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Review

Lysophosphatidylinositols, from Cell Membrane Constituents to GPR55 Ligands

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Lysophosphatidylinositols (LPIs) are membrane constituents that alter the properties of said membranes. However, recent data showing that the once orphan receptor, GPR55, can act as a receptor for LPIs has sparked a renewed interest in LPIs as bioactive lipids. As evidence supporting the importance of LPIs and/or GPR55 is continuously accumulating and because LPI levels are altered in a number of pathologies such as obesity and cancer, the coming years should bring new, exciting discoveries to this field. In this review, we discuss the recent work on LPIs and on their molecular target, the GPR55 receptor. First, we summarize the metabolism of LPIs before outlining the cellular pathways activated by GPR55. Then, we review the actions of LPIs and GPR55 that could have potential pharmacological or therapeutic applications in several pathophysiological settings, such as cancer, obesity, pain, and inflammation.

Highlights

After years of testing cannabinoid ligands, GPR55 pharmacology finally distinguishes itself from the pharmacology of the cannabinoid receptors with the characterization of ligands that bind GPR55 but not the CB_1 and CB_2 cannabinoid receptors.

Thanks to the increasing number of studies, it is progressively apparent that GPR55 activation will lead to beneficial or detrimental effects, depending on the pathophysiological situation. In this perspective, developing selective agonists as well as selective antagonists is of high interest.

Besides cancer, the role of GPR55 and LPIs in obesity and metabolic diseases is emerging as a topic of interest. Similarly, the interest for GPR55 and LPIs in the central nervous system is increasing in recent years.

Lysophosphatidylinositols as Bioactive Lipids

Lysophospholipids have long been considered as simple membrane components whose only role was to alter the mechanical properties of said membranes. Indeed, lysophospholipids alter the spontaneous curvature of the membrane, hence modulating the function of membrane proteins (Box 1) [1]. However, over the years lysophospholipids have come to be regarded as 'bioactive lipids'. Among these lysophospholipids, some, such as lysophosphatidic acid, have been extensively studied while others, such as lysophosphatidylinositols (LPIs), are much less explored [2].

LPIs consist of a glycerol backbone, an acyl chain on the sn-1 or sn-2 position and a myo-inositol head group (Box 1 and Figure 1). Interestingly, some acyl chains are more frequent at one position compared with the other. As an example, arachidonoyl LPI and stearoyl LPI are more often detected as 2-acyl and 1-acyl LPI, respectively. LPIs are found in relatively large amounts in the murine brain (20–40 nmol/g of tissue), with stearoyl LPI being the most abundant, followed by arachidonoyl LPI [3,4]. Nevertheless LPIs are found ubiquitously and at similar levels in the body [4].

As we will discuss here, the recent work on LPIs and on their molecular target, the GPR55 receptor, support the bioactivity of this lipid family. Therefore, we will first summarize the metabolism of LPIs before outlining the pharmacology of GPR55 as well as the non-GPR55-mediated effects of LPI. Finally, we will review the pathophysiological actions described for LPIs and GPR55.

Metabolism and Efflux of LPIs

Biosynthesis of LPIs

As mentioned, LPIs exist as sn-1 or sn-2 lysophospholipids. These two **isomers** (see Glossary) share the same phosphatidylinositol (PI) precursors but are produced by distinct phospholipases (Figure 1). Phospholipase A_1 (PLA_1) enzymes will cleave at the sn-1 position of PIs,

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leading to the production of 2-acyl LPIs. Phospholipase A_2 (PLA_2) enzymes will produce 1-acyl LPIs, following hydrolysis of the sn-2 acyl chain of PIs.

1-acyl LPIs are essentially generated by the activity of cytosolic PLA_2 (group IV PLA_2) enzymes, and more specifically $cPLA_{2\alpha}$ [5]. Intracellular Ca^{2+} levels and phosphorylation regulate the activity of this enzyme. Upon activation, $cPLA_{2\alpha}$ translocates from the cytosol to intracellular membranes and releases arachidonic acid (its major product) from PIs and consequently LPIs [6].

PLA_1 is a large family of enzymes that can be classified depending on their distribution (intracellular or extracellular). While extracellular PLA_1 has been implicated in the production of some lysophospholipids in mammals (lysophosphatidic acid and lysophosphatidylserine), the intracellular PLA_1 DDHD domain containing 1 (DDHD1) is involved in the biosynthesis of LPIs [7]. Although this enzyme, first identified as PA-PLA_1, is not specific for LPI biosynthesis, it clearly hydrolyzes PIs into LPIs. Moreover, since 1-stearoyl-2-arachidonoyl PI is very abundant in mammalian tissues, DDHD1 seems to play an important role in the synthesis of arachidonoyl LPI [7].

Catabolism of LPIs

While the production of LPIs appears quite straightforward, their catabolism can take several routes (Figure 1) as reviewed by Yamashita *et al*. [8]. Indeed, LPIs are not only hydrolyzed by several phospholipases but they can also be reacylated into PIs.

LPIs can be deacylated by lysophospholipase A (lyso-PLA) to generate glycerophosphoinositol and a fatty acid. Another catabolic pathway involves a phospholipase C activity (LPI-specific phospholipase C, lysoPI-PLC) which was described as having high affinity for LPI, with very little hydrolysis of other lysophospholipids observed. This lysoPI-PLC hydrolyzes both 1-acyl and 2-

Box 1. Non-receptor-mediated effects of LPI

Lysophospholipids can modify the properties of cell membranes by altering membrane curvature. Indeed, while phospholipids are essentially of cylindrical shape (except for phospholipids with short alkyl chains, which can be considered as lysophospholipids in terms of shape), most lysophospholipids, including LPIs, are considered to have an inverted cone shape (Figure I). This is due to the large head group relative to the smaller hydrophobic domain. This structure leads to a stabilization of convex surfaces and favors the formation of micelles rather than lipid bilayers, as is the case with phospholipids. Therefore, lysophospholipids are considered as non-bilayer-forming lipids [99]. However, when lysophospholipids are mixed with phospholipids, they lead to a modification of the physical properties of the bilayer by introducing lateral stress or strain within this bilayer. Insertion of lysophospholipids with large head groups creates a positive membrane curvature, which could negatively impact membrane invagination, while facilitating membrane bending towards the cell exterior [100]. Data obtained using fluorescent membrane dyes suggest that LPIs change the intrinsic properties of the plasma membrane; this effect is not observed with lysophospholipids with smaller head groups such as lysophosphatidylethanolamines [101]. This effect of LPIs on plasma membrane lipid packing translates into a shift towards a liquid disordered phase in the outer leaflet and can modulate binding to the cell surface. In this case, the authors showed that stearoyl-LPI inhibited the binding of the shiga toxin to the neutral glycosphingolipid Gb3 at the cell surface [101].

Changes in membrane conformation can also result in changes in protein properties (ion channels for instance) and therefore lead to cellular effects that are not mediated by direct binding of LPIs to a receptor. Indeed, lysophospholipids such as lysophosphatidylcholines and LPIs can produce reversible activations of the K^+ channels TREK-1 and TRAAK [71]. Moreover, LPI was shown to inhibit Na/K-ATPase and to activate large- and intermediate-conductance Ca^{2+}-dependent potassium channels (BK_{Ca} and IK_{Ca}) independently of GPR55 [102–104].

Finally, it was also shown that lysophospholipids with large head groups can regulate the kinetics and properties of clathrin-dependent endocytosis. In this case, stearoyl-LPI reduced the number of invaginated clathrin-coated pits at the plasma membrane, leading to fewer endocytic events [100].

