GPR55: a new promising target for metabolism?

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Abstract

GPR55 is a G-protein-coupled receptor (GPCR) that has been identified as a new cannabinoid receptor. Given the wide localization of GPR55 in brain and peripheral tissues, this receptor has emerged as a regulator of multiple biological actions. Lysophosphatidylinositol (LPI) is generally accepted as the endogenous ligand of GPR55. In this review, we will focus on the role of GPR55 in energy balance and glucose metabolism. We will summarize its actions on feeding, nutrient partitioning, gastrointestinal motility and insulin secretion in preclinical models and the scarce data available in humans. The potential of GPR55 to become a new pharmaceutical target to treat obesity and type 2 diabetes, as well as the foreseeing difficulties are also discussed.

The endocannabinoid system in energy balance

The endocannabinoid system (ECS) is involved in a wide range of biological processes that include psychological, cardiovascular or respiratory functions. However, one of the aspects that have attracted more attention over the last years is undoubtedly its ability to modulate energy metabolism. The best-characterized endocannabinoids are N-arachidonoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), which exert their effects by binding to the specific G-protein-coupled receptors CB1 and CB2 (Ligresti et al. 2016). These receptors are abundantly expressed throughout the central nervous system (CNS), including areas that control food intake and energy expenditure (hypothalamus and brainstem) and reward-related responses (nucleus accumbens), as well as in peripheral tissues affecting metabolic homeostasis, such as the gastrointestinal (GI) tract, adipose tissue, liver and muscle (Ligresti et al. 2016).

A significant amount of research has demonstrated that activation of the CB1 receptor by cannabinoid ligands stimulates food intake (Di Marzo & Matias 2005), reduces GI motility (Calignano et al. 1997) and increases lipogenesis and steatosis in the liver (Osei-Hyiaman et al. 2008). In addition, activation of CB1 increases lipogenesis and inflammation and reduces lipolysis, in the white adipose tissue (WAT) (Silvestri & Di Marzo 2013).

Furthermore, CB1 activation impairs insulin sensitivity, as evidenced by reduced glucose uptake in the skeletal muscle (Esposito et al. 2008). CB1 is the main receptor of the ECS involved in metabolic functions. Hence, selective inhibitors for this receptor have been developed, with the
CB1 inverse agonist SR141716A pyrazole (Rimonabant) approved as anti-obesity therapy, although it was later discontinued because of its psychiatric side effects.

**GPR55: a putative cannabinoid receptor**

Although there is a good understanding of the consequences of CB1 and CB2 activation, studies using either CB1 and CB2 agonists and knock-out mice for CB1 and/or CB2 have shown that there is an additional cannabinoid receptor that responds to the cannabinoid stimulus (Begg *et al.* 2005, Baker *et al.* 2006, Ryberg *et al.* 2007). The seven-transmembrane G-protein-coupled receptor 55 (GPR55) was initially identified as a novel cannabinoid receptor when *in silico* screening of patents by GlaxoSmithKline and AstraZeneca revealed that it interacts with some cannabinoid receptor agonists and antagonists (Baker *et al.* 2006). GPR55 couples to G$_\alpha_{12/13}$ and G$_q$ proteins and is widely expressed in both brain and peripheral tissues (Ross 2009) (for details, see 'Distribution of GPR55 in humans and rodents' section), suggesting its involvement in multiple biological actions. The sequence of GPR55 only exhibits 13% and 14% homology with CB1 and CB2, respectively (Elbegdorj *et al.* 2013). However, it has the ability to interact with and be modulated by endogenous (endocannabinoids), plant (phytocannabinoids) and small synthetic cannabinoid ligands; therefore, GPR55 could be responsible for the non-CB1/CB2 receptor effects of those ligands (Baker *et al.* 2006, Ryberg *et al.* 2007).

**Distribution of GPR55 in humans and rodents**

The distribution of Gpr55 mRNA expression has been identified in detail (Fig. 1); however, there is less information regarding GPR55 protein levels. In addition, rat and mouse Gpr55 gene sequence share 75% and 78% homology with the human sequence, respectively (Ryberg *et al.* 2007), so the results derived from different species need to be carefully considered as some differences have been reported.

