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.

Key Words

- ▶ GPR55
- ▶ adipose tissue
- ▶ diabetes II
- ▶ cannabinoids

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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-arachidonylethanolamide (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, Sylantsev *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
GPR55 distribution and function in main human and rodent metabolic tissues.

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 *GPR55* 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]GTP γ S-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, Waldeck-Weiermair *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, Andradás *et al.* 2016) and GI disorders (Hasenoehl *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.

Animal model	Drug	Action on GPR55	EC ₅₀ /IC ₅₀ values for GPR55, CB1 and CB2	Dose/route administ.	Main outcomes	Ref.
CD1 mice	AM-251	Agonist	39 nM, –, –	0.1 mg/kg, i.p.	No effects on whole gut transit or colonic propulsion	Li et al. (2013)
CD1 mice	CBD	Antagonist	0.445, 3.35, 27.5 µM	0.5 mg/kg, i.p.	No effects on whole gut transit or colonic propulsion	Li et al. (2013)
CD1 mice	O-1602	Agonist	13, >30,000, >30,000 nM	10 mg/kg, i.p.	Slowed whole gut transit and colonic bead expulsion, no effect on gastric emptying	Li et al. (2013)
CD1 mice	O-1602	Agonist	13, >30,000, >30,000 nM	10 mg/kg, i.p. or 10 µg i.c.v.	No effects on locomotor activity	Li et al. (2013)
GIP ^{-/-} mice	Abn-CBD	Agonist	2.5, >30, >30 µM	0.1 µmol/kg, i.p.	Increased GSIS and improved glucose tolerance	McKillop et al. (2016)
GLP-1 ^{-/-} mice	Abn-CBD	Agonist	2.5, >30, >30 µM	0.1 µmol/kg, i.p.	No effects on GSIS or glucose tolerance	McKillop et al. (2016)
GPR55 ^{-/-} mice					Unchanged energy intake and feeding pattern, increased fat mass and insulin resistance, decreased physical activity, and normal glucose tolerance and GSIS	Meadows et al. (2016)
GPR55 ^{-/-} mice					Small impairment of glucose tolerance	Song et al. (2012)
GPR55 ^{-/-} mice					Less severe inflammation during dextran sulfate sodium (DSS) colitis	Stančić et al. (2015)
GPR55 ^{-/-} mice	O-1602	Agonist	13, >30,000, >30,000 nM	200 µg/kg, i.p.	Increased food intake	Diaz-Arteaga et al. (2012)
GPR55 ^{-/-} mice	O-1602	Agonist	13, >30,000, >30,000 nM	10 mg/kg, i.p.	No changes in gut transit and colonic bead expulsion	Li et al. (2013)
GPR55 ^{-/-} mice	O-1602	Agonist	13, >30,000, >30,000 nM	0.1–30 µmol/L	Reduced inhibition of neurogenic contractions	Ross et al. (2012)
GPR55 ^{+/+} mice	CID16020046	Antagonist	0.21 µM, –, –	20 mg/kg, i.p.	Improved intestinal inflammation, decreased proinflammatory cytokines and leukocyte recruitment, unchanged locomotor activity	Stančić et al. (2015)
NIH Swiss mice	Abn-CBD	Agonist	2.5, >30, >30 µM	0.1 µmol/kg, i.p.	Increased GSIS and improved glucose tolerance	McKillop et al. (2013)
NIH Swiss mice	AM-251	Agonist	39 nM	0.1 µmol/kg, i.p.	Increased GSIS and improved glucose tolerance	McKillop et al. (2013)
NIH Swiss mice	O-1602	Agonist	13, >30,000, >30,000 nM	0.1 µmol/kg, i.p.	Increased GSIS and improved glucose tolerance	McKillop et al. (2013)

(Continued)

Table 1 Continued.

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

Endocannabinoids The main endocannabinoids AEA 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 Ca²⁺ 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 Ca²⁺ 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 pharmacologically active component of cannabis, Δ⁹-tetrahydrocannabinol (Δ⁹-THC), acts as a GPR55 agonist mediating intracellular Ca²⁺ 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 Δ^9 -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 Rimnabant agonist activity (Kapur *et al.* 2009). However, it has also been reported that Rimnabant 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 β_2 -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).

Lysophosphatidylinositol (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 oncogene 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), modulation 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 (PPAR γ) 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 WAT 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 (Stančić *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 (Stančić *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 Ca^{2+} 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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