Off-Target Cannabinoid Effects Mediated by GPR55

Christopher M. Henstridge

Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

Key Words

GPR55 • Lysophosphatidylinositol • Cannabinoid

Abstract

Given the vast therapeutic potential of the endocannabinoid system, the revelation of a novel cannabinoid-sensitive target was treated with great excitement. The orphan G-protein coupled receptor 55 (GPR55) was initially touted as a novel cannabinoid target in early industrial patent literature. Consequently, numerous studies have revealed GPR55 expression in a diverse array of cells and tissues, regulating various physiological and pathological processes. Although a confusing cannabinoid profile has prevented its classification as a cannabinoid receptor, the therapeutic potential of the receptor cannot be denied, with roles in cancer progression, bone resorption and analgesia. This commentary aims to summarize GPR55 expression data and speculate on potential therapeutic exploitation of this enigmatic orphan receptor.

Copyright © 2012 S. Karger AG, Basel

Introduction

Consisting of two specific receptor targets (CB₁ and CB₂), two endogenous ligands (anandamide and 2-arachidonoylglycerol) and an increasing number of enzymes

involved in their production and degradation, the endocannabinoid system contains numerous potential drugable targets capable of modulating its physiology. However, pharmacological evidence and the use of knockout animals suggest that this picture is somewhat simplified, as cannabinoid ligands also interact with non-cannabinoid targets [1]. Given the vast therapeutic potential of the endocannabinoid system, considerable effort has been spent in both academic and industrial labs to identify these novel cannabinoid-sensitive targets, which revealed an exciting new candidate, the orphan G-protein-coupled receptor 55 (GPR55). Initially, GPR55 cannabinoid sensitivity was described in two industrial patents [2] but was soon reinforced by a paper in 2007 from AstraZeneca showing potent activation of GPR55 by numerous diverse cannabinoid ligands, including endocannabinoids [3]. Since this early study, GPR55 research has rapidly expanded in a somewhat erratic fashion, leading to a confusing and sometimes contradictory pharmacology [reviewed extensively elsewhere; ref. 4–7]. Although this has resulted in a perplexing cannabinoid ligand profile for GPR55, it is clear that the receptor is activated by numerous cannabinoid ligands in a variety of paradigms. GPR55 expression has now been described in an assortment of cells and tissues, regulating diverse physiological and pathophysiological processes. Furthermore, recent studies are beginning to shed light on early inconsistencies and describe the exciting therapeutic potential of this re-

ceptor. Thus it appears that a number of off-target effects of cannabinoid ligands may be regulated by GPR55 and this must be considered when approaching new cannabinoid-based therapeutics.

GPR55 Structure

Human GPR55 is a 319-amino acid protein and was first isolated and cloned in 1999 and mapped to human chromosome 2q37 [8]. Interestingly, GPR55 is phylogenetically distinct from CB₁ and CB₂, showing low amino acid identity (CB₁, 13.5% and CB₂, 14.4%) and the closest homologues are the purinergic receptor P2Y₅ (29%), the purinoceptor-like orphan receptors GPR23 (30%) and GPR35 (27%) and the chemokine receptor CCR4 (23%).

Although several cannabinoid ligands can activate GPR55, it lacks the classical 'cannabinoid binding pocket' present in both CB₁ and CB₂ [9]. Homology models of GPR55 in both its active and inactive state were constructed to examine the ligand binding pocket of GPR55 [10]. The active conformation model of GPR55 revealed a deep, vertical and highly hydrated binding pocket consisting of many hydrophilic residues, which is in contrast to the highly hydrophobic CB₁ and CB₂ receptor binding pockets. Furthermore, the third extracellular loop of GPR55 is significantly longer than CB₁ and CB₂ and contains many charged amino acids, which taken together suggests that GPR55 is structurally distinct from the traditional cannabinoid receptors [10–14].