Glossary

Allosteric modulator: a ligand that increases, or decreases, the action of an agonist (or antagonist) by binding to a different location on the receptor than the orthosteric site.
Biased agonism: this notion covers the fact that some agonists are able to preferentially activate one (or several) intracellular pathways over the other potential pathways for a given receptor.
Deorphanization: the identification of endogenous ligands for an orphan receptor (i.e., a receptor that has a similar structure to other identified receptors but whose endogenous ligand has not yet been identified).
Endocannabinoid: an endogenous molecule able to bind and activate either or both cannabinoid receptors (i.e., CB_1 and CB_2). To date the canonical endocannabinoids are 2-arachidonoylglycerol and *N*-arachidonoylethanolamine.
Isomer: two (or more) molecules sharing the same formula but having different arrangement of the atoms in their structure. In the context of LPI, sn1 and sn2 LPI are called positional isomers.
Cross-antagonism: As GPCRs heteromerize, the ligand of one receptor can antagonize its own receptor but also modulate the other partner of the heteromer. So if receptors A and B heteromerize, a selective antagonist of receptor A could, via cross-antagonism, prevent the activation of receptor B although it does not bind to it. A similar phenomenon is possible for agonists (cross-agonism).

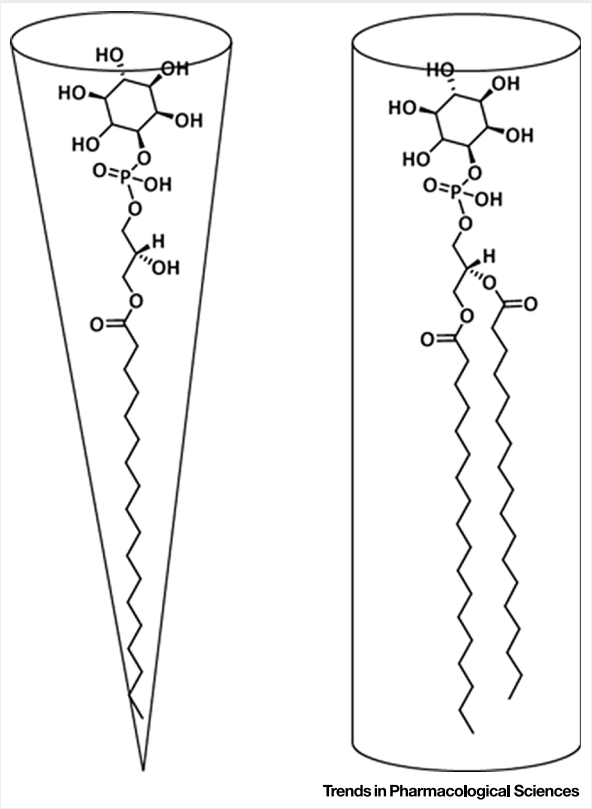

Figure I. Structure of Inverted Cone-Shaped 18:0 LPI and Cylindrical 18:0/18:0 PI. Lysophosphatidylinositols (LPIs), constituted by a large head group and a small hydrophobic domain with only one acyl chain, are structured as inverted cone-shapes, while phosphatidylinositols (PIs), with two acyl chains, are organized as cylindrical shapes, leading to the formation of micelles and lipid bilayers, respectively.

acyl LPI into acylglycerol and inositolphosphate [8]. An additional hydrolytic route of LPIs involves Autotaxin, a lysophospholipase D (lyso-PLD) which converts lysophospholipids into lysophosphatidic acid. Finally, α/β hydrolase domain 6 (ABHD6) was recently shown to hydrolyze lysophospholipids, including LPIs, into fatty acids, thus exhibiting an LPA activity [9].

LPIs can also be reacylated into PIs and are therefore involved in the so-called 'remodeling' of PIs. Indeed, LPIs are considered as acyl-acceptor molecules leading to phospholipid production [10]. This system is very important for the balance of fatty acids and has a crucial impact on phospholipid homeostasis. Moreover, fatty acids in cellular phospholipids have an asymmetrical distribution: generally, saturated fatty acids are esterified at the sn-1 position while polyunsaturated fatty acids (and mainly arachidonic acid for PI) are esterified at the sn-2 position. This point is important for the ability of acyltransferases to remodel PIs from fatty acids and LPIs [8].

Membrane-bound O-acyltransferase 7 (MBOAT7) is a LPI-specific acyltransferase (LPIAT), able to add a fatty acid to the free alcohol of the glycerol to generate the corresponding PI. MBOAT7 is required for the incorporation of polyunsaturated fatty acids into PIs, mainly in the sn-2 position [11]. This enzyme plays a crucial role in brain development. Indeed, MBOAT7$^{-/-}$ mice show reduced arachidonic acid content in PIs, as well as atrophy of the cortex and the hippocampus and a lifespan of 1 month [12].

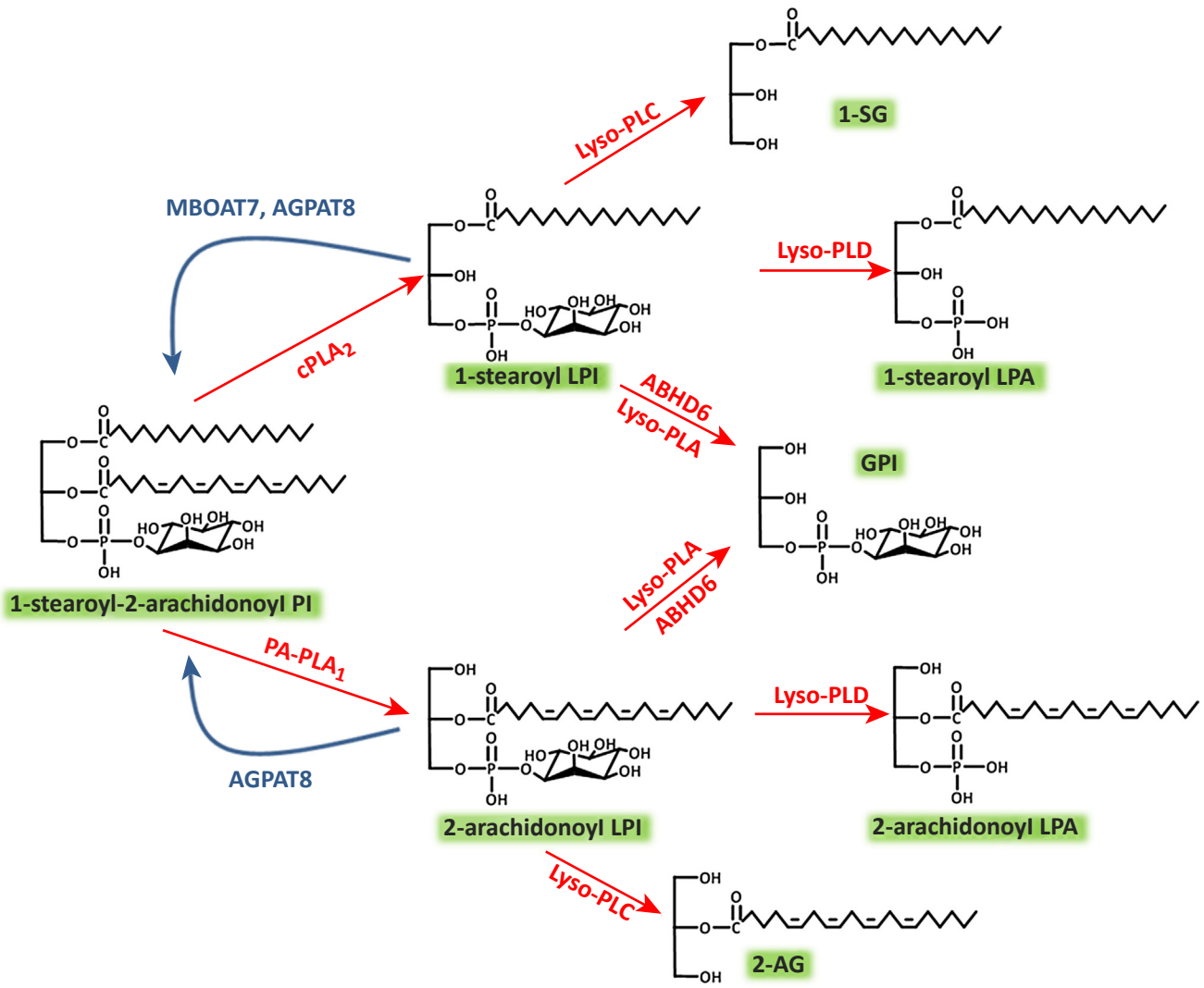

Figure 1. Schematic View of LPI Metabolism. Phosphatidylinositols (PIs) release lysophosphatidylinositols (LPIs) from cell membranes via the actions of phospholipase A_2 ($cPLA_2$) and phospholipase A_1 ($PA\text{-}PLA_1$) generating 1-acyl LPI and 2-acyl LPI, respectively, Here, as an example, the hydrolysis of 1-stearoyl-2-arachidonoyl PI (one of the most abundant PIs) will lead to 1-stearoyl LPI and 2-arachidonoyl LPI. These LPIs can be metabolized by several enzymes: lysophospholipase A (Lyso-PLA), lysophospholipase C (Lyso-PLC), and lysophospholipase D (Lyso-PLD) giving glycerophosphoinositol (GPI), acylglycerols (here, 2-arachidonoylglycerol, 2-AG and 1-stearoylglycerol, 1-SG), and lysophosphatidic acid (LPA), respectively. LPI can be reacylated into phospholipids via acyltransferase activities: MBOAT7 and AGPAT8 reacylate 1-acyl LPI but only AGPAT8 is able to reacylate 2-acyl LPI into the parent PI.

LPIs can also be esterified by the enzyme 1-acylglycerol-3-phosphate acyltransferase 8 (AGPAT8). Contrary to MBOAT7, AGPAT8 seems involved in the sn-1 fatty acid remodeling of PI and has 2-acyl LPIs as physiological substrates [13,14]. However, AGPAT8 has a wide range of physiological substrates and is involved in the sn-2 acylation of lysophospholipids [15].

A rather classical way of interfering with bioactive lipid signaling is to inhibit the enzymes responsible for their biosynthesis or degradation. Therefore, such inhibitors could be considered as potential drugs. However, this approach is quite complex in the case of LPIs. Indeed, if we consider the biosynthetic enzymes, $cPLA_{2\alpha}$ and DDHD1 are also involved in the production

of arachidonic acid and lysophosphatidic acid, respectively. The acyltransferases are essential for the remodeling of PIs and therefore will influence the composition of membranes. Therefore inhibiting these enzymes to affect LPI levels will also have several 'off-target' effects. Moreover, as reported above, it is likely that other yet unidentified enzymes are also implicated in the catabolism of LPIs, thus making it difficult to control LPI levels through enzyme inhibition.

Efflux of LPIs

The metabolism or actions of LPIs could also be dependent on their transport across membranes [16]. However, little is known about the existing export system for LPIs. One study reported the implication of the ATP-binding cassette transporter (ABCC1) in the export of LPIs into the extracellular medium in the PC-3 human prostate cancer cell line [17]. While the exact mechanisms are still unknown, the authors proposed an autocrine pathway that could regulate proliferation in cancer cells where 1-acyl LPI is synthetized by $cPLA_2$, released by ABCC1, and activates a membrane receptor to stimulate cell proliferation [17].

The GPR55 Receptor

Although LPIs were identified for the first time in the 1960s, and the first evidence for receptor-mediated effects of these lipids was reported in 1995 [18], it was their identification as the endogenous ligands of the G protein-coupled receptor GPR55 in 2007 that sparked a renewed interest in the study of these lipids [19].

However, this receptor was initially reported as a putative receptor for **endocannabinoids**, another family of bioactive lipids [20]. Indeed, GPR55 was first shown to respond to some cannabinoid ligands, some more consistently than others. Therefore many of the reported ligands are cannabinoid-related, which raises issues in terms of selectivity when interpreting their effects as GPR55-mediated (see Table 1 for a summary of the studied ligands and Table S1 in the online supplementary material for a more comprehensive list of tested compounds). However, LPIs, and especially arachidonoyl LPI, are clearly agonists of GPR55 [3,21,22]. Indeed, several LPI species were found to induce dose-dependent ERK phosphorylation in GPR55-expressing HEK293 cells [19]. LPIs also induce rapid transient increase of intracellular Ca^{2+} in the same cells and stimulate [^{35}S]GTPγS binding. Depending on the fatty acid, the potencies of LPIs for ERK phosphorylation were different, with 2-arachidonoyl LPI showing the highest level of activity and a reported EC_{50} of 30 nM, and 1-palmitoyl LPI being a weak partial agonist [3]. Accordingly, the International Union of Basic and Clinical Pharmacology lists LPIs as the endogenous agonists of GPR55 but this receptor still retains its orphan status due to the complex reported pharmacology and insufficient *in vivo* data.

To date, there is no specific radioligand for GPR55 and no routine binding assay described. Therefore, most of the available data was obtained through functional assays in transfected cells monitoring intracellular calcium fluxes, ERK phosphorylation, β-arrestin recruitment, or receptor internalization in transfected cells (see Box 2 for an overview of the signaling pathways). Moreover, while some studies compared the effects of LPIs with the different cannabinoid ligands in the same assay and validated their results by using untransfected cells, this is not the case for all the studies describing agonist or antagonist ligands of GPR55.

Apart from the different assays used, **biased agonism** could also explain the inconsistent pharmacology of GPR55. Indeed, one study compared the effect of LPIs and Virodhamine in HEK293 cells stably transfected with human GPR55. They found that the effect of both ligands on intracellular Ca^{2+} was blocked by CID16020046, a GPR55 antagonist. However, the effect of Virodhamine was mediated by $G_{\alpha 13}$, Rho, ROCK, and PLCε while the effect of LPI

Table 1. Ligands and Putative Ligands of the GPR55 Receptor. This table lists the most representative ligands of the GPR55 receptor, along with their functionality and potency (when available). Some ligands once thought to be GPR55 ligands are also present (e.g., PEA). Because biased agonism is well described for GPR55, several assays are listed for the same ligand. A more comprehensive version of this table [including additional compounds (e.g., CP-55,940, Δ^9-THC, 2-AG, AM281, etc.) and additional assays (e.g., CREB or NFAT activation)] is available in online supplementary material Table S1

Ligand	Cell type	Output	Agonist/antagonist	EC_{50} or IC_{50}
Lysophospholipids				
LPI	hGPR55-U2OS	β-arrestin	Agonist [24]	EC_{50} = 1.2 μM [24]
		Rc internalization		ND
	hGPR55-HEK293	Luciferase reporter	Agonist [109]	EC_{50} = 3.6 μM [109]
		GTPγS	Agonist [19]	ND
		$[Ca^{2+}]_i$	Agonist [19,21,106,110]	EC_{50} = 0.05 μM [106]
		ERK phosphorylation	Agonist [19,106,107,111]	EC_{50} = 0.074–1 μM [106,107,111]
		Rc internalization	Agonist [21,106]	ND
1-palmitoyl LPC	hGPR55-HEK293	ERK phosphorylation	No effect [3]	ND
		$[Ca^{2+}]_i$	No effect [3]	ND
	PC3 cells	$[Ca^{2+}]_i$	Agonist (blocked by ML193 and CID16020046) [112]	ND
1-oleoyl LPC	PC3 cells	$[Ca^{2+}]_i$	Agonist (blocked by ML193 and CID16020046) [112]	ND
1-palmitoyl LPG	hGPR55-HEK293	ERK phosphorylation	Agonist [3]	ND
		$[Ca^{2+}]_i$	Agonist [3]	ND
'Cannabinoid-derived' ligands				
N-arachidonoylethanolamine (anandamide)	hGPR55-U2OS	β-arrestin	No effect [24]	
			Partial agonist/antagonist [113]	Inhibits response to LPI (IC_{50} = 5.91 μM – I_{max} = 20%) and SR1 (IC_{50} = 12 μM – I_{max} = 65%) [113]
		Rc internalization	Decreases agonists-induced receptor internalization [113]	ND
	hGPR55-HEK293	GTPγS	Agonist [29,114]	EC_{50} = 18 nM [29]
		ERK phosphorylation	No effect [3,19]	
		$[Ca^{2+}]_i$	No effect [3,21]	
			Agonist [22]	ND
		β-arrestin	Agonist [109]	ND
		Luciferase reporter	No effect [109]	
N-palmitoylethanolamine (PEA)	hGPR55-U2OS	β-arrestin	No effect [24]	
	hGPR55-HEK293	ERK phosphorylation	No effect [19]	
		GTPγS	Agonist [29,114]	EC_{50} = 4 nM [29]
		$[Ca2+]_i$	No effect [22]	
NAGly	hGPR55-CHO	ERK phosphorylation	Agonist [115]	ND
		$[Ca2+]_i$	Agonist [115]	ND
Virodhamine	hGPR55-U2OS	β-arrestin	Partial agonist/antagonist [113]	Inhibits response to LPI (IC_{50} = 6.57 μM – I_{max} = 46%) and SR1 (IC_{50} = 9.44 μM – I_{max} = 68%) [113]
		Rc internalization	Decreases agonists-induced Rc internalization [113]	ND