The GPR55 receptor is present in multiple areas of the human brain including hypothalamus, nucleus accumbens, caudate nucleus, striatum and putamen (Sawzdargo *et al.* 1999, Henstridge *et al.* 2011). Studies in rodent CNS also showed a wide expression Gpr55 mRNA in hippocampus, thalamus, forebrain, frontal cortex, hypothalamus, cerebellum and striatum (Chiba *et al.* 2011, Sylantyev *et al.* 2013, Wu *et al.* 2013, Méndez-Díaz *et al.* 2016), and GPR55 mRNA has also been found in monkey striatum (Martinez-Pinilla *et al.* 2014). In addition, there is evidence that GPR55 is expressed at the

![Figure 1](https://www.jme.endocrinology-journals.org/article-pdf/58/3/192/2690468/jme_58_3_0192.pdf)
protein level in the dorsal root ganglia neurons, where its activation enhances the excitability of sensory neurons (Lauckner et al. 2008).

At the peripheral level, Gpr55 is widely distributed. Several reports detected Gpr55 expression throughout the GI in both human (Henstridge et al. 2011) and rodents (Ryberg et al. 2007, Lin et al. 2011, Schicho et al. 2011, Schicho & Storr 2012, Li et al. 2013). The gene expression pattern differs between areas, with higher levels in mouse jejunum and ileum than those in colon or stomach (Ryberg et al. 2007). Apart from being found in the submucosa (Lin et al. 2011), Gpr55 is also expressed in neurons from the myenteric plexus (Lin et al. 2011, Li et al. 2013) pointing to a possible role of Gpr55 in GI physiological functions such as motility and secretion. Gpr55 mRNA has also been described in the liver of humans (Moreno-Navarrete et al. 2012) and mice (Romero-Zerbo et al. 2011); however, the hepatic function of the LPI/GPR55 system is still unknown. In the pancreas, both human (Song et al. 2012) and rodent (Romero-Zerbo et al. 2011, McKillop et al. 2013) islets express GPR55 as indicated from gene and protein expression studies. Furthermore, immunohistochemical analysis of rat and mouse pancreas sections showed GPR55 expression specifically in insulin-secreting β-cells, but not in glucagon- or somatostatin-releasing α- and δ-cells, respectively (Romero-Zerbo et al. 2011, McKillop et al. 2013). Very recently, the presence of GPR55 in few mouse glucagon-secreting cells and in a major portion of human α-cells has been reported (Liu et al. 2016). Regarding the adipose tissue, both GPK5 protein and mRNA have been detected in human and rodents. The expression of GPR55 in human WAT was firstly reported by Moreno-Navarrete and coworkers (Moreno-Navarrete et al. 2012). When comparing obese and lean subjects, the authors observed increased GPR55 expression in visceral adipose tissue (VAT) from obese patients, with the highest level in those who also had type 2 diabetes (T2D). A similar pattern was detected in human subcutaneous adipose tissue (SAT). Accordingly, plasma LPI levels were also higher in obese patients compared to those in lean subjects and correlated with fat percentage and BMI in women. A recent study reported that a number of factors regulate rodent WAT Gpr55 expression. Imbernon and coworkers showed increased WAT Gpr55 after fasting, which was reversed after refeeding and also after leptin treatment (Imbernon et al. 2014). The measurements of circulating LPI showed the inverse pattern to that observed for the receptor expression. Augmented WAT Gpr55 expression was also detected throughout lifespan, as well as in ovariectomized rats. In contrast, WAT Gpr55 decreased during gestation, possibly as a result of enhanced leptin levels, as well as after orchidectomy (Imbernon et al. 2014). Thus, rodent WAT Gpr55 expression is modulated by nutritional status, gonadal steroids, gestation, postnatal development and pituitary factors. GPR55 is also expressed in both human and mice osteoclasts and osteoblasts, and GPR55 agonists favor in vitro osteoclasts resorption (Whyte et al. 2009). In addition, GPR55 is also present in cell lines derived from human bone and cartilage sample (Henstridge et al. 2011). Human immune cells express GPR55, indicating it could be implicated in chemotaxis, although this is still under investigation (Balenga et al. 2011, Henstridge et al. 2011). GPR55 has also been detected in other organs and tissues such as placenta, proximal tubule cells of kidney and mast cells (with unknown functions) (Henstridge et al. 2011).