GPR55 Pharmacology

In line with the structural differences, the most consistent agonist described for GPR55 to date is a non-cannabinoid lipid called lysophosphatidylinositol (LPI), for which GPR55 appears structurally receptive [10, 11]. All GPR55 studies that have tested LPI, have described this lipid as a potent agonist. Although GPR55's cannabinoid pharmacology is particularly contentious with ligands reported as agonists, antagonists or being inactive, GPR55 does exhibit cannabinoid sensitivity in a multitude of cell lines and assays [reviewed extensively elsewhere; ref. 4–7]. One potential issue may be the lack of ligand specificity. Many cannabinoid ligands interact not only with CB₁ and CB₂ but other non-cannabinoid targets such as transient receptor potential vanilloid (TRPV) channels and peroxisome proliferator-activated receptors (PPARs) [15] and differences in the expression of these other targets

between studies may explain some of the discrepancies. However, the situation is improving, with recent studies describing new specific ligands for GPR55 [10, 16, 17]. Brown et al. [16] reported a number of structurally related benzylpiperazine compounds with activity at GPR55, which were inactive at CB₁ and CB₂. Kotsikorou et al. [10] recently mapped the putative binding site of GPR55 using novel GPR55-specific compounds. LPI was shown to interact with the K2.60 residue at the extracellular side of transmembrane domain 2 via its electronegative head group and the authors discuss the significance of this residue as all ligands tested interacted with this lysine [10]. Importantly, the authors showed that although the ligands used in their study differed from those in the Brown et al. [16] study, they adopted similar three-dimensional conformations and interacted with the residues critical for ligand-induced activation [10]. Furthermore, they also reported that the cannabinoid antagonist AM251 adopts a similar conformation as these compounds and interacts with the same critical residues, reinforcing the idea that AM251 is indeed a GPR55 agonist (as previously reported) [3, 18–21].

Intriguingly, a recent study suggests that certain cannabinoid ligands may inhibit or enhance LPI-induced activation of GPR55 [22]. The authors describe the possibility of an orthosteric and allosteric binding site on GPR55 as some ligands acted as agonists when applied alone, but inhibited LPI-induced activation in a non-competitive manner when applied in tandem. This finding is particularly important as it may help to resolve some of the early pharmacological inconsistencies in the field as it shows that ligands may be bitopic in the same assay, acting as both an agonist and an antagonist. This is especially relevant for the arylpyrazole ligands (AM251 and rimonabant) which have already been shown to be agonists in some assays and antagonists in others.

Classification of GPR55 as a cannabinoid receptor is prevented by the evidence that the most potent endogenous ligand is the non-cannabinoid lipid LPI and that the only cannabinoid ligands with any real affinity for GPR55 are synthetic. However, it is clear that cannabinoid interactions with GPR55 are relevant yet complex and require careful consideration when assessing novel cannabinoid therapeutics. Another confounding issue is that some GPR55 ligands have other non-cannabinoid targets. For example the cannabidiol analogue O-1602 is inactive at CB₁ and CB₂ and is often used as a 'GPR55 ligand', yet has clear GPR55-independent effects [23, 24]. Therefore, further work is required before certain molecules can be categorized as GPR55 ligands.

GPR55 (Patho)physiology and Therapeutic Potential

1. GPR55 in the Central Nervous System

CB₁ is the highest expressed G-protein-coupled receptor in the brain and is involved in the fundamental regulation of neuronal transmission by controlling presynaptic neurotransmitter release [25]. CB₂ expression in the central nervous system (CNS) is still controversial, although recent evidence suggests functional CB₂ receptors may exist in certain brain regions [26]. Furthermore, cannabinoid receptors are also expressed in glial cells where they not only directly regulate distinct glial processes but may indirectly regulate neuronal communication [27, 28]. However, pharmacological evidence and knockout animals suggest the presence of novel cannabinoid-sensitive sites within the brain [1, 2, 29]. For example, numerous groups have reported a WIN55212-sensitive site in the brain of CB₁ knockout mice and anandamide has been shown to stimulate GTPγS binding in brain homogenates from both wild-type and CB₁ knockout mice [30–33]. In addition, primary microglia and astrocytes express unidentified cannabinoid targets that regulate cellular responses to excitotoxicity, cell migration and cytokine release [27].