Table 1. (continued)

Ligand	Cell type	Output	Agonist/antagonist	EC_{50} or IC_{50}
	hGPR55-HEK293	ERK phosphorylation	No effect [19]	
		GTPγS	Agonist [29,114]	EC_{50} = 12 nM [29]
		$[Ca2+]_i$	No effect [22]	
O-1602	hGPR55-U2OS	β-arrestin	No effect [24]	
	hGPR55-HEK293	ERK phosphorylation	No effect [3]	
		$[Ca^{2+}]_i$	No effect [3]	
		GTPγS	Agonist [29,36]	EC_{50} = 1.4–13 nM [29,36]
		β-arrestin	No effect [109]	
Cannabidiol	hGPR55-U2OS	β-arrestin	No effect [24]	
	hGPR55-HEK293	GTPγS	Antagonist [29,114]	IC_{50} = 354 nM [29]
		$[Ca2+]_i$	No effect [22]	
		ERK phosphorylation	No significant effect on LPI effect [107]	
SR141716A	hGPR55-U2OS	β-arrestin	Agonist [24]	EC_{50} = 3.9 μM [24]
		Rc internalization	Agonist [24]	ND
	hGPR55-HEK293	β-arrestin	Agonist [109]	EC_{50} = 9.3 μM [109]
		Luciferase reporter	Agonist [109]	EC_{50} = 10.9 μM [109]
		$[Ca^{2+}]_i$	Agonist [106]	EC_{50} = 1.14 μM [106]
			Agonist [110]	ND
			Antagonist [22]	ND
		ERK phosphorylation	Agonist [106]	EC_{50} = 0.64 μM [106]
			No effect [19]	
			Agonist when tested alone and reduces LPI Emax when tested with LPI [107]	ND
		Rc internalization	Agonist [106]	ND
		GTPγS	Agonist [114]	ND
AM251	hGPR55-U2OS	β-arrestin	Agonist [24]	EC_{50} = 9.6 μM [24]
		Rc internalization	Agonist [24]	ND
	hGPR55-HEK293	β-arrestin	Agonist [109]	EC_{50} 2.7–3.1 μM [109]
		Luciferase reporter	Agonist [109]	EC_{50} = 3.4 μM [109]
		$[Ca^{2+}]_i$	Agonist [21,106]	EC_{50} = 0.63 μM [21,106]
			Agonist [110]	ND
		ERK phosphorylation	Agonist [106]	EC_{50} = 0,54 μM [106]
			Agonist when tested alone and reduces LPI Emax when tested with LPI [107]	EC_{50} = 2.34 μM [107]
		Rc internalization	Agonist [106]	ND
		GTPγS	Antagonist [29]	IC_{50} = 39 nM [29]
GW405833	hGPR55-HEK293	ERK phosphorylation	Partial agonist when tested alone and enhances effect of LPI when tested with LPI [107]	EC_{50} = 1.87 μM [107]

Table 1. (continued)

Ligand	Cell type	Output	Agonist/antagonist	EC_{50} or IC_{50}
O-1918	hGPR55-U2OS	β-arrestin	No effect [24]	
	endothelial cells (human)	$[Ca2+]_i$	Antagonist of AEA' effects [26]	
Oher ligands				
Agonists				
CID1792197	hGPR55-U2OS	β-arrestin	Agonist [116]	EC_{50} = 0.11 μM [116]
		ERK phosphorylation	Agonist [116]	ND
CID1172084 (analog of ML185)	hGPR55-U2OS	β-arrestin	Agonist [116]	EC_{50} = 0.16 μM [116]
		ERK phosphorylation	Agonist [116]	ND
CID2440433 (ML184)	hGPR55-U2OS	β-arrestin	Agonist [116]	EC_{50} = 0.26 μM [116]
		ERK phosphorylation	Agonist [116]	ND
GSK494581A	hGPR55-yeast		Agonist [110]	EC_{50} = 0.79 μM [110]
	hGPR55-HEK293	$[Ca2+]_i$	Agonist [110]	EC_{50} = 0.16 μM [110]
GSK319197A	hGPR55-yeast		Agonist [27]	EC_{50} = 0.40 μM [27]
	hGPR55-HEK293	$[Ca2+]_i$	Agonist [27]	EC_{50} = 6.3 μM [27]
Compound 17l in ref. [117][a]	GPR55- CHO	β-arrestin	Agonist [117]	EC_{50} = 0.17 μM [117]
	hGPR55-HEK293	$[Ca2+]_i$	Agonist [117]	EC_{50} = 0.002 μM [117]
Compound 14b in ref. [118][b]	hGPR55-HEK293	xCELLigence	Partial agonist [118]	EC_{50} = 0.006 μM [118]
Antagonists				
PSB-SB-489	hGPR55-CHO	β-arrestin	Antagonist [119]	IC_{50} = 1.77 μM [119]
PSB-SB-115	hGPR55-CHO	β-arrestin	Antagonist [119]	IC_{50} = 3.45 μM [119]
PSB-SB-1203	hGPR55-CHO	β-arrestin	Antagonist [119]	IC_{50} = 0.26 μM [119] (CB_1: Ki = 0.24; CB_2: Ki = 0.21 [119])
CID16020046	hGPR55-HEK293	$[Ca2+]_i$	Antagonist [120]	IC_{50} = 0.21 μM vs. LPI [120]
		NFAT activation	Antagonist [120]	IC_{50} = 0.48 μM vs. LPI IC_{50} = 0.31 vs. GSK [120]
		SRE induction	Antagonist [120]	IC_{50} = 1.99 μM vs. LPI IC_{50} = 1.48 vs. GSK [120]
		NF-kB activation	Antagonist [120]	IC_{50} = 0.71 μM vs. LPI IC_{50} = 0.64 vs. GSK [120]
		ERK phosphorylation	Antagonizes LPI and GSK [120]	ND
		Rc internalization	Antagonizes LPI [120]	ND
CID23612552 (ML191)	hGPR55-U2OS	β-arrestin	Antagonist [121]	IC_{50} = 1.08 μM vs. LPI IC_{50} = 1.03 μM vs. ML186 [121]
		ERK phosphorylation		IC_{50} = 0.4 μM vs. LPI [121]
CID1434953 (ML192)	hGPR55-U2OS	β-arrestin	Antagonist [121]	IC_{50} = 0.7 μM vs. LPI IC_{50} = 0.29 μM vs. ML186 [121]
		ERK phosphorylation		IC_{50} = 1.1 μM vs. LPI [121]
CID1261822 (ML193)	hGPR55-U2OS	β-arrestin	Antagonist [121]	IC_{50} = 0.22 μM vs. LPI IC_{50} = 0.12 μM vs. ML186 [121]
		ERK phosphorylation		IC_{50} = 0.2 μM vs. LPI [121]

[a]17l: N-((4-(N-(Furan-2-ylmethyl)sulfamoyl)phenyl)carbamothioyl)-[1,1′-biphenyl]-4-carboxamide.
[b]14b: 2,4-Dihydro-7-methoxy-2-{2-[4-(2-methoxyphenyl)- piperazinyl]acetamidomethyl}-4,4-dimethylchromeno[4,3-c]-pyrazole.

Box 2. GPR55 Signaling

GPR55 leads via $G\alpha_{12/13}$ and $G\alpha_q$ to the activation of several downstream pathways (Figure I), most of which were characterized with LPI as ligand.