The complex pharmacology of GPR55 (ligands and interactions with CB1/CB2 agonists/antagonists) (special focus on LPI, the endogenous ligand)

Although we are starting to learn the biological functions of GPR55, achieving a good understanding is quite difficult due to the lack of functional selectivity within the pharmacology of this receptor (Ross 2009) (Table 1). The effects of cannabinoids through GPR55 have been investigated using different pharmacological approaches such as the [35S]GTPγS-binding assay (measurement of GPR55 agonist-induced GDP-GTP exchange as an indicator of receptor activation), intracellular Ca2+ transients, phosphorylation of ERK or activation of GTPase proteins and β-arrestin recruitment models (Oka et al. 2007, 2009, Ryberg et al. 2007, Lauckner et al. 2008, Waldbeck-Weitkamp et al. 2008, Henstridge et al. 2009, Kapur et al. 2009, Ross 2009, 2011, Pertwee et al. 2010, Whyte et al. 2015, Zeng et al. 2015). Moreover, in vivo studies have allowed a better knowledge of the physiological relevance of GPR55 signaling in processes such as energy metabolism (Romero-Zerbo et al. 2011, Diaz-Arteaga et al. 2012, Moreno-Navarrete et al. 2012, Imbernon et al. 2014), inflammation (Staton et al. 2008), vascular function (Daly et al. 2010), bone physiology (Whyte et al. 2009), cancer (Perez-Gomez et al. 2012, Andradas et al. 2016) and GI disorders (Hasenoehrl et al. 2016). Taking into account the large amount of physiological systems in which GPR55 is involved, the design of potent and tissue-specific GPR55 ligands remains an important field to develop new drugs.
Table 1  Effects of GPR55 agonists/antagonists on different metabolic parameters from *in vivo* studies.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Drug</th>
<th>Action on GPR55</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt;/IC&lt;sub&gt;50&lt;/sub&gt; values for GPR55, CB1 and CB2</th>
<th>Dose/route administ.</th>
<th>Main outcomes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1 mice</td>
<td>AM-251</td>
<td>Agonist</td>
<td>39 nM, −, −</td>
<td>0.1 mg/kg, i.p.</td>
<td>No effects on whole gut transit or colonic propulsion</td>
<td>Li et al. (2013)</td>
</tr>
<tr>
<td>CD1 mice</td>
<td>CBD</td>
<td>Antagonist</td>
<td>0.445, 3.35, 27.5 µM</td>
<td>0.5 mg/kg, i.p.</td>
<td>No effects on whole gut transit or colonic propulsion</td>
<td>Li et al. (2013)</td>
</tr>
<tr>
<td>CD1 mice</td>
<td>O-1602</td>
<td>Agonist</td>
<td>13, &gt;30,000, &gt;30,000 nM</td>
<td>10 mg/kg, i.p.</td>
<td>Slowed whole gut transit and colonic bead expulsion, no effect on gastric emptying</td>
<td>Li et al. (2013)</td>
</tr>
<tr>
<td>CD1 mice</td>
<td>O-1602</td>
<td>Agonist</td>
<td>13, &gt;30,000, &gt;30,000 nM</td>
<td>10 mg/kg, i.p. or 10 µg i.c.v.</td>
<td>No effects on locomotor activity</td>
<td>Li et al. (2013)</td>
</tr>
<tr>
<td>GIP&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>Abn-CBD</td>
<td>Agonist</td>
<td>2.5, &gt;30, &gt;30 µM</td>
<td>0.1 µmol/kg, i.p.</td>
<td>Increased GSIS and improved glucose tolerance</td>
<td>McKillop et al. (2016)</td>
</tr>
<tr>
<td>GLP-1&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>Abn-CBD</td>
<td>Agonist</td>
<td>2.5, &gt;30, &gt;30 µM</td>
<td>0.1 µmol/kg, i.p.</td>
<td>No effects on GSIS or glucose tolerance</td>
<td>McKillop et al. (2016)</td>
</tr>
<tr>
<td>GPR55&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>O-1602</td>
<td>Agonist</td>
<td>13, &gt;30,000, &gt;30,000 nM</td>
<td>200 µg/kg, i.p.</td>
<td>Increased food intake</td>
<td>Diaz-Arteaga et al. (2012)</td>
</tr>
<tr>
<td>GPR55&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>O-1602</td>
<td>Agonist</td>
<td>13, &gt;30,000, &gt;30,000 nM</td>
<td>10 mg/kg, i.p.</td>
<td>No changes in gut transit and colonic bead expulsion</td>
<td>Li et al. (2013)</td>
</tr>
<tr>
<td>GPR55&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>O-1602</td>
<td>Agonist</td>
<td>13, &gt;30,000, &gt;30,000 nM</td>
<td>0.1–30 µmol/L</td>
<td>Reduced inhibition of neurogenic contractions</td>
<td>Ross et al. (2012)</td>
</tr>
<tr>
<td>GPR55&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>CID16020046</td>
<td>Antagonist</td>
<td>0.21 µM, −, −</td>
<td>20 mg/kg, i.p.</td>
<td>Improved intestinal inflammation, decreased proinflammatory cytokines and leukocyte recruitment, unchanged locomotor activity</td>
<td>Stančić et al. (2015)</td>
</tr>
<tr>
<td>NIH Swiss mice</td>
<td>Abn-CBD</td>
<td>Agonist</td>
<td>2.5, &gt;30, &gt;30 µM</td>
<td>0.1 µmol/kg, i.p.</td>
<td>Increased GSIS ad improved glucose tolerance</td>
<td>McKillop et al. (2013)</td>
</tr>
<tr>
<td>NIH Swiss mice</td>
<td>AM-251</td>
<td>Agonist</td>
<td>39 nM</td>
<td>0.1 µmol/kg, i.p.</td>
<td>Increased GSIS ad improved glucose tolerance</td>
<td>McKillop et al. (2013)</td>
</tr>
<tr>
<td>NIH Swiss mice</td>
<td>O-1602</td>
<td>Agonist</td>
<td>13, &gt;30,000, &gt;30,000 nM</td>
<td>0.1 µmol/kg, i.p.</td>
<td>Increased GSIS ad improved glucose tolerance</td>
<td>McKillop et al. (2013)</td>
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Table 1  Continued.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Drug</th>
<th>Action on GPR55</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt;/IC&lt;sub&gt;50&lt;/sub&gt; values for GPR55, CB1 and CB2</th>
<th>Dose/route administ.</th>
<th>Main outcomes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH Swiss mice</td>
<td>OEA</td>
<td>Agonist</td>
<td>0.44, 30, 30 µM</td>
<td>0.1 µmol/kg, i.p.</td>
<td>Increased GSIS and improved glucose tolerance</td>
<td>McKillop et al. (2013)</td>
</tr>
<tr>
<td>NIH Swiss mice</td>
<td>PEA</td>
<td>Agonist</td>
<td>4, 30,000, 19,800 nM</td>
<td>0.1 µmol/kg, i.p.</td>
<td>No significant differences in either GSIS or glucose tolerance</td>
<td>McKillop et al. (2013)</td>
</tr>
<tr>
<td>STZ-mice</td>
<td>Abn-CBD</td>
<td>Agonist</td>
<td>2.5, &gt;30, &gt;30 µM</td>
<td>0.1 µmol/kg/day, oral</td>
<td>Reduced food intake, no changes in body weight, lowered blood glucose and plasma glucagon, increased plasma insulin and pancreatic insulin content, improved glucose tolerance and insulin sensitivity, and decreased cholesterol and triacylglycerol</td>
<td>McKillop et al. (2016)</td>
</tr>
<tr>
<td>Sprague–Dawley rats</td>
<td>O-1602</td>
<td>Agonist</td>
<td>13, &gt;30,000, &gt;30,000 nM</td>
<td>0.1–0.5 µg, i.c.v.</td>
<td>Increased food intake, decreased CART mRNA and protein levels</td>
<td>Diaz-Arteaga et al. (2012)</td>
</tr>
<tr>
<td>Sprague–Dawley rats</td>
<td>O-1602</td>
<td>Agonist</td>
<td>13, &gt;30,000, &gt;30,000 nM</td>
<td>2–200 µg/kg, i.p.</td>
<td>Transiently increased food intake</td>
<td>Diaz-Arteaga et al. (2012)</td>
</tr>
<tr>
<td>Sprague–Dawley rats</td>
<td>O-1602</td>
<td>Agonist</td>
<td>13, &gt;30,000, &gt;30,000 nM</td>
<td>0.04–0.4 µg/h, i.c.v.</td>
<td>Reduced WAT GLUT1 and GLUT4 mRNA levels, increased fat mass change</td>
<td>Diaz-Arteaga et al. (2012)</td>
</tr>
<tr>
<td>Sprague–Dawley rats</td>
<td>O-1602</td>
<td>Agonist</td>
<td>13, &gt;30,000, &gt;30,000 nM</td>
<td>0.1 mg/kg/day, i.p.</td>
<td>Unchanged food intake and body weight gain, increased fat mass, decreased WAT ATGL and increased WAT IL-6 mRNA levels</td>
<td>Diaz-Arteaga et al. (2012)</td>
</tr>
<tr>
<td>Wistar rats</td>
<td>O-1602</td>
<td>Agonist</td>
<td>13, &gt;30,000, &gt;30,000 nM</td>
<td>0.2 mg/kg, i.p.</td>
<td>Improved glucose tolerance, increased GSIS</td>
<td>Romero-Zerbo et al. (2011)</td>
</tr>
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</table>