GPR55 mRNA expression has been described in numerous CNS-derived cells and tissues [reviewed in ref. 34] and the receptor appears to be expressed in both neurons and glia [35]. Pietr et al. [35] used real-time PCR to confirm the expression of GPR55 in primary microglial cells, signifying a potential role for GPR55 in neuroimmunological regulation. GPR55 protein localization in the CNS is limited; however, it has been described in mouse dorsal root ganglia [36] and data presented at the 2011 Society for Neuroscience conference, suggest that GPR55 protein may also be found in the hippocampus [37, 38].

The endocannabinoid system is believed to be critical for successful brain development and shaping neuronal connectivity [39, 40]. Interestingly, a recent study in a neuroendocrine cell line (PC12 cells) reported that GPR55 activation led to neurite retraction, suggesting a role for GPR55 in neurite dynamics [41]. This may indicate that GPR55 plays a role in neurite growth and subsequent network formation, however, a recent study by Wu et al. [42] assessing the role of cannabinoid receptors in the development of corticothalamic and thalamocortical axonal projections (CTA and TCA, respectively), found that removal of CB₁ receptor significantly disrupted correct path finding and fasciculation of CTAs and TCAs, although the lack of GPR55 had no effect. However, GPR55

expression in the thalamus and cortex may be very low [34] and thus exerts no effect on these networks. Therefore, further work is required to assess the potential of GPR55 in neuronal network formation in other regions of the brain.

An exciting new avenue of GPR55 research is the potential role for this receptor in regulating neurotransmitter release. Given that GPR55 expression has been determined in PC12 cells [41], it is interesting to note that the GPR55 ligand LPI can induce exocytosis and subsequent catecholamine release from these cells [43]. LPI application resulted in a significant intracellular Ca²⁺ rise, due to release from intracellular stores, which is a well-described downstream signalling readout of GPR55 activation. The effects were not observed with the closely related lipid molecules (lysophosphatidylcholine and lysophosphatidylserine), potentially suggesting that GPR55 activation in vitro leads to increased transmitter release. This intriguing finding has been tantalizingly reinforced by preliminary data presented in abstract form at the 2011 Society for Neuroscience conference [37, 38]. Using immunohistochemistry and fluorescent ligand binding, the authors report the presence of GPR55 in the hippocampus. In hippocampal CA3-CA1 circuits, GPR55 agonists increased spontaneous release probability and increased evoked synaptic events, in a manner dependent upon the presence of GPR55 and intact calcium stores [37, 38]. Thus, it appears that GPR55 may be expressed in certain neuronal circuits to increase presynaptic transmitter release, with a strikingly distinct function from CB₁, which normally acts to decrease release. This may be particularly important for the failure of certain cannabinoid therapeutics, such as rimonabant (Acomplia®), which have detrimental psychotropic side effects. Rimonabant is a CB₁ antagonist, which can increase transmitter levels in the brain [44]. However, rimonabant has also been described as a GPR55 agonist [reviewed in ref. 5] and thus at synapses containing GPR55 and CB₁, the drug may dually increase transmitter release by blocking CB₁ and activating GPR55, leading to greatly increased synaptic activity and potentially psychosis. However, given the large difference in potency of rimonabant at these two receptors, correct dosage would rule out a GPR55 effect unless the drug can accumulate to high levels in the brain.

GPR55 protein has also been located in mouse large diameter dorsal root ganglia neurons [36]. Cannabinoid-induced activation of GPR55 significantly inhibited the potassium M current, which the authors suggest may enhance the excitability of sensory neurons. Therefore, cur-

rent evidence in the CNS suggests that GPR55 may act to potentiate synaptic communication, in an opposite manner to CB₁.

Although the precise function of GPR55 in the CNS has yet to be fully elucidated, some interesting clues are beginning to emerge. However, with greater access to GPR55 antibodies and new selective ligands, plus extensive evaluation of the knockout mice, the true significance and therapeutic potential of GPR55 in the nervous system will be ascertained.

2. GPR55 in Vasculature and Blood

Numerous studies exploiting rat mesenteric arterial preparