review), and given the wide distribution of these lipids in tissues and cells, and their involvement in a very different range of physiological and pathophysiological processes, it is likely that the number of GPCR transducing LPLs signals will increase. As far as SIP is concerned, five mammalian GPCRs ($S1P_{1-5}$) have been identified so far [22] (Table 1). The aminoacidic sequence reveals around 50% of identity among the LPA receptors, and a homology of about 35% between the LPA and SIP receptors [23]. Another common feature between the LPA and S1P receptors is the high expression levels and distribution in diverse organs and cell types where they regulate a wide range of functions.

The characterisation of some of these receptors started back in the 90s when the first receptors for LPA and for S1P were identified. As discussed above LPA is a ligand for at least six different receptors: LPA_{1-6}. These receptors are involved in processes such as proliferation and survival (LPA_1 and LPA_2), brain development (LPA_1), T-cells activation (LPA_3) and stress fibre formation (LPA_4 and LPA_5); and some of them are ubiquitously expressed (LPA_1, LPA_2 and LPA_3) [24–26]. S1P binds

Table 1
G protein-coupled receptors associated to lysophospholipids.

Receptor	Ligand	Signalling	Main expression	References
LPA_1 (Edg2)	LPA	G_i, G_q, $G_{12/13}$	Brain, heart, gut, kidney, stomach	[24–26,127]
LPA_2 (Edg4)	LPA	G_i, G_q, $G_{12/13}$	Heart, lung, gut, stomach, brain	[24,25,127,128]
LPA_3 (Edg7)	LPA	G_i, G_q	Testis, kidney, lung, brain	[25,128–130]
LPA_4 (P2Y9, GPR23)	LPA	G_q, $G_{12/13}$	Uterus, ovary, placenta, platelets, mesenchymal cells	[131–133]
LPA_5 (GPR92)	LPA	G_q, $G_{12/13}$	Small intestine, peripheral nervous system dorsal root ganglia	[134,135]
LPA_6 (P2Y5)	LPA	G_q, G_s, $G_{12/13}$	Henle and Huxley layers of scalp hair follicles	[26,136]
$S1P_1$ (Edg1)	S1P	$G_{i/o}$	Brain, lung, spleen, cardiovascular system and kidney	[25,137–140]
$S1P_2$ (Edg 5)	S1P	$G_{i/o}$, G_q, $G_{12/13}$, G_s	Widely expressed	[25,137,138,140]
$S1P_3$ (Edg 3)	S1P	$G_{i/o}$, G_q, $G_{12/13}$, G_s	Cardiovascular system, lungs, kidney, intestines, spleen, and cartilage	[137,140–142]
$S1P_4$ (Edg6)	S1P	$G_{i/o}$, $G_{12/13}$, G_s	Thymus, spleen, bone marrow, appendix, and peripheral leukocytes	[138,143,144]
$S1P_5$ (Edg 8)	S1P	$G_{i/o}$, $G_{12/13}$	Brain, spleen, white matter tracts, oligodendrocytes, skin, nervous tissue	[138,145–147]
GPR119	LPI	G_s	Pancreas, gastrointestinal tract and brain	[69]
GPR55	LPI	$G_{i/o}$, G_q, $G_{12/13}$	Brain, gastrointestinal tract, testis, adrenal glands, dorsal root ganglion, cancer cells, endothelial cells	[27,39,116,124]

mainly to five different GPCRs ($S1P_{1-5}$), although its ability to bind to other receptors has been reported (GPR3, GPR6, GPR12 and GPR45). The receptors $S1P_{1-5}$ are involved in processes such as migration ($S1P_1$, $S1P_2$ and $S1P_4$), proliferation ($S1P_1$, $S1P_4$ and $S1P_5$), survival ($S1P_1$ and $S1P_5$), angiogenesis ($S1P_1$) and vascular development ($S1P_3$); similar to LPA receptors some of them are ubiquitously expressed ($S1P_1$, $S1P_2$ and $S1P_3$). These five receptors, together with LPA_{1-3}, belong to the Edg (Endothelial Differentiation Gene) family, and both subfamilies share a 35% of identity at the amino acid level. With regard to LPI, one receptor, G protein-coupled receptor 55 (GPR55), has been described to be the functional receptor of this lipid. GPR55 is a member of the rhodopsin family of GPCRs and is involved in processes such as migration, proliferation, bone physiology and inflammatory and neuropathic pain, and in terms of expression it is mainly found in regions of the CNS, adrenal glands and gastrointestinal tract [27]. GPR55 has been initially proposed as a cannabinoid receptor responsible for cannabinoid responses not mediated by the classical cannabinoid receptors type 1 and type 2 (CB1 and CB2) [28], however its classification as a cannabinoid receptor has been recently reconsidered [28,29]. Indeed, both pharmacology and signalling data of GPR55 are quite distinct compared to classical cannabinoid receptors as well as its structure homology to CB1 and CB2. Furthermore, despite the fact that certain endocannabinoids, phytocannabinoids and synthetic cannabinoids are GPR55 receptor agonist or antagonist LPI is the most consistent and potent agonist characterised so far [30]. Nevertheless, it is worth to note that a final consensus has not been reached on whether GPR55 is a LPI-specific receptor or the putative cannabinoid receptor CB3.

4. Lysophosphatidylinositol (LPI)

4.1. LPI biology and history

Although much less characterised than LPA and S1P, the lysophospholipid LPI has been shown to be present in different range of cells (Table 2) and to be involved in very different processes. In the earliest studies, LPI was considered as a secondary product of the arachidonic acid pathway upon PLA_2 agonist stimulation. Very little was known about the biological function of LPI. The first evidence showing a possible physiological role for this LPL came with two papers published in the mid-80s showing a stimulatory effect of LPI in the release of insulin by pancreatic islets [31,32]. Soon after that, four papers reported that transformation of NIH 3T3 fibroblasts and cell lines derived from the rat thyroid line FRTL5 cells with the oncogene Ras, a key regulator of growth and differentiation, increased the activity of the enzyme PLA_2 and resulted in accumulation of arachidonic acid and glycerophosphoinositol [33–36]. We then reported for the first time that transformation of epithelial thyroid cells with Ras led to the synthesis and accumulation of high levels of LPI [37].

Table 2
Cell types producing LPI.

Cell type	Species	References
Platelets	Human	[40]
BALB/3T3 fibroblasts	Mouse	[41]
FRT-Fibro (thyroid fibroblasts)	Rat	[89]
Endothelial cells	Human, porcine and bovine	[63,100–104]
RAW 264.7 macrophages	Mouse	[148]
Adrenal capillary endothelial (BACE) cells	Bovine	[99]
Peripheral blood neutrophils	Human	[149]
Ovarian cancer cells	Human	[93–95]
Dorsal root ganglion X neurotumour hybrid, F-11	Mouse and rat hybrid	[150]
PC-3 prostate cancer	Human	[68]

The accumulation of LPI as a consequence of the malignant cell transformation, together with previous demonstrations showing the mitogenic effect of LPA [6], prompted us to investigate a possible effect of LPI as a regulator of cell proliferation. Our work clearly identified LPI as a potent mitogenic factor [37,38]. A clear limitation on LPI research at this stage, contrary of other LPLs such as LPA and S1P, was that a receptor for this LPI was not known, even though evidence clearly suggested the existence of a specific receptor for LPI on the plasma membrane [37,38]. First, the synergistic effect of LPI and insulin in promoting cell proliferation on thyroid cells FRTL5 suggested the involvement of a tyrosine kinase receptor. Second, LPI increased in a dose-dependent manner the incorporation of [^{3}H]thymidine and cell number of FRLT5 cells and FRLT5 cells transformed with Ras (kiki cells), whereas LPA did not have the same effect on kiki cells. This evidence ruled out the possibility that LPI shared the same receptor as LPA or that the mitogenic effect of LPI in FRLT5 cells was due to its conversion to LPA. Third, concentrations of LPI that stimulated cell proliferation also induced an increase in cytosolic Ca^{2+} levels in a reversible manner [37]. A receptor for LPI was finally identified in 2007 when it was reported that LPI, and not other LPLs, induced ERK1/2 phosphorylation through the activation of GPR55 [39]. The discovery that GPR55 may be the specific and functional receptor for LPI has fuelled novel interest on the biological role of this LPL in diverse cell functions. Indeed, from the first evidence for a biological role of LPI in insulin release and cell proliferation, many other functions for this lipid are now known (Table 3), and they will be discussed in more detail below.

4.2. LPI synthesis

LPI is mainly generated by the action of PLA_2 that catalyses the hydrolysis of phosphatidylinositol (PI) at the acyl group on ester position sn-2 [40] (Fig. 2). This hydrolysis generates LPI and free arachidonic acid (AA), which is important for the synthesis of ecosanoids and prostaglandins. PLA_2 has been shown to be involved in conversion of PI to LPI in platelets, stimulated either with thrombin or ionophore A23187 [40]. These results corroborated earliest evidence indicating

Table 3
LPI effects.

Activity	Model	References
Insulin release	Pancreatic islets	[31,32]
Proliferation	FRTL5 epithelial thyroid cells	[37,38]
Intracellular calcium mobilisation	Endothelial cells, hGPR55-HEK293, hepatocytes, rat mesenteric arteries, k-ras transformed thyroid cells, pancreatic islets, DRG neurones, PC-3	[38,39,61,63,68,72,74–77,80,118]
PLA_2 activation	k-ras transformed thyroid cells	[38]
PLC activation	k-ras transformed thyroid cells	[38]
Store-operated Ca^{2+} entry (SOCE)	Rat astrocytes, rat coronary smooth muscle cells (SMCs), cerebellar granule neurones	[49–51]
TRP channels activation	CHO, PC-3	[70,78,79]
Exocytosis	Neuroendocrine cells (PC12)	[65]
K^+ channels	Neurones, endothelial cells	[58,60,66,151]
Migration	MDA-MB-231, PC-3, smooth muscle cells (SMC), neutrophils, endothelial cells (inhibition)	[70,84,85,87,88]
L-type calcium channel currents	Primary pituitary cells	[62]
ERK1/2 phosphorylation	hGPR55-HEK293, U2OS-GPR55, human osteoclasts, DU145, PC-3, OVCAR3	[39,61,68,114,152,153]
Akt phosphorylation	PC-3, DU145	[68]
p38 phosphorylation	hGPR55-HEK293, IM-9	[118]
Rho activation	hGPR55-HEK293, mouse osteoclasts, OVCAR3, DU145	[68,114,118,124,153]
Inhibition of osteoclast formation	Mouse and human osteoclasts	[114]
Transcription factors activation: NFAT, CREB, NF-κB, ATF-2	hGPR55-HEK293, IM-9	[118,124,152]

that LPI can be synthesised by PLA_2 activity in BALB/3T3 mouse transformed cells [41]. A study on rat liver and brain lysosomes suggested that both phospholipases PLA_1 and PLA_2 can be involved in the synthesis of LPI [42]. This idea was further supported by other studies reporting LPI synthesis by the hydrolytic activity of the Ca^{2+}-independent PLA_1 (Fig. 2) in plasma membranes from rat and bovine brain [43–45].

4.2.1. PLA_1

The PLA_1 is a family of enzymes that can be classified in extracellular and intracellular enzymes. The extracellular members have been shown to be involved in the synthesis of lysophosphatidylserine (LPS) and LPA. Among the intracellular members, PLA_1 DDHD domain containing 1 (DDHD1) is involved in the synthesis of LPI [46]. In HEK293 cells overexpressing DDHD1, this enzyme shows a PI–PLA_1 activity hydrolysing PI in response to ionomycin stimulation and generating LPI but also LPA, LPC and lysophosphatidylethanolamine (LPE). This PI–PLA_1 activity occurs naturally in the neuroblastoma cell line SK-N-SH which expresses DDHD1 endogenously. Very interestingly, the catalytic activity of this enzyme is modulated by PA, generated through phospholipase D (PLD) activation [46], indicating a PA–PLA_1 activity and suggesting the existence of a LPI regulatory system in neuroblastoma cells.

4.2.2. PLA_2

The PLA_2 enzymes are classified into three major categories: the $sPLA_2$ (secreted PLA_2), the Group IV calcium-dependent [2,3], $cPLA_2$ (cytosolic PLA_2) and the Group VI cytosolic $iPLA_2$ (calcium-independent PLA_2) [47]. The group IV calcium-dependent PLA_2 is the main responsible for LPI synthesis [40,41,48], although LPI has been described to be also a product of $iPLA_2$ [49–51]. The $cPLA_2$ has a very well characterised C2 domain which is involved in phospholipids binding as well as calcium ions binding, resulting in a calcium-dependent activation of the enzyme [52]. This enzyme is the most ubiquitously expressed of the group and its function is involved in a wide spectrum of cellular processes controlling cellular homeostasis. An important role for $cPLA_2$ has also been demonstrated in pathological conditions such as inflammation and cancer [53].

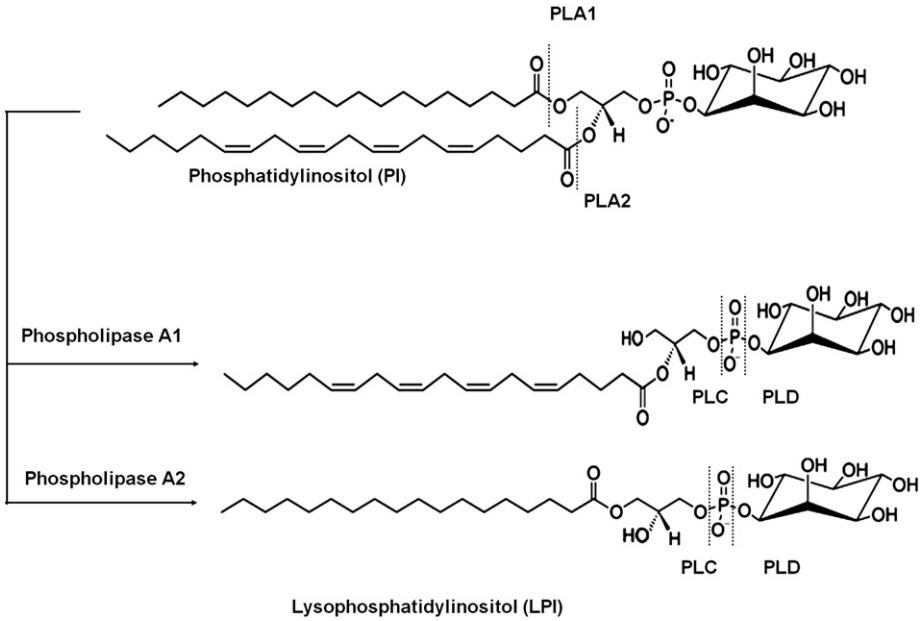

Fig. 2. Lysophosphatidylinositol synthesis and degradation: The phospholipases A1 and A2 synthesize LPI through the hydrolysis of phosphatidylinositol (PI) at the acyl group on ester positions sn-1 and sn-2. LPI can be further degraded by the action of the phospholipases C and D.

4.3. Degradation pathway of LPI: LysoPI-PLC and lysoPLD

In porcine platelets membranes, it was originally reported that part of LPI was deacylated on sn-1 by a lysophospholipase (lysoPLA), generating aqueous metabolites identified as glycerophosphoinositol [45]. In addition to this pathway of degradation or removal of LPI, these authors and others identified the activity of a phospholipase C (PLC) with specificity for LPI (Fig. 2). The specific isoform of PLC with affinity for the lyso form of PI, called lysoPI-PLC, was shown to degrade LPI in porcine platelets membranes and synaptic plasma membranes from rat brain [43,44,54,55]. The specificity of this enzyme for LPI seemed to be high since very little hydrolysis of LPC, LPE and LPS was observed [43]. This specific isoform is different from PI-PLC and seems to have hydrolytic activity at both acyl chains sn-1 and sn-2. The existence of this lysoPI-PLC suggests that LPI acts as an intermediate metabolite of the PI pathway at the level of synaptic membranes. Another enzyme involved in LPI metabolism is the lysophospholipase lysoPLD (Fig. 2). The LPI produced by activated platelets was demonstrated to be converted to LPA in a lysoPLD dependent manner [56]. Following platelets activation LPLs, and in particular LPI, can be released into the plasma where they are metabolised and converted to LPA by lysoPLD present in blood plasma and serum. This lysoPLD has been shown to be autotaxin [57], the main enzyme involved in LPA synthesis.

4.4. Biological activities of LPI

Our understanding of the biological activities of LPI is limited compared to the extensive literature gathered for LPA and S1P. The early evidence of LPI actions in the literature is a consequence of studies where LPI has been compared with other LPLs. The LPI biological activities can be divided as non-receptor and receptor-mediated.

4.4.1. Non-receptor mediated effects of LPI

As mentioned before, LPLs can modify the properties of membranes by altering membrane curvature. Based on this, the effects of LPLs as regulators of membrane proteins have been well characterised. A good example of this is represented by the demonstration that LPI can activate the 2-pore domain K^+ channels TREK-1 and TRAAK [58]. These channels are important regulators of synaptic function by regulating the neurone membrane potential [59], suggesting a potential role for LPI as a modulator of synaptic function at nervous system. The authors show the dependence of this effect on the length of the carbonyl chain and the presence of a large polar head but not on the charge of the molecule [58,59]. A similar study carried on TREK channels demonstrated that LPI also activates bTREK-1 and bKv1.4 K^+ currents [60]. The authors conclude that the activation of native bTREK-1 channels by LPLs does not seem to involve GPCR and suggest a direct interaction with the channel. These observations are interesting since it is known that brain tissue has considerable levels of LPI [61]. Another effect of LPI at the plasma membrane is the inhibition of L-type calcium channel currents in primary pituitary cells [62]. This effect is due to the partition of LPI in the plasma membrane and dependent on the negative charge of the polar head. In a similar way, LPI is involved in the regulation of Ca^{2+} channels at the plasma membrane and mitochondrial membrane. In endothelial cells, LPI stimulation induced the mobilisation of intracellular Ca^{2+} accompanied by an initial membrane hyperpolarisation and followed by a long lasting sustained depolarisation. The first two effects are receptor-mediated whereas the sustained depolarisation of the membrane is independent of the receptor, suggesting the possible interaction of LPI on the plasma membrane cation channels [63]. The depolarisation of the membrane is a consequence of intracellular Na^+ loading due to inhibition of Na/K-ATPase and direct activation of non-selective cation channels. LPI and other LPLs play also a role at the level of the mitochondrial membrane by inhibiting Ca^{2+} uptake and inducing Ca^{2+} release from the mitochondria through physical interactions with the lipid bilayer. This interaction is speculated to modify the mitochondrial membrane potential causing the final Ca^{2+} efflux [64]. The role for LPI at the level of the plasma membrane as a regulator of the exocytosis process in neuroendocrine cells has also been demonstrated [65]. External infusion of LPI in rat PC12 cells, but not LPC or LPS, induced vesicle fusion, clearly indicating exocytosis. This effect was shown to be dependent of intracellular Ca^{2+}. It is not clear whether this effect is receptor-mediated or due to the physical properties of the lipid. In addition to acting on the plasma membrane, LPI, as already described for LPA and S1P, can also function inside the cell, such as our reported effect on Ras GAP activity [65] or the modulator activity on Ca^{2+}-activated large-conductance K^+ channels in endothelial cells [65]. Indeed, it has been recently demonstrated a receptor-independent effect of LPI on the Ca^{2+}-activated K^+ channels single-channel activity of endothelial cells, suggesting that LPI may be a potent intracellular messenger capable of modulating electrical responses in the vasculature [66].

4.4.2. Receptor mediated effects of LPI

Accumulating data have now demonstrated that most of the biological effects of LPI are mainly mediated by the receptor GPR55. This receptor was cloned in 1999 as an orphan GPCR and it was identified as a receptor for LPI in 2007. In this original paper the authors overexpressed the human GPR55 in HEK293 cells and compared the potency of different ligands to activate the receptor. Among different LPLs tested, LPI was the only one able to induce ERK1/2 phosphorylation through GPR55 [39]. Consistent with this, LPI stimulation of HEK293 cells expressing GPR55 induced a transient increase in intracellular Ca^{2+} and stimulated the binding of [^{35}S]GTPs to the cell membranes. These actions were specific to LPI since none of the LPI degradation products had a stimulatory effect. This study represented the first clear demonstration of LPI as endogenous ligand for the receptor GPR55. The possibility that LPI can bind to other different GPCR receptors has also been suggested very recently [67,68]. Indeed, it was recently demonstrated that LPI, as well as other LPLs, can also activate GPR119 in RH7777 rat hepatoma cells stably expressing human GPR119 [69]. Moreover, an effect on prostate cancer cells migration induced by LPI and mediated by TRPV2 channel has been also described [70]. Therefore, these data strongly suggest that LPI may regulate very different cellular processes through the activation of diverse signalling pathways.

4.4.3. LPI and intracellular calcium

Ca^{2+} represents a key intracellular messenger for many signalling transduction pathways in the cells. This ion is a key player in the maintenance and function of a diverse number of physiological intracellular processes. LPI has a well established role in regulating intracellular Ca^{2+} homeostasis, and this effect has been related to processes such as insulin release from pancreatic islets, artery contraction and cell proliferation and migration [71]. Early data showed that LPI administration to hepatocytes induced Ca^{2+} mobilisation [72]. An effect of LPI on Ca^{2+} mobilisation has also been shown in rat liver mitochondria where LPI regulates calcium transport inducing both uptake and release of Ca^{2+} [64,73]. In rat mesenteric arteries LPI increases Ca^{2+} sensitivity of contraction upon stimulation with contraction agonist [74]. Moreover, LPI induces intracellular Ca^{2+} release in rat and mouse pancreatic islets and this action is related, at least in part, with promoting insulin secretion [75,76]. Although it has not been determined yet, the LPI-induced insulin release could be related with its capability to induce the exocytosis process as it has been very recently shown in PC12 neuroendocrine cells [65]. The exocytosis processes in these cells are Ca^{2+}-dependent, and it is believed that the increase in intracellular Ca^{2+} induced by LPI could trigger exocytosis. It is not clear whether this effect is mediated by GPR55, or by its physical properties. However, a LPI GPR55-mediated effect on calcium has been reported in endothelial cells [63,77]. LPI induces Ca^{2+} mobilisation

from intracellular stores and membrane hyperpolarisation. These effects are inhibited in presence of a GPR55 antagonist and in cells upon down regulation of GPR55. The mobilisation of cytosolic Ca^{2+} mediated by LPI has also been linked to cell proliferation. Stimulation of k-ras transformed thyroid cells with LPI leads to PLC activation, mobilisation of cytosolic Ca^{2+} and PLA_2 activation, resulting in a mitogenic stimulus [38]. Several studies have shown that LPI regulates Ca^{2+} influx via store operated Ca^{2+} entry channels in rat astrocytes, rat coronary smooth muscle cells and cerebellar granule neurones [49–51]. Specifically it was suggested that activation of $iPLA_2$, and therefore LPI synthesis, induces Ca^{2+} influx through SOCE at the plasma membrane of astrocytes and neurones [49,50]. Very recently, three studies have demonstrated the LPI-induced Ca^{2+} influx through channels belonging to the transient receptor potential (TRP) family. The first reports showed the activation of the transient receptor potential (melastatin)-8 (TRPM8) [78,79]. In Chinese hamster ovary (CHO) cells expressing mouse TRPM8, LPI evoked an increase in intracellular Ca^{2+} with no effect in untransfected control cells [78]. TRPM8 is a thermosensor activated by cold and mainly expressed in a subpopulation of small cold-sensitive dorsal root ganglion (DRG) neurones which also express GPR55; interestingly LPI is able to induce an increase in intracellular Ca^{2+} in these cells probably by binding GPR55 [80]. The third study shows that LPI induces a calcium influx via TRPV2 channel [70]. This effect was observed in CHO cells stably expressing mouse TRPV2, HEK293 cells expressing human TRPV2, and in the human prostate cancer cell line PC-3, which expresses TRPV2. Very interestingly, this effect was accompanied by an increase in the migration of PC-3 cells [70], therefore demonstrating the existence of a link between LPI-induced Ca^{2+} mobilisation and cell migration in cancer cells. We have recently shown an effect of LPI in mobilisation of intracellular calcium in PC-3, which is dependent on GPR55 activation and linked to cell proliferation [68]. Altogether these data demonstrate the role of LPI as a regulator of Ca^{2+} mobilisation and its role on diverse physiological and pathophysiological processes.

4.4.4. LPI effects on cell migration

Cell migration is a complex process involved in physiological and pathophysiological conditions. It is essential for normal embryonic development, function of immune system, inflammation, wound healing, and angiogenesis but it can also facilitate cancer progression and metastasis [81,82]. LPLs are well known regulators of cell migration, and their effect is exerted on several cell types. For instance, LPA and S1P regulate cell migration of endothelial cells, lymphocytes and fibroblasts, but also of ovarian and breast cancer cells. Similarly, evidence of a role for LPI in migration of both normal cells and cancer cells has been reported. For instance in sperm cells, LPI induces an increase in motility (sperm capacitation) and acrosome reaction, both processes involved in egg fertilisation [83]. Another pro-migratory effect of LPI has been reported in human coronary artery smooth muscle cells (SMC) where both LPC and LPI stimulate cell migration [84]. Interestingly, LPI can also inhibit cell migration. For instance another comparative study showed that the activity of LPI as a negative regulator of migration on endothelial cells was higher than LPC and other LPLs [85]. In addition to these data, it has been shown that LPI and other LPLs stimulate the expression of adhesion molecules on endothelial cells (EC) [86], such as VCAM-1 and ICAM-1 in rabbit aortic endothelial cells and human umbilical vein cells respectively [86]. Taken together, the evidence observed on EC suggests a role for LPI and LPLs as regulators of endothelium function.

It has been recently shown that LPI activation of GPR55 induces a directional migration of human peripheral blood neutrophils [87]. Interestingly, a coordinate mechanism has been unveiled between GPR55 and the cannabinoid receptor CB2, with synergistic activation of neutrophils Rho signalling and directed migration towards inflammatory sites.

As mentioned before, LPI can also regulate migration of cancer cells. Activation of TRPV2 channels by LPI (and also LPC) in PC-3 leads to an increase in cell migration [70]. LPI and LPC stimulation induces phosphoinositide 3-kinase (PI3K) activation, translocation of TRPV2 from cytoplasm to the plasma membrane and Ca^{2+} influx, resulting in an increase in cell migration. Based on these data, the authors indicate that the mechanism of action of LPLs is mediated by the action of a GPCR, although a specific receptor has not been identified. Indeed it was shown that the effect of LPC on Ca^{2+} was mediated by G_i and G_o, but the signalling for LPI was not determined. Migration of metastatic breast cancer cells is also regulated by LPI [88]. Stimulation of MDA-MB-231 cells with LPI increases the chemotaxis of these cells to 10% serum, however LPI itself did not have chemotactic effect. This migratory response was sensitive to cannabidiol (CBD), a well recognised GPR55 antagonist, indicating a potential role for GPR55 as the receptor mediating the effect. Moreover, LPI also affected the elongation and polarisation of breast cancer cells [88]. Both processes are intimately linked with cell migration which indicates that LPI induces a migratory phenotype on breast cancer cells. Taken possibly together these results suggest a possible regulatory role of LPI and GPR55 on cancer metastasis.

4.4.5. LPI and cell proliferation

The first indirect evidence of a role for LPI in cell proliferation came from a study showing that transformation of rodent fibroblasts with cytoplasmic and membrane associated oncogenes was followed by an intracellular increase on the levels of glycerophosphoinositol (GPI) [34]. GPI is generated by the enzyme PLA_2 as a result of the deacylation of LPI. Interestingly, the authors showed that the accumulated levels of GPI are correlated with cell proliferation and transformation, although indirectly, these data implied that LPI is a potential mediator in the cascade activated by oncogenes and therefore in the proliferation state of the cells. Subsequently, we were the first to demonstrate that LPI could act as a mitogen inducing cell proliferation. A study performed in FRTL5 differentiated epithelial thyroid cells showed that LPI increased [^{3}H]thymidine incorporation and cell number [37]. This result demonstrated that LPI itself is a mitogen. Moreover we demonstrated that, in a cell line stably transformed with the k-ras oncogene (KiKi cells) derived from the FRTL5 differentiated thyroid cells, the increased activity of the enzyme PLA_2 led to LPI synthesis [37]. The comparison of LPI levels between KiKi cells and the differentiated FRTL5 cells showed that the levels were increased in the k-ras transformed cell line. This increase in LPI is a consequence of the activation of enzymes involved in the Ras pathway, and not a consequence of transformation [89]. Interestingly, LPI also exerted a mitogenic effect in KiKi cells whereas LPA did not affect the incorporation of [^{3}H]thymidine of these cells. We also showed that LPI is able to potentiate the proliferative effect induced by insulin in FRTL5 cells [37] as reported for KiKi cells. LPI was detected in the extracellular medium of ras-transformed fibroblasts, indicating that these cells not only synthesise the lipid but they are also able to release it, as it was previously shown for LPA in stimulated platelets [16]. Almost undetectable levels of LPI were observed in normal fibroblasts. Similarly k-ras transformed KiKi cells, showed increased intracellular and extracellular levels of LPI. The mechanism by which these cells release LPI was unknown at the time. Recently we have reported that LPI export is mediated by the ATP binding cassette transporter ABCC1 (MRP1) in PC-3 cells [68] indicating that cancer cells have the ability to promote their proliferation by synthesising and releasing LPI. Taking together, these data indicate that LPI is a mitogen factor regulating the proliferation of normal and oncogene-transformed cells. Other authors have also reported the involvement of LPI in cell proliferation. NIH3T3 fibroblasts stably transfected with the phosphatidylinositol transfer protein alpha (PI-TPalpha), a protein involved in the transfer of phospholipids between membranes *in vitro*, have an increased rate of cell growth [90]. It was observed that the levels of LPI are increased in these cells, compared to wild type fibroblasts, due to the action of PLA_2. The authors suggested that LPI could be responsible for the increased rate of cell growth observed. Similarly, primary human dermal microvascular endothelial cells stimulated with LPI showed increased

proliferation through a mechanism involving the activation of GPR55. The role of GPR55 in endothelial function has been partially characterised and it has been related to processes such as endothelial tube formation and angiogenesis [91,92], which suggest that LPI is a regulator of endothelial function. The link between the axis LPI/GPR55 and cell proliferation has also been recently corroborated in cancer cells as discussed below.

4.5. LPI and cancer

Early data published by us in the 90s, suggested a potential role for LPI in cancer [37,89]. The fact that malignant transformation of epithelial thyroid cells and fibroblasts with the oncogene Ras led to the synthesis and release of LPI, together with the fact that LPI was a mitogen in these cells inducing cell proliferation, clearly suggested an involvement of LPI in Ras-dependent tumours. This was later supported by clinical evidences showing that patients with ovarian cancer, or peritoneal cancer had higher accumulated levels of LPI in blood and ascites than healthy controls [93–95]. Alongside LPI, levels of LPA were also found to be elevated in these patients. It is important to mention that the high levels of LPLs were not due to an increase in the total levels of phospholipids since no differences were found in total lipids in the ascites of these two populations. Therefore, LPLs and in particular LPI can be used for the diagnosis of ovarian cancer as they represent useful biomarkers for the disease [96,97]. As already discussed, LPI has a role in migration, orientation and polarisation of human breast cancer cells through GPR55 activation [88]. Effect on migration of the highly metastatic MDA-MB-231 cells, as well as MCF-7 cells overexpressing GPR55 was detected. Furthermore LPI activates TRPV2 channels and stimulates migration of PC-3 [70]; this report did not study the possible involvement of GPR55 although it is mentioned that the mechanism of action of LPI could be mediated by the action of a GPCR. Therefore, LPI has a stimulatory effect on the migration of cancer cells and this action is probably mediated by the activation of GPR55. Another strong link between LPI and cancer has been recently established. We have shown that LPI, together with GPR55, is involved in an autocrine loop regulating the proliferation of prostate and ovarian cancer cells [68] (see Fig. 3). In these cells, the enzyme $cPLA_2$ synthesises a pool of intracellular LPI that is released by the transporter ABCC1. Once in the extracellular media, LPI activates GPR55 and signalling cascades downstream the receptor stimulating cell proliferation. Mobilisation of intracellular Ca^{2+}, Akt activation and ERK1/2 phosphorylation downstream Rho/ROCK pathway were observed in ovarian cancer cells OVCAR3 and prostate cancer cells PC-3 and DU145, which express endogenous GPR55. Downregulation of either $cPLA_2$ or ABCC1 by siRNA leads to a decrease in the proliferation of PC-3 cells. Moreover, downregulation of GPR55 induces a complete inhibition of the proliferation of OVCAR3 and PC-3 cells, which cannot be resumed by addition of LPI. This fact suggests that these cells may contribute to increase the levels of LPI in the plasma of patients with prostate cancer, in the same way as it has been shown in patients with ovarian cancer [93]. It is then plausible to speculate that LPI may be used as a biomarker, at least for patients with ovarian and prostate cancer, or even for the prognosis of these cancers. Another group has reported the importance of GPR55 for cancer cells growth. Analysis of GPR55 expression on breast cancer tissues has shown a correlation between receptor expression, tumour aggressiveness, and proliferative index assessed by the percentage of Ki-67 positive cells [98]. Similar correlation has been found by analysing published microarray data on pancreatic tumours and glioblastomas. Overexpression of GPR55 in different cell lines from breast cancer, pancreatic adenocarcinoma and glioma enhanced cell viability [98]; and HEK293 cells overexpressing GPR55 showed an increase in proliferation dependent of $cPLA_2$, suggesting a role of LPI on proliferation. A role for GPR55 *in vivo* was also determined. Knock down of GPR55 in subcutaneous tumours generated in mice with T98G glioma led to a reduced growth of these tumours [98]. Taking together, these data indicate that LPI and its receptor GPR55 are key regulators of cell proliferation in many types of cancer [67,68,98]. There is no doubt that future work will shed further light into the role of LPI in several cancer types since GPR55 is expressed in many other human cancer cell lines such as neuroglioma, astrocytoma, melanoma, cervix adenocarcinoma, hepatocellular carcinoma, T cell leukaemia and B lymphoblastoid myeloma [98].

4.6. Other biological functions of LPI

4.6.1. LPI and endothelium

Given that ECs are able to synthesise and release LPI [99–104], and that these cells express the LPI receptor GPR55 [77], it is not surprising

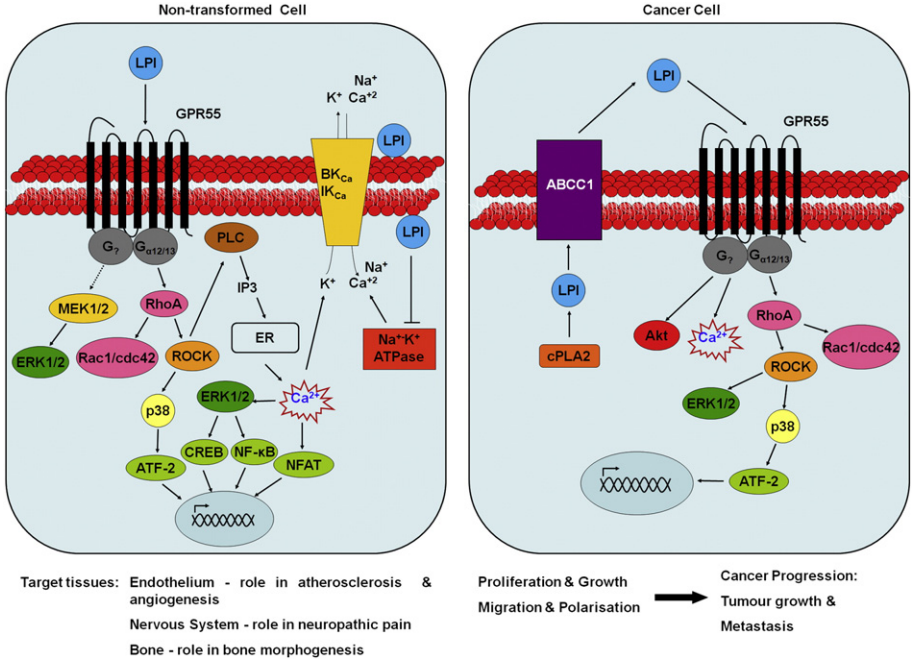

Fig. 3. Lysophosphatidylinositol signalling in non-transformed and cancer cells: LPI activates common intracellular targets downstream GPR55 involved in the regulation of diverse physiological and pathophysiological processes as depicted in non-transformed and cancer cells.

that this lipid has a crucial role in endothelial function. The effect of LPI as modulator of ionic homeostasis is well known. Micromolar concentrations of LPI activate GPR55 and induce an increase in intracellular Ca^{2+}, through large-conductance Ca^{2+} and voltage-gated potassium (BK_{Ca}) channels, a K^+ current and membrane hyperpolarisation [63]. LPI activates non-selective cation channels in a GPR55-independent way, resulting in intracellular Na^+ loading, and inhibition of the Na^+/K^+ ATPase [63,66]. The stimulatory effect of LPI on these BK_{Ca} channels has been previously reported in different studies [105,106]. Moreover, activation of the 2-pore domain K^+ channels TREK-1 and TRAAK by LPI has been reported in endothelial cells [58]. A potential role for LPI as regulator of angiogenesis and atherosclerosis has been suggested according to reports showing that the migration of ECs is negatively regulated by LPI [85], and that LPI induces the expression of adhesion molecules (VCAM-1 and ICAM-1) [86]. It is worth mentioning that the adhesion molecules are intimately related with the adhesion of circulatory monocytes to the endothelium, a critical step on the development of atherogenesis. Another link with this pathology has been established in a report showing that LPI has an inhibitory effect on endothelium-dependent hyperpolarisation induced by acetylcholine, a process mediating vascular relaxation [107]. This means that plasmatic LPI could be implicated in the development of endothelial dysfunction and atherosclerosis.

In agreement with these actions of LPI, GPR55 has been shown to be involved in angiogenesis. N-arachidonoyl serine (ARA-S), a pro-angiogenic factor, induces EC migration and tube formation through the activation of GPR55. Reduced expression of GPR55 by siRNA significantly inhibits these two processes [91,92]. Interestingly, when GPR55 was knocked down, in the absence of ARA-S, tube formation was also reduced, clearly suggesting a role for the receptor in angiogenesis; and more important, LPI stimulated the proliferation of endothelial cells [92]. Furthermore, GPR55 has been also suggested to be involved in angiogenesis as a mediator of the effects of cannabinoid ligands which cannot be explained through the activation of CB1 and CB2 receptors [77,108,109], although some contradictory data have been reported [110,111].

Although there are clear data indicating that LPI and GPR55 are regulators of endothelial function under physiological and pathophysiological conditions, further studies are needed to clarify their specific role.

4.6.2. LPI and the nervous system

Although the research in this field is not very extensive, evidence suggests a role for LPI in the nervous system. Brain tissue accumulates considerable levels of LPI (37.5 nmol/g tissue); and GPR55 is expressed at high levels in the central nervous system. Hitherto, LPI has been involved in neuropathic pain and cerebral ischemia.

4.6.2.1. LPI and pain. A role for LPI has been reported at the level of CNS. It has been shown that LPI stimulation increases intracellular Ca^{2+} in DRG neurones [80] and that LPI can activate TRPM8 channel, which is highly expressed in DRG neurones. Therefore LPI is able to modulate the function of this specific group of neurones, the large-diameter DRG neurones, that are involved in nociception, associated to states of neuropathic or inflammatory pain and which poses high levels of GPR55 [80]. The development of the GPR55 knockout mice ($GPR55^{-/-}$) has shed some light into this aspect [112]. Impairment in the response to models of inflammatory mechanical hyperalgesia achieved by the injection of Freund's complete adjuvant and partial nerve ligation has been described in these mice. This effect was attributed to the increase in the level of some anti-inflammatory and other cytokines observed in knockout mice [112]. The data clearly indicate that GPR55 could be a potential target to treat inflammatory and neuropathic pain.

4.6.2.2. LPI and cerebral ischemia. As reported before LPLs, and in particular LPI, can activate TREK and TRAAK K^+ channels. These channels have an important regulatory role on the control of neurone membrane potential [59], and it has been suggested that they can have a role in neuroprotection. These results suggest that LPI may also have a neuroprotective effect. It has been demonstrated that cultures of cerebral granules treated with LPI were protected against cell death induced by glutamate *in vitro* [113]. Moreover, a protective effect of LPI was seen in a model of cerebral ischemia *in vivo*. Injection of LPI 30 min before induction of ischemia resulted in a strong inhibition of neurone loss. In the same line, LPI prevented the death of hippocampal neurones induced by transient global forebrain ischemia [113]. The authors suggest that these neuroprotective effects may be mediated by the action of LPI on TREK-1 and TRAAK. These data imply a potential therapeutic use of LPI as an anti-ischemic target.

4.6.3. LPI and bone physiology

A role for LPI and its receptor GPR55 has been described in bone morphogenesis [114]. The study reported that LPI is able to inhibit the formation of mouse osteoclasts *in vitro*. When macrophages obtained from the bone marrow of $GPR55^{+/+}$ mice were cultured for 5 days in presence of LPI, an inhibition in osteoclasts formation was seen as indicated by the percentage of tartrate-resistant acid phosphatase positive mononuclear cells. Interestingly, this effect was not seen using macrophages from $GPR55^{-/-}$ mice. Moreover, LPI treatment of human osteoclasts for 5 days increased the resorption area; and this effect seems to involve the activation of Rho and ERK1/2. Treatment of both mouse and human osteoclasts with LPI activated Rho and stimulated ERK1/2 phosphorylation. Activation of these proteins was not observed in osteoclasts from $GPR55^{-/-}$ mice, and it was inhibited by the GPR55 antagonist cannabidiol (CBD) [114]. As mentioned above, $GPR55^{-/-}$ mice failed to develop mechanical hyperalgesia in a model of neuropathic hypersensitivity [112]. These mice also have a phenotype at the level of bone mass. Consistent with the inhibition in osteoclast formation induced by LPI, the number of osteoclasts observed in male $GPR55^{-/-}$ was increased compared to $GPR55^{+/+}$ mice; however, the functionality of these cells was impaired. These osteoclasts were inactive with an increase in the volume and thickness of trabecular bone, and in the cartilage remnants within the trabecular bone as a consequence of impairment in bone resorption [114]. Therefore, these studies show a role for GPR55 in neuropatic pain and bone resorption, and indicate that GPR55 could be use as a potential therapeutic target in the treatment of osteoporosis and neuropathic pain [27].

4.7. LPI and GPR55 interaction/recognition

Several molecular species of LPI can be naturally found in mammalian tissues, however their quantity and activity are still unknown. In the original paper identifying GPR55 as the LPI receptor, the LPI used in these experiments was obtained by treatment of soybean PI, which in mammalian tissues mainly consist of stearic acid esterified at the sn-1 position and arachidonic acid esterified at the sn-2 position [39]. The LPI generated through the PLA_2 enzymatic activity had palmitic acid (74%) followed by stearic acid (12%), oleic acid (5%) and linoleic acid (9%) as main fatty acyl moieties [61]. Later on, the same authors analysed the levels as well as the different species of LPI present in rat brain and showed that rat brain contains 37.5 nmol/g tissue of LPI [61]. The stearic acid-containing species (18.9 nmol/g tissue; 50.5%), is the most abundant although a significant amount of arachidonic acid-containing species (8.3 nmol/g tissue; 22.1%) was also found, together with palmitic acid-containing species (4.8 nmol/g tissue; 12.8%), oleic acid-containing species (5.0 nmol/g tissue; 13.3%) and palmitoleic acid-containing species (0.5 nmol/g tissue; 1.3%). Interestingly, when the effect of the different LPI species was compared, using as a read out the activation of ERK1/2 and the

mobilisation of intracellular Ca^{2+} levels in GPR55 expressing HEK293 cells, it was observed that molecular species of LPI had different potencies, with the 2-arachidonoyl LPI (2-AGPI) being the most active (EC50 was 30 nM). There is currently no information on the molecular species of LPI present in tissues expressing GPR55, such as spleen, tonsils, thymus, testes, ileum and brain [115–118]. Furthermore, although the presence of LPI in plasma and body fluids is well known, very little is known about the different species of LPI and which of those reaches the diverse tissues where GPR55 is expressed. It would be important to identify the molecular species of the lipid present in these tissues [119].

It has been suggested that LPI and 2-AGPI are able to assume different conformations within the lipid bilayer, regulating the interaction with the receptor [120]. Moreover, these authors suggest the existence of a lipid pathway for endogenous ligand entry in order to interact with the receptor [120]. Computational analysis of Molecular Dynamics simulations in a fully hydrated bilayer of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) of LPI and 2-AGPI has shown specific behaviours of these lipids affecting their interaction with GPR55. The results indicate that LPI and 2-AGPI can adopt a tilted headgroup conformation which may provide a low enough profile in the lipid bilayer for LPI and 2-AGPI to enter GPR55 via the putative transmembrane helix 6/7 (TMH6/7) entry port. This TMH6/7 lipid pathway for ligand entry was previously shown to be necessary for the interaction with the cannabinoids receptors CB1 and CB2 [121]. This study represents the first evidence of the physical interaction of LPI with GPR55. More recently a study has came out reporting the development of a computer model of GPR55 in an activated state in order to compare binding conformations (ligand binding pocket), and ligand potency [122]. GPR55 belongs to the rhodopsin-like (Class A) GPCR, and within these group of receptors only few of them have been crystallised. These analyses have revealed that GPR55 shares common crystal features such as an extracellular N terminus, seven transmembrane alpha helices (TMHs) connected by loops and an intracellular C terminus. Based on these data and the sequence of GPR55 the authors have generated a homology model of the GPR55 activated state, and studied the interaction of the receptor with LPI and other synthetic compounds identified as GPR55 agonists. LPI and GPR55 form a complex in which the binding site for the lipid locates between the transmembrane helices 2, 3, 6 and 7, corroborating, at least partially, the results obtained by Kotsikorou et al. Moreover, activation of GPR55 results in a binding site able to accommodate ligands with inverted-L shapes or T shapes in a vertical position deep inside the binding pocket. Interestingly, this analysis revealed that among the agonist studied, LPI is the ligand with the best interaction energy.

4.8. Signalling pathways activated by LPI

It was originally reported that LPI can regulate PLC, PLA_2, adenylyl cyclase and intracellular Ca^{2+} mobilisation [123]. Since the identification of GPR55, our understanding of the signalling cascades regulated by LPI has increased. It is now well established that upon LPI stimulation, GPR55 couples to $G_{\alpha 12/13}$ and $G_{q/11}$, initialising signalling cascades (Fig. 3). The main intracellular target activated by LPI/GPR55 is ERK1/2 which can be phosphorylated upon LPI stimulation in diverse cellular models, such as HEK293 overexpressing GPR55 [39], PC-3 and DU145 prostate cancer cells [68], osteoclasts [114], OVCAR3 ovarian cancer cells [68], EVSA-T breast cancer cells and T98G glyoma cells [98]. Another main intracellular target of LPI, downstream GPR55, is the small GTPase Rho, and this protein has been involved in some of the physiological effects of LPI. Treatment of cells with LPI leads to the activation of Rho, Rho-associated protein kinase (ROCK) and mobilisation of intracellular Ca^{2+}, as shown in HEK293 cells expressing recombinant human GPR55 [124] and in prostate and ovarian cancer cells [68]. Moreover, LPI also activates Rho in human and mouse osteoclasts inducing polarisation and resorption of these cells which express endogenous GPR55 [114]. Rho and ROCK are also involved in the activation of the MAPK p38. Interestingly, activation of p38 has been described in HEK293 cells overexpressing GPR55 and in IM-9 lymphoblastoid cells which express endogenous level of the receptor [118], and it is linked to the phosphorylation and activation of the transcription factor ATF-2. Similarly, it was previously reported that LPI activates the transcription factor NFAT, inducing its nuclear localisation in HEK293 expressing human recombinant GPR55. This effect is also mediated by the activation of Rho [124]. Nevertheless, it should be noted that studies on cell overexpressing a receptor while studying constitutive signalling pathways might be of limited impact and potentially misleading.

4.9. GPR55 receptor heterodimerization

Several GPCRs have been shown to form complexes with themselves (homodimers) or with other receptors (heterodimers). Cannabinoid receptor CB1 has been shown to form both homodimers and heterodimers with dopamine receptor D2 and opioid receptors [125,126]. Emerging evidence suggest that GPR55 may also form heterodimers with integrin and CB receptors.

A recent study in human umbilical vein endothelial cells has shown that CB1 and GPR55 trigger distinct signalling pathways, whereby one exhibits negative feedback on the other, depending on the status of integrin configuration [77]. In the presence of extracellular Ca^{2+} anandamide binding to CB1 results in G_i protein-mediated activation of Syk and its subsequent signal transduction pathway, resulting in activation of NFκB. In addition, Syk inhibits PI3K that represents a key signalling protein in the transduction of GPR55-originated signalling. However, once integrins are clustered, as a consequence of extracellular Ca^{2+} removal or addition of Mn^{2+}, Syk does not further inhibit GPR55-triggered signalling. Subsequently, the latter causes intracellular Ca^{2+} mobilisation from the ER via a PI3K–Bmx–PLCγ pathway and, in turn, activation of Ca^{2+}-activated NFAT.

More recently, an intriguing study provided evidence that GPR55 modulates CB2-mediated responses in human blood neutrophils [87]. Indeed in the latter cells GPR55 increases the migratory response towards the CB2 agonist 2-arachidonoylglycerol, while inhibiting neutrophil degranulation and reactive oxygen species production. Using HEK293 and HL60 cell lines, along with primary neutrophils, it has been shown that GPR55 and CB2 interfere with each other's signalling pathways at the level of small GTPases, such as Rac2 and Cdc42. This ultimately leads to cellular polarisation and efficient migration as well as abrogation of degranulation and ROS formation in neutrophils. Ultimately, GPR55 limits the tissue-injuring inflammatory responses mediated by CB2, while it synergizes with CB2 in recruiting neutrophils to sites of inflammation.

5. Concluding remarks

Research of the past decades has demonstrated that many important physiological and pathophysiological processes are regulated by LPLs. Among these, LPI has been shown to play a role both in physiological conditions and in disease, even though numerous open questions remain unanswered. For instance it is still not clear which enzymes are responsible for the abnormal levels of LPI detected in different diseases. The identification of these critical enzymes will be crucial in order to develop novel therapeutic strategies. Furthermore, LPI receptors need to be further characterised on ligand specificity and response, as well as for pharmacological inhibition. The specific role of LPI in pathological conditions needs further characterisation as suggested by several *in vitro* results that link LPI to diverse pathologies. In addition, it should be taken into account the complexity of different LPI species potentially present in cells, characterised by different fatty acid chain length and degree of saturation, associated with different biological functions. This is a rather unexplored field that requires further

investigation. Despite several studies have suggested that LPLs and the enzymes producing LPLs are potential biomarkers that may help with early diagnosis, many scientific and technical challenges need to be resolved. Finally, the search of novel GPR55 antagonists will certainly be an emerging field of LPI based drug target development in diseases and a novel exciting field could be the development of novel antagonists based on the LPI structure.

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