$G\alpha_q$ was shown to stimulate PLC activity, inducing Ca^{2+} release from the endoplasmic reticulum and activation of various protein kinase C (PKC) isoforms [22,24]. $G\alpha_{12/13}$ leads to the activation of small GTPases, such as Ras homologue gene family member A (RhoA), resulting in the activation of Rho-associated protein kinase (ROCK) leading to PLC activation [21,22,42]. ROCK activation can catalyze the phosphorylation of intracellular proteins such as p38 mitogen-activated protein kinase (MAPK) [105]. Extracellular signal regulated kinase (ERK) kinase 1/2 can also be activated following ligand binding to GPR55 leading, along with p38, to the activation of transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) or activating transcription factor 2 (ATF-2) [24,28,105]. 3′-5′-cyclic adenosine monophosphate response element (CREB) activation was found in HEK293 cells transfected with human GPR55, but not in human endothelial colony-forming cells [57,106]. ROCK activation and the increased intracellular Ca^{2+} were also shown to activate the nuclear factor of activated T cells (NFAT) transcription factor [21,106].

GPR55 activation was also shown to activate mitogen-activated protein kinase kinase (MEK) 1/2, leading to ERK1/2 activation in HEK293 cells transfected with GPR55 [24,107], protein kinase B (Akt) phosphorylation in several human cancer cell lines, and activation of the transcription factor ETV4 in MDA-MB-231 cells [17,62].

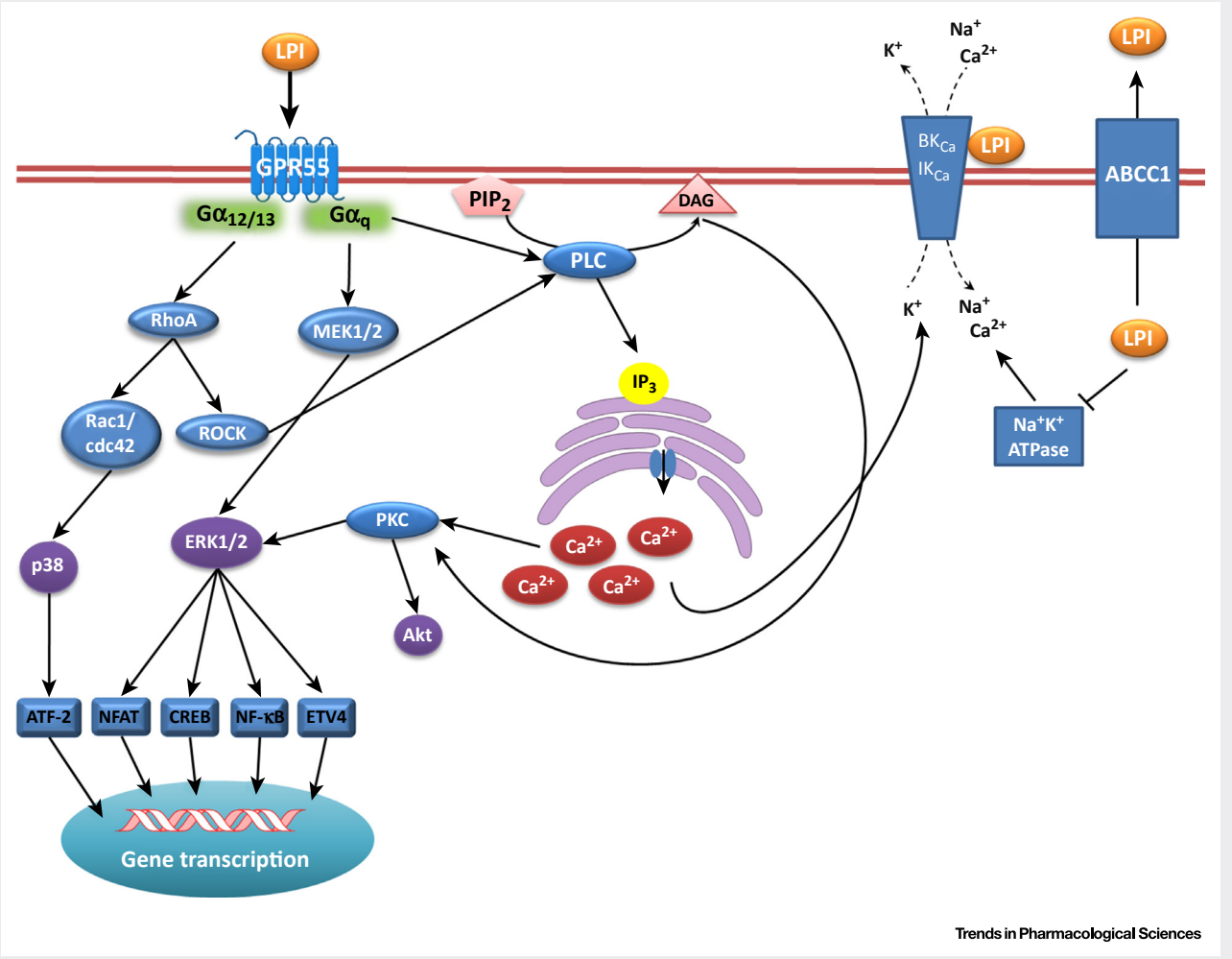

Figure I. Cellular Signaling of GPR55 and lysophosphatidylinositol (LPI).

was more dependent on $G\alpha_q$ and PLCβ [23]. However, in another study with HEK293 cells transfected with human GPR55, LPIs activated the $G\alpha_{13}$, Rho, ROCK pathway [21]. Another example of potentially biased agonism comes from a study that found that LPIs, but not the cannabinoid receptor antagonists SR141716A and AM251, could induce ERK phosphorylation in

hGPR55-transfected U2OS cells, despite all three ligands inducing β-arrestin recruitment and receptor internalization [24].

Additionally, some cannabinoids have been suggested to be **allosteric modulators** of GPR55, which could explain the complex pharmacology of cannabinoids at GPR55. Indeed, allosteric modulation is often dependent on the probe, system, and assay used, which could explain the inconsistent data [25].

While it is gradually emerging that GPR55 is not a cannabinoid receptor, there is a clear crosstalk between GPR55 and the cannabinoid receptors (CB_1 and CB_2), either through modulation of integrin clustering [26] or through heteromerization [27,28]. Indeed, like numerous GPCRs, GPR55 was found to oligomerize, notably with the cannabinoid receptors, leading to alterations in signaling. However the consequences of this interaction were different depending on the cannabinoid receptor considered (Box 3).

This crosstalk between receptors, while not uncommon in GPCR signaling, increases the complexity of delineating a clear pharmacological picture for GPR55. Developing a binding assay will provide valuable and complementary information to the functional assays used so far. The new synthetic ligands recently developed, and especially the most potent, could serve as a template for the development of a radioligand.

Pathophysiological Actions of LPIs and GPR55

GPR55 is ubiquitously expressed [29–35], therefore, despite its relatively recent **deorphanization** and complex pharmacology, it is implicated in many physiological and pathological processes, including inflammation, nociception, cancer, bone development, metabolic disturbances, synaptic transmission, and anxiety (Figure 2, Key Figure). In the following section, we discuss the effects of either LPIs or GPR55 in several settings. Some studies in the literature attribute effects to GPR55 following the use of atypical cannabinoid ligands, such as

Box 3. GPR55 Receptor Oligomerization

Like many GPCRs, GPR55 was shown to form heteromers with the cannabinoid receptors CB_1 and CB_2. This leads to alterations in signaling and the consequences of these alterations are dependent on the cannabinoid receptor considered.

For instance, NFAT and ERK1/2 activation by LPI were significantly reduced when both CB_1 and GPR55 were expressed in HEK293 cells compared with GPR55 alone. Conversely, CB_1 signaling was enhanced by the presence of GPR55 [27]. Heteromers of GPR55 and the CB_2 receptor were also reported in HEK293 cells transfected with both receptors. In this case, heteromerization also led to a reduction in GPR55-mediated NFAT (along with CREB and NF-κB) activation. However, this was accompanied by a potentiation of GPR55-mediated ERK1/2 activation. Conversely, ERK1/2 activation by CB_2 is reduced when GPR55 is coexpressed in the cells [28]. In both these studies, the authors also transfected the cells with CCR5, another Gi-coupled constitutively expressed GPCR, in order to assess whether the effects observed were due to the cannabinoid receptors in particular or merely to the expression of a Gi-coupled GPCR [27,28]. Cross-antagonism and a negative crosstalk between the two receptors when activated was observed by another group using HEK293 cells cotransfected with GPR55 and CB_2 [75].

Beyond transfected cells, heteromers of GPR55 and CB_1 were put forth in rat brain slices and in the caudate and putamen nuclei of a non-human primate [108]. A further interaction between CB_1 and GPR55 was shown in human endothelial cells where, depending on integrin clustering, CB_1 signaling either inhibits or does not inhibit GPR55 signaling [26]. Heteromers of GPR55 and CB_2 were put forth in a human glioblastoma cell line that endogenously expresses both receptors [75]. GPR55 activation was also shown to modulate the effect of CB_2 activation by the endocannabinoid 2-arachidonoylglycerol (2-AG) in human neutrophils and differentiated HL60 cells [77]. It was, however, not investigated if that was an interaction at the level of the intracellular pathways or if receptor heteromers were involved.

Key Figure

Effects of the LPI/GPR55 Axis and Their Potential Therapeutic Modulation

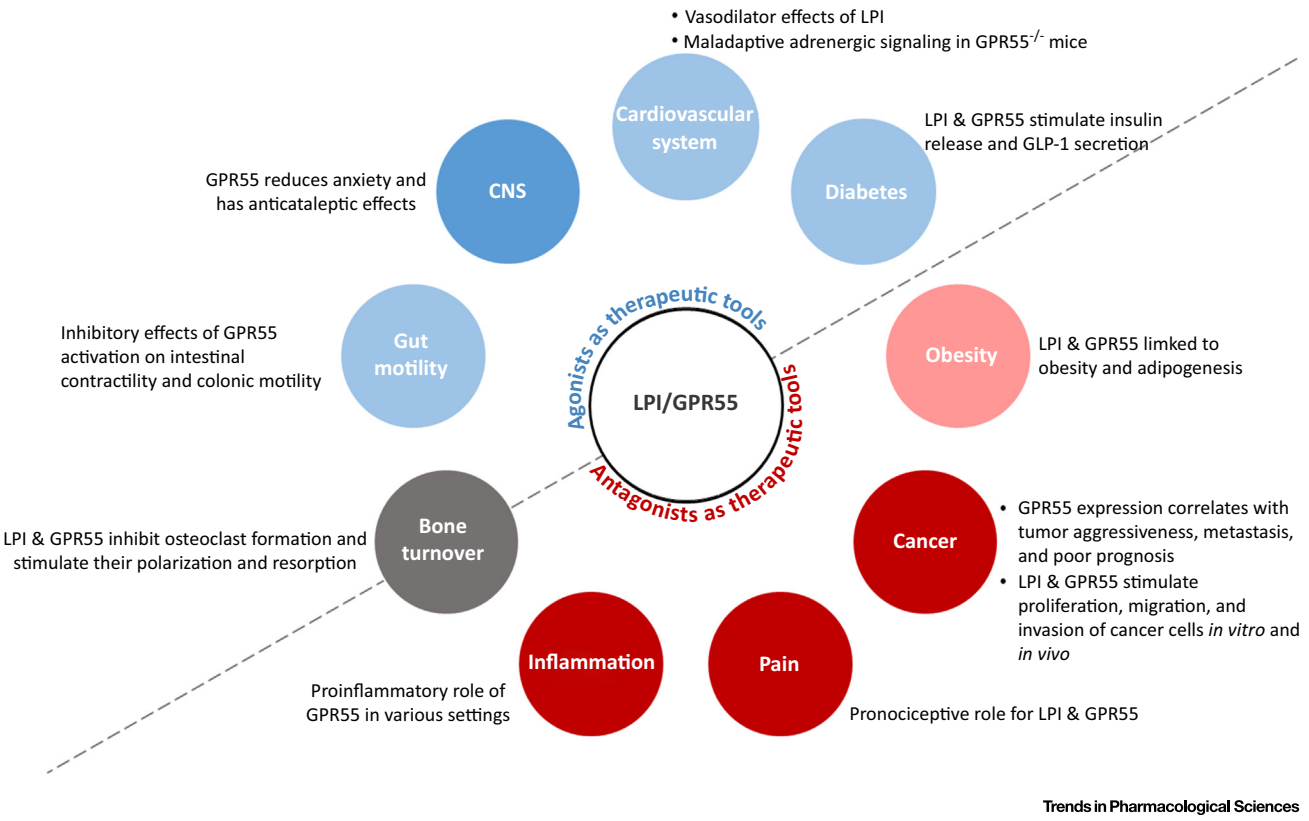

Figure 2. The main effects of GPR55 and lysophosphatidylinositol (LPI) discussed in this paper are represented in the figure. Diseases or conditions where LPI and GPR55 had deleterious effects, and therefore where antagonists of GPR55 could be promising therapeutic tools, are represented in red. In blue are the conditions where GPR55 and LPI had beneficial effects and thus agonists could be used. The lighter colors represent situations where more studies are needed to confirm the data. Bone turnover is in grey, as only one paper reported effects of LPI and GPR55 in that setting, and it is unclear yet whether they could be involved in osteoporosis or possibly cancer-associated bone metastases or pain.

oleoylethanolamide, palmitoylethanolamide, O-1602, and abnormal cannabidiol or the blockade of some effects by putative antagonists such as O-1918. However, as mentioned previously, there is no consensus as to whether some of these compounds are indeed GPR55 ligands and if they are, their selectivity is questionable. They were shown to have GPR55-independent targets and to retain their effects in GPR55$^{-/-}$ mice [36–38]. Therefore we elected to review the effects directly attributable to GPR55, as well as the effects of LPIs, and discuss if the latter were GPR55-dependent or not.

Cardiovascular Diseases

A potential role for GPR55 has been suggested in the cardiovascular system. GPR55$^{-/-}$ mice exhibit ventricular remodeling, maladaptive adrenergic signaling and systolic dysfunction in comparison with wild type mice, thus potentially implicating this receptor in the pathogenesis of

heart failure [39]. Moreover, LPI levels were increased in a rat model of asphyxia-induced cardiac arrest and decreased following cardiopulmonary resuscitation [40]. Whether these altered levels support the implication of LPIs in cardiovascular disorders remains to be shown. However, from a translational point of view, it is noteworthy that LPI levels were also elevated in the plasma of acute coronary syndrome patients [41].

LPIs have vasodilator effects in isolated rat small mesenteric arteries and human pulmonary arteries [42,43]. LPIs also inhibit vasopressor responses to sympathetic stimulation or noradrenaline injection in rats [44], as well as endothelial hyperpolarization to acetylcholine and histamine in mouse endothelial cells and excised mouse aorta [45]. Some of these effects were GPR55-dependent and involved intracellular Ca^{2+}, PPARγ, nitric oxide, and calcium-activated potassium channels [43–45].

Energy Homeostasis and Metabolic Disorders

Obesity and Body Weight

GPR55 is likely to be implicated in human obesity, as GPR55 mRNA and protein expression were increased in the visceral adipose tissue of obese individuals and even more so in diabetic obese individuals [30]. However, GPR55 expression in visceral fat correlated with increased body weight only in female patients [30]. Moreover, a missense polymorphism of GPR55 (Gly195Val), leading to decreased GPR55 function, was associated with increased incidence of anorexia nervosa in a cohort of Japanese women [46].

However, GPR55 might be differentially regulated during obesity in human and rodents. Indeed, studies in rats fed a high-fat diet and in mice lacking leptin (ob/ob mice) showed the opposite trend, with decreased GPR55 expression in visceral adipose tissue compared with lean animals [30]. Moreover, there was no difference in body weight, energy intake, and feeding patterns between wild type mice and $GPR55^{-/-}$ mice fed standard chow and even mice fed a high-fat diet [34,47,48]. However, energy expenditure, motor activity, and adiposity were not as consistent. One study found that $GPR55^{-/-}$ mice exhibited increased fat-mass and insulin resistance, as well as a decreased locomotor activity [48], while another study reported a nonsignificant trend towards increased adiposity with an increase in motor activity during the first part of the night and a decrease in energy expenditure [47]. The difference in age between the two cohorts of mice (5-month-old versus 9-week-old mice) could be one factor explaining the differences observed. It is also important to consider differences in generating the various $GPR55^{-/-}$ strains used. Moreover, gender differences as well as methodological factors (temperature, age, animal housing, etc.) and possible compensatory adjustments could account for some of the discrepancies observed with different strains of $GPR55^{-/-}$ mice.

Feeding also modulated GPR55, as its expression in rat white adipose tissue was increased, while circulating LPI levels were decreased, after fasting and returned to normal following feeding or leptin treatment [49]. Other factors, such as gestation, postnatal development, and gender were also implicated in the regulation of GPR55 expression [49].

A role for LPI in adipogenesis and diabetes has also been suggested. Indeed, circulating LPI levels were increased in obese patients compared with lean controls, and also, in this case, correlated with increased body weight in females [30]. In the same study, incubation of human visceral adipose tissue explants with LPIs upregulated mRNA expression of GPR55 and of genes involved in fatty acid synthesis and adipocyte differentiation, such as fatty acid synthase, acetyl coA carboxylase, and PPARɣ [30]. LPI levels were also increased in individuals with type 2 diabetes in comparison with controls [50]. A possible role for LPIs in type 1 diabetes has also

been suggested, as significantly higher circulating LPI levels were found in a mouse model of type 1 diabetes compared with control animals [51].

These studies suggest that GPR55 and LPIs could be linked to increased weight in humans and that its pharmacological modulation could help in controlling obesity and associated disorders. However, differences between human and rodent studies suggest potential species differences in the metabolic roles of GPR55. Understanding these differences as well as the potential similarities between humans and rodents, while keeping in mind potential compensatory mechanisms in knock-out mice, would help in advancing this field forward.

Insulin Secretion and Glucose Metabolism

LPIs mobilize Ca^{2+} in pancreatic islets and promote insulin release [35,52]. While the expression of GPR55 in insulin-secreting pancreatic islets [33] suggests an effect of the LPI/GPR55 axis, insulin-stimulating effects of LPIs were also found in islets from $GPR55^{-/-}$ mice [35].

In the same setting, the reported GPR55 antagonist cannabidiol also stimulated insulin release in a GPR55-independent manner [35]. Conversely, the effects of O-1602 on insulin release were blunted in islets from $GPR55^{-/-}$ mice, suggesting that the effects of O-1602 on insulin release are, at least in part, mediated by GPR55 [33,35]. GPR55 expression was also reported in human pancreatic α-cells [35]. However, whether GPR55 or LPIs are implicated in glucagon secretion remains to be unraveled.

Finally, LPIs increased intracellular Ca^{2+} and induced glucagon-like peptide-1 (GLP-1) secretion from GLUTag cells and primary murine small intestinal cells in a RhoA and PLC-dependent manner. These effects of LPI were blunted by a GPR55 siRNA and involved TRPV2 channel activation [53]. Collectively, these studies implicate LPIs and GPR55 in the control of glucose homeostasis. However, glucose tolerance and glucose-induced insulin secretion were not affected in $GPR55^{-/-}$ mice compared with wild type littermates [48]. Thus, further research *in vivo* is needed to clarify the involvement of LPIs and GPR55 in metabolic diseases and diabetes. In this context, one study suggested that GPR55 was implicated in the improvement of plasma glucose and insulin resistance in mice with streptozocin-induced diabetes [54]. However, this was done following administration of abnormal cannabidiol, which was shown in another study to have effects in $GPR55^{-/-}$ mice, despite stimulating GPR55-dependent GTPγS activation [36].

Cell Migration and Proliferation

A role for LPIs in cancer has been postulated for years, as LPIs stimulate proliferation of differentiated and transformed thyroid cells and are released by cancer cells [17,55–57]. Moreover, LPI levels were increased in the blood and ascites of patients with ovarian or peritoneal cancer, and in the blood of patients with colon cancer, compared with healthy individuals [58–60]. In breast and colorectal cancers, GPR55 expression is correlated with tumor aggressiveness, metastases, and poor prognosis [61–63]. The importance of GPR55 in tumorigenicity is also apparent from studies in non-small cell lung cancer, where it was shown that a microRNA targeting GPR55 (miR-675-5p) was decreased in tumors compared with adjacent tissue and negatively correlated with metastasis. Accordingly upregulation of miR-675-5p downregulated GPR55 and decreased cell growth, proliferation, migration, and invasiveness [64].

GPR55, which is expressed in many human cancer cell line models, and LPIs were reported to be involved in proliferation, migration, and invasion of breast, ovarian, prostate, and glioblastoma cancer cells *in vitro* [17,60,61,65,66]. Accordingly, knockdown of GPR55 decreases cell

proliferation and migration, while its overexpression or incubation with LPIs have the opposite effect [17,60–63,65,66]. LPIs also stimulate angiogenesis in a GPR55-dependent manner [57]. Studies also point to a role of GPR55 in cancer progression *in vivo*. For instance, GPR55 knockdown reduces tumor growth in xenografted nude mice [61]. Moreover, GPR55-deficient mice were more resistant to carcinogen-induced carcinoma formation, azoxymethane-induced colorectal cancer, and colitis-associated colorectal cancer compared with wild type mice [63]. Mice injected with MDA-MB-231 breast cancer cells that endogenously express GPR55 had more lung metastases when treated with LPIs than vehicle, an effect that was modulated when GPR55 was knocked down or overexpressed in the injected cells [62]. However, while most of these studies point to a procarcinogenic role of GPR55 activation, Anandamide and O-1602 were shown to have antiproliferative effects in cholangiocarcinoma in a GPR55-dependent manner [67].

Based on these data, it will be interesting to see whether the novel GPR55 antagonists are able to decrease the development of cancer in preclinical models *in vivo*. In light of the data obtained by knocking down GPR55, colon cancer and breast cancer models, as well as the metastasization processes, appear to be interesting starting points.

Pain

A pronociceptive role for GPR55 was put forward: GPR55 activation by LPI in periaqueductal gray neurons reduces the thermal nociceptive threshold, an effect blocked by the selective GPR55 antagonist ML193 [68]. It is worth noting, however, that depending on the dose and force of mechanical stimulation, LPI-induced nociceptive hypersensitivity (following intraplantar administration) was abrogated, reduced, or unchanged in GPR55$^{-/-}$ mice [69]. In the same setting, LPI-induced tactile allodynia was more affected by deletion of $G\alpha_q$ and $G\alpha_{12/13}$ from sensory neurons than by GPR55 deletion, suggesting that GPCRs other than GPR55 could also be involved in its effects [69]. Indeed, LPI was proposed as a ligand for GPR119 [70] and has non-GPCR targets, such as potassium channels, that could also be implicated in nociception [45,71].

Loss of GPR55 also affects thermal nociception, although it is unclear in which direction. Indeed, male GPR55$^{-/-}$ mice display mild thermic hyperalgesia [47], whereas the opposite was observed for female GPR55$^{-/-}$ mice but only at 50°C [34,72,73].

Opposing results on mechanical hyperalgesia were also reported in GPR55$^{-/-}$ mice. One study found that deletion of GPR55 abolished mechanical hyperalgesia following inflammatory or neuropathic insults [72], while another study found no differences [73]. It is noteworthy that in the latter study, the authors used several models of inflammatory and neuropathic pain and different outputs to measure hyperalgesia [73]. Besides the use of different knock-out mice, the differences between studies could also be due to the different methods used to measure hyperalgesia.

Finally, the GPR55 antagonists ML193 and CID16020046 blocked the analgesic effects of the endocannabinoid 2-AG in a model of antiretroviral-induced neuropathic pain in mice [74]. Whether this is due to 2-AG acting at the GPR55 receptor or to the **cross-antagonism** between cannabinoid receptors and GPR55 [75] remains to be elucidated.

Inflammation

GPR55 is expressed in immune cells [76–79] and its mRNA expression in these cells is modulated by inflammatory stimuli, which suggests a role for GPR55 in inflammation [76].

In support of this hypothesis, murine studies suggest a proinflammatory role of GPR55 in gastrointestinal inflammation, as experimental colitis was less severe in GPR55$^{-/-}$ mice compared with wild type mice [37,80]. Accordingly, the GPR55 antagonist CID16020046 reduced colon inflammation in murine colitis and inhibited lymphocyte and macrophage infiltration into the colon lamina propria. This effect could be due to decreased macrophage activation, as CID16020046 decreased migration and CD11b mRNA expression in J774 macrophages. Conversely, infiltration of neutrophils into the lamina propria was enhanced by the GPR55 antagonist. The opposite was observed *in vitro* where CID16020046 decreased the migration of human neutrophils [80]. Accordingly, activation of GPR55 modulates the effects of CB_2 activation and induces migration in human neutrophils [77].

The modulation of immune cell activation by GPR55 and its ligands could also have an effect in the central nervous system. Indeed, while LPI alone induced microglia chemotaxis, it inhibited ATP-induced microglia migration and exerted neuroprotective effects in rat hippocampal slices exposed to NMDA. The neuroprotective effects of LPI were absent in the presence of GPR55 siRNA [81]. Contrasting with these neuroprotective effects of GPR55 activation, but consistent with the proinflammatory role of GPR55, C57BL/6 GPR55$^{-/-}$ mice developed less severe experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis. This was, however, dependent on the background of the mice and no impact of knock-out was observed in the ABH mouse background [82]. Thus, the evidence available points to a rather proinflammatory role of GPR55; whether this is confirmed and translated into GPR55-antagonists as anti-inflammatory drugs remains to be explored.

Central Nervous System Function

The role of GPR55 in central nervous system (CNS) functions has recently attracted interest. GPR55 boosts neurotransmitter release in rat hippocampal slices and affects GABAergic transmission, dopamine activity, and hippocampal synaptic plasticity in rodents, suggesting a role for GPR55 in synaptic transmission, social interaction, and memory processing [83–86]. Indeed, the GPR55 antagonist CID16020036 impaired learning of rats in the T maze paradigm [87]. However, knockdown of GPR55 did not affect synaptic transmission, short-term and long-term synaptic plasticity, conditioned fear learning and memory, gross motor skills, or anxiety and behavior, but it impaired more challenging motor coordination responses [34].

GPR55 also plays a role in modulation of anxiety-like behavior in rodents, with GPR55 agonists and antagonists exhibiting anxiolytic and anxiogenic effects, respectively [88,89].

GPR55 was also suggested to play a role in axonal navigation and refinement, notably during development. Indeed, lysophosphatidyl-β-D-glucoside GPR55-dependently mediates guidance of nociceptive afferent axons in the spinal cord [90]. Moreover, LPIs cause neurite retraction in differentiated PC12 cells in culture and modulates axon growth and target innervation of retinal projections *in vitro* and *in vivo* via GPR55 [91,92].

Finally, a potential role for GPR55 in Parkinson's disease treatment was suggested, as GPR55 expression was decreased in the striatum in a murine model of Parkinson's disease. Accordingly, GPR55 agonists had an anticataleptic effect in mice treated with haloperidol to mimic the symptomatic motor difficulties of Parkinson's disease patients [93]. However, while most of these studies point to a beneficial role of GPR55 activation in the CNS, GPR55 antagonists had beneficial effects on seizures and social deficits in a mouse model of Dravet syndrome, a form of childhood epilepsy [94].

Other Effects

Gut Movement

A role for GPR55 in gut motility was suggested when lipopolysaccharide-induced septic ileus, which slows upper gastrointestinal transit, increased GPR55 expression in the ileum [95]. However, the authors reported similar effects in that study for O-1602 and cannabidiol, reported respectively as an agonist and antagonist of GPR55, which complicates the interpretation of the data [95]. Since then, two other studies reported inhibitory effects of O-1602 on intestinal contractility and colonic motility in mice that were blunted in $GPR55^{-/-}$ mice [96,97], suggesting that GPR55 could be a target for the treatment of colonic motility disorders.

Bone Turnover

One of the first reported roles of the LPI/GPR55 axis has been described in bone morphogenesis [98]. GPR55 activation (with LPI and O-1602) inhibited osteoclast formation while stimulating their polarization and resorption *in vitro*. This effect was attenuated in osteoclasts from $GPR55^{-/-}$ mice. Consistent with the *in vitro* data, long bones from male $GPR55^{-/-}$ mice showed increased numbers of inactive osteoclasts, unresorbed cartilage, and increased trabecular bone volume and thickness. However, no effect on bone volume and thickness was observed in female $GPR55^{-/-}$ mice who, while also presenting increased unresorbed cartilage, had a reduced number of osteoclasts [98]. Further studies will have to determine whether the LPI/GPR55 axis could be an interesting target in osteoporosis.

Concluding Remarks and Future Perspectives

While it is now widely accepted that LPIs are GPR55 ligands and that some of the cannabinoid ligands are also GPR55 ligands, many of the compounds tested in the literature as GPR55 ligands lack selectivity and have GPR55-independent effects. This has led to contradictory observations and possible misinterpretations and hinders the discovery of the pathophysiological roles of GPR55 (see Outstanding Questions). Moreover, it is possible that GPR55 could have other endogenous ligands than LPIs and that LPIs have other targets than GPR55. Despite these hurdles, GPR55 was implicated in many physiological and pathological processes that deserve further exploration. The recent development of selective agonists and antagonists of this receptor could help in moving the field forward. For instance, using antagonists to confirm the effects obtained with knock-out animals would provide further evidence of the druggability of GPR55 in inflammation and cancer. Another potential hurdle in the way to understanding the role of GPR55 in human physiology is the difference in results between humans and rodents, notably when it comes to obesity and metabolic diseases. Finally, it should be noted that while GPR55 activation seems beneficial in some settings, such as obesity and diabetes, it is rather deleterious in other settings, such as cancer. Therefore, antagonists of GPR55 might prove effective as cancer treatments. However, as GPR55 seems to be implicated in the control of mood and behavior, caution should be taken with the development and use of compounds targeting GPR55 and their potential side effects. Therefore, as it is often the case with receptors having multiple functions, delineating which diseases could actually benefit from a modulation of LPI and GPR55 will be a key question in the future.

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Supplemental Information

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Outstanding Questions

Does GPR55 have other endogenous ligands as well as LPIs? As mentioned lysophosphatidyl-β-D-glucoside (LysoPtdGlc), differing from LPI by the sugar moiety, was recently reported to be a ligand for GPR55. Some lysophosphatidylcholines were also suggested to activate GPR55. Future studies will have to confirm this.

What are the other receptors for LPIs? The studies discussed here suggest that, besides GPR55, additional receptor(s) might mediate LPIs' effects. The closest receptor in terms of homology is GPR35, however 2-arachidonoyl LPI did not mediate GPR35-dependent changes in Ca^{2+}. This leaves the question open for future research.

LPIs are both bioactive lipids and intermediates in membrane remodelling. To what extent the changes in LPI levels measured in a given pathophysiological condition are due to changes in 'bioactive' LPI levels remains to be answered.

Selective GPR55 ligands can be potential therapeutic tools; however, the selectivity of GPR55 ligands remains an issue, notably with the proposed cannabinoid ligands.

- No binding assay so far to screen for GPR55 ligands is available. While the absence of a high-affinity (relatively) selective ligand precluded the development of such an assay, the new ligands should be amenable to radiolabelling.
- Complex pharmacology of the identified ligands, with biased agonism, allosteric modulators, and partial agonists is an opportunity to fine-tune GPR55's effects but clearly complicates the development of these drugs.
- Because of the poor selectivity of most GPR55 ligands reported so far, the use of a putative GPR55 agonist should be validated by using a different agonist, blocking with a selective antagonist, and eventually using siRNA or knock-out mice in order to properly dissect the pathophysiological roles of GPR55.

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