**Endocannabinoids**  The main endocannabinoids AEA (PEA, virodhamine and oleoylethanolamide (OEA)) and 2-AG, that bind to both CB1 and CB2 receptors, are synthesized and released ‘on demand’, a process that can be regulated by both physiological and pathological conditions (Fowler et al. 2005). The activation of GPR55 by the ‘classical’ endocannabinoids shows controversial results, and there is no consensus in how AEA modulates GPR55 signaling due to the diversity of pathways that can be activated by this ligand (Ross 2009). In human embryonic kidney 293 (HEK293) cells transiently expressing GPR55, AEA induces Ca<sup>2+</sup> mobilization (Lauckner et al. 2008, Waldeck-Weiermair et al. 2008). However, the concentration of AEA necessary to activate GPR55 is much higher than normally required to activate the CB1 receptor (Pertwee 2007, Brown & Robin Hiley 2009, Ross 2009). Noladin-ether, palmitoylethanolamine (PEA), virodhamine and oleoylethanolamide (OEA) are endogenous lipids that have also been reported to modulate GPR55 signaling (Ryberg et al. 2007). Yet, even though PEA and virodhamine display high affinity for GPR55 (Ryberg et al. 2007), they failed to elicit Ca<sup>2+</sup> responses (Lauckner et al. 2008).

**Phytocannabinoids and synthetic canna­binoids**  Phytocannabinoids (cannabinoids naturally occurring in the cannabis plant) do not only exert their actions through CB1 and CB2 receptors. Reportedly, the main pharmacoactive component of cannabis, Δ⁹-tetrahydrocannabinol (Δ⁹-THC), acts as a GPR55 agonist mediating intracellular Ca<sup>2+</sup> transients (Ryberg et al. 2007, Lauckner et al. 2008), although another report showed no activation of ERK signaling by Δ⁹-THC (Oka et al. 2007).
This discrepancy also occurs with the abnormal non-psychoactive cannabidiol (Abn-CBD); two studies reported GPR55 activation by this compound (Johns et al. 2007, Ryberg et al. 2007), whereas it failed to mobilize Ca\(^{2+}\) in a different one (Lauckner et al. 2008).

Due to the complexity of the GPR55-binding pocket structure and signaling, it is difficult to synthesize specific agonists or antagonists. In this sense, there are some studies trying to develop new drugs targeting GPR55 using different pharmacological approaches (Brown et al. 2011, Heynen-Genel et al. 2011, Morales et al. 2016). Most of those new synthetic compounds (reviewed in Morales & Jagerovic 2016) are helping to elucidate the structural motifs involved in the ligand–GPR55 interaction and contribute to the rational design of new GPR55 ligands. Several studies have reported GPR55 agonist activity for the synthetic Δ⁹-THC analogue HU210, CBD and its synthetic analogue O-1602 (Ryberg et al. 2007, Waldeck-Weiermair et al. 2008, Oka et al. 2009). However, two other studies suggested that CBD may act as GPR55 antagonist (Whyte et al. 2009, Li et al. 2013). Other synthetic cannabinoids with potent effects over CB1 and/or CB2 receptors also seem to modulate the GPR55 signaling. The CB2 receptor agonist JWH015 activates GPR55 signaling in different cell types, modulating intracellular Ca\(^{2+}\) transients (Oka et al. 2007, Lauckner et al. 2008); however, its structural analogue WIN55212 failed to activate GPR55 (Oka et al. 2007, Ryberg et al. 2007, Lauckner et al. 2008). Finally, the synthetic cannabinoids pyrazoles, strong antagonists of the CB1 receptor, show diverse effects through GPR55. AM251 is a high-affinity ligand of GPR55 and modulates Ca\(^{2+}\) transients (Ryberg et al. 2007, Henstridge et al. 2009), whereas AM281 has no affinity for GPR55 (Ryberg et al. 2007). The outcomes obtained when using a β-arrestin–green fluorescent protein biosensor conferred Rimonabant agonist activity (Kapur et al. 2009). However, it has also been reported that Rimonabant is capable of blocking the GPR55 signaling responses induced by other agonist compounds such as JWH015 and AEA, which for instance trigger intracellular Ca\(^{2+}\) transients (Lauckner et al. 2008, Waldeck-Weiermair et al. 2008). CID-16020046 is a recently synthesized compound that selectively inhibits GPR55 without inducing any effect via CB1 or CB2, in both yeast and HEK293 cells expressing human GPR55 (Kargl et al. 2013). In addition, a β2-adrenergic agonist, (R,R′)-40-methoxy-1-naphthylfenoterol (AMF), has been described as a potent GPR55 inhibitor, blocking both O-1602- and AM251-induced GPR55 signaling in cancer cell lines (Paul et al. 2014) and reducing the chemoresistance of carcinogenic cells (Singh et al. 2016).

**Lyosphatidylinositol (LPI): the endogenous ligand of GPR55** LPI is a lipid signaling molecule generated by phosphatidylinositol hydrolysis via the action of the Ca\(^{2+}\)-dependent phospholipase A2 (19) and Ca\(^{2+}\)-independent phospholipase A1 (Billah & Lapetina 1982) and interacts with specific G-protein-coupled receptors (Ishii et al. 2004, Arifin & Falasca 2016). Recently, LPI has been proposed as the endogenous ligand of GPR55 based on in vitro and in vivo studies. In 2007, Oka and coworkers detailed that LPI induced rapid phosphorylation of ERK and increased intracellular Ca\(^{2+}\) in GPR55-expressing HEK293 cells (Oka et al. 2007), a finding that was corroborated in subsequent studies (Lauckner et al. 2008, Henstridge et al. 2009, Kapur et al. 2009, Oka et al. 2009). Similar effects of LPI/GPR55 on intracellular Ca\(^{2+}\) metabolism, requiring Ras homologue A protein (RhoA) activation and ERK phosphorylation, have also been described in cells expressing human recombinant GPR55 (Ryberg et al. 2007, Henstridge et al. 2009, Oka et al. 2009) and in cultured human and mouse osteoclasts expressing native GPR55 (Whyte et al. 2009).

Previous to the description of LPI as the endogenous ligand of GPR55, relevant biological functions had been associated to LPI such as being an inducer of insulin release (Metz 1986), a mediator of hepatic vitamin D3-modulating Ca\(^{2+}\) metabolism (Baran & Marie Kelly 1988) and a mitogenic modulator in both neuron and endothelial cells (Falasca et al. 1995, Corda et al. 2002). Nowadays, how GPR55 contributes to these LPI-mediated effects is an object of research.

Recent evidence reveals that both LPI and GPR55 are main factors in driving cell proliferation and migration (Ross 2011). Clinical data showed a positive correlation between the malignancy of ovarian cancer and the LPI content in ascites and serum (Sutphen et al. 2004). On the contrary, there is a negative correlation between GPR55 expression in human breast and glioblastoma tumors and their aggressiveness, and patient survival is lower in patients whose glioblastomas express higher levels of GPR55 (Andradas et al. 2011). Cells transformed by the ras oncopogene are capable of releasing LPI (Falasca et al. 1998), and there is a close relation between the LPI/GPR55 system and cell migration and proliferation; therefore, it has been postulated that LPI might serve as an autocrine factor in cancer cells (Ross 2011), with special relevance in bone cancer (Tonyali et al. 2010, Pineiro et al. 2011, Ross 2011).
Given the increasing evidence linking LPI/GPR55 and obesity and cancer (Calle & Kaaks 2004, Basen-Engquist & Chang 2011), it will be important to know the precise mechanisms regulated by the GPR55 pharmacology in both diseases.

Metabolic actions of GPR55

Metabolic actions of GPR55 in the CNS

Given its extensive distribution in the brain, GPR55 participates in a number of physiological processes that include movement coordination and motor activity (Wu et al. 2013, Bjursell et al. 2016), nociception (Deliu et al. 2015), modulaion of anxiety-like behaviors (Rahimi et al. 2015), as well as energy expenditure (Bjursell et al. 2016, Meadows et al. 2016) (Fig. 1). Energy homeostasis is mainly determined by the balance between energy intake (from food ingestion) and energy expenditure (from basal metabolic rate, thermogenesis and physical activity). To decipher the effects of central GPR55 signaling on food consumption, a number of pharmacological studies have been performed in animal models; however, many of them employed cannabinoid receptor/lipid biased compound libraries, which lead to lack of receptor selectivity (Kotsikorou et al. 2013). For instance, rats that followed a treatment with CBD displayed reduced body weight gain and food intake; however, those effects were not detected when animals also received AM630, a selective CB2 antagonist, suggesting that the CB2 receptor might be involved in the outcomes previously observed (Ignatowska-Jankowska et al. 2011). Similarly, although the intracerebroventricular (i.c.v.) administration of the atypical cannabinoid O-1602 acutely stimulates food intake in rats, a parallel experiment performed in GPR55−/− mice revealed that effect of O-1602 on food consumption was independent of GPR55 (Diaz-Arteaga et al. 2012). Moreover, Gpr55−/− mice did not exhibit different food intake compared to their control littermates (Bjursell et al. 2016, Meadows et al. 2016). Nonetheless, the fact that a dysfunctional alteration of the Gpr55 gene is associated to increased vulnerability to anorexia nervosa in Japanese women, suggests that Gpr55 plays a role in the regulation of feeding behavior (Ishiguro et al. 2011).

Although the extent to which GPR55 modulates food consumption is unclear, phenotyping studies using Gpr55−/− mice showed decreased energy expenditure and spontaneous locomotor activity, which results in increased fat mass and leptin levels, as well as in insulin resistance (Meadows et al. 2016) (Table 1), a trend toward obesity. In addition, the chronic i.c.v. infusion of O-1602 increases adiposity in rats by inhibiting lipolytic genes and independently of food intake (Diaz-Arteaga et al. 2012), although it remains unclear whether this effect is mediated by GPR55 or by another unidentified receptor.

Metabolic actions of GPR55 in WAT

Due to the high activity of the ECS in VAT of T2D patients (Di Marzo 2008), the LPI/GPR55 system has been studied in detail in WAT. Reportedly, LPI induced the expression of lipogenic genes and peroxisome proliferator-activated receptor γ (PPARγ) in human VAT explants, and further experiments with differentiated adipocytes from VAT of obese patients displayed increased intracellular Ca2+ in response to LPI (Moreno-Navarrete et al. 2012), which could be related to increased lipogenic activity (Gericke et al. 2009). All these results suggest that GPR55 signaling is positively associated with human obesity; hence, pharmacological blockade of GPR55 could be a therapeutic approach to control excessive weight gain.

In contrast to the observations made in humans, studies performed in animal models show a different participation of GPR55 in adipose tissue metabolism (Table 1). Genetic GPR55 ablation leads to increased adiposity (Meadows et al. 2016) and, accordingly, obese rodent models including mice lacking leptin (ob/ob mice) and rats fed a high-fat diet (HFD) showed decreased VAT GPR55 expression (Moreno-Navarrete et al. 2012). Such potentiation of adiposity may result, at least in part, from reduced physical activity (Meadows et al. 2016); however, other factors might also be involved. Diaz-Arteaga and coworkers showed decreased mobilization of fatty acids after either central or peripheral sub-chronic O-1602 treatment in rats (Diaz-Arteaga et al. 2012). In agreement, O-1602 increased the intracellular Ca2+ levels and lipid accumulation in 3T3-L1 cells (Diaz-Arteaga et al. 2012). Given that O-1602 is capable of triggering signals through various receptors, it remains to be determined whether GPR55 mediated such effects.

Metabolic actions of GPR55 in the gastrointestinal (GI) tract

Physiological studies show that activation of GPR55 resulted in the inhibition of the neurogenic contractions in the mouse intestine (Ross et al. 2012) and are also involved in colon motility (Lin et al. 2011, Li et al. 2013). For instance, O-1602 reduced contractions in muscle
strips from the colon and ileum, which slowed whole gut transit and colonic bead expulsion; effects that were reversed by CBD and did not occur in mice lacking GPR55 (Li et al. 2013). In addition, intraperitoneal and i.c.v. injections of O-1602 slowed whole gut transit and colonic bead expulsion in WT mice but not in Gpr55−/− mice (Li et al. 2013). Moreover, O-1602 was found to reduce neurogenic contractions in the gut through GPR55 and independently of CB1 and CB2 (Ross et al. 2012). A role of GPR55 in GI inflammation has also been proposed from the observations that Gpr55−/− mice displayed less susceptibility to intestinal inflammation compared to their control littermates (Stančič et al. 2015). In addition, the administration of the GPR55 antagonist CID-16020046 was shown to improve intestinal inflammation and to decrease proinflammatory cytokines and leukocyte recruitment to the colon (Stančič et al. 2015). Although O-1602 has been shown to be protective against experimentally induced colitis, it was determined that its effects were independently of the CB1, CB2 and GPR55 receptors (Schicho et al. 2011). Unfortunately, despite the observations from rodent studies suggesting a role of GPR55 in inflammation (Table 1), which could be useful for the development of new therapeutic strategies to treat inflammatory bowel disease, the function of GPR55 in human GI is still unknown.

**Metabolic actions of GPR55 in the islets of Langerhans**

The presence of GPR55 in β-cells suggest a potential role of GPR55 in the regulation of insulin secretion, and consequently, in glucose homeostasis. In fact, LPI-induced insulin secretion had been previously reported (Metz 1986), but only recently it was demonstrated that GPR55 mediates such effect. The exposure of rodent islets to GPR55 agonists increases intracellular Ca2+ concentration and cAMP levels, resulting in enhanced insulin secretion (Romero-Zerbo et al. 2011, McKillop et al. 2013). The stimulation of human islets with GPR55 ligands also potentiates insulin levels during both the first and second phases (Song et al. 2012). In agreement, glucose-responsive BRIN-BD11 cells stimulated with a range of GPR55 agonists at different concentrations display greater insulin release, whereas inhibition of insulin secretion is detected after exposure of the cells to a GPR55 antagonist (McKillop et al. 2013). In line with this, daily oral administration of Abn-CBD, a potent selective agonist for GPR55 (McKillop et al. 2013), lowered blood glucose and plasma glucagon and increased plasma insulin and pancreatic insulin content in streptozotocin-induced diabetic mice (McKillop et al. 2016). After long-term administration of Abn-CBD, glucose tolerance and insulin sensitivity were markedly improved, and total cholesterol and triacylglycerol were decreased (McKillop et al. 2016). In this report, the GPR55 agonist decreased food consumption, though no effect on overall body weight was detected (McKillop et al. 2016).

The participation of GPR55 in the outcomes obtained was confirmed in islets from Gpr55−/− mice, which failed to increase glucose-stimulated insulin secretion (GSIS) after stimulation with O-1602 (Romero-Zerbo et al. 2011). In addition, acute intraperitoneal administration of GPR55 agonists stimulated insulin secretion after a glucose load and improved glucose tolerance in rodents (Romero-Zerbo et al. 2011, McKillop et al. 2013). However, studies employing Gpr55−/− mice report different effects of GPR55 on glucose metabolism (Table 1). Mice globally lacking Gpr55 showed slightly impaired glucose tolerance (Song et al. 2012), whereas a more recent report showed normal glucose tolerance in Gpr55−/− mice with no differences in GSIS (Meadows et al. 2016). The lack of methodological information impedes to decipher the factor/s (i.e. age, gender, strain...) responsible for the differences reported in these animals.

**Future perspectives**

Given the clinical relevance of the endocannabinoid system in terms of metabolic syndrome, the discovery of an alternative cannabinoid receptor has logically aroused great interest. However, the initial enthusiasm is being challenged by the difficulties that imply targeting GPR55. The main reasons for this are likely explained because we are facing a G-protein-coupled receptor that first, has an extremely complicated pharmacology and second, the fact that it is regulated in a species-dependent manner, makes it difficult to understand the potential translation of preclinical data. These difficulties are reflected by the fact that supposed specific GPR55 agonists/antagonists do not show a consistent action on food intake, gastrointestinal motility or body weight and, even though sometimes they affect these parameters, the mechanisms may be independent of GPR55. Indeed, a great progress in the development of new compounds has been made during the last years, but it seems that most, if not all, the compounds that are assumed to be specific for GPR55, may play actions in both a GPR55-dependent and -independent manner.

Although findings obtained in mice lacking Gpr55 show different results (Romero-Zerbo et al. 2011, Song et al. 2012, McKillop et al. 2013, 2016), the most solid
data are probably coming from the pancreas, where GPR55 is expressed in both human and rodent β-cells and GPR55 agonists have an insulinotropic action. This consistency is strengthened by the fact that the LPI/GPR55 system has a potential role in human type 2 diabetes (Moreno-Navarrete et al., 2012). Thus, the therapeutic potential of agonizing GPR55 for the treatment of T2D is currently very attractive. However, we must take these emerging data with caution because even if GPR55 agonists have positive effects on insulin secretion and sensitivity, those compounds will target many different tissues due to the wide expression of GPR55. For instance, GPR55 agonists will reach a large number of brain areas, and therefore, it is plausible to hypothesize that these compounds will exert off-target effects. Thus, the design and validation of GPR55 agonists with tissue-specific action might be an important issue to investigate in further studies. In any case, the full characterization of the different actions of GPR55 in energy balance and glucose metabolism is prompting a new and exciting, but also complex, pharmaceutical target.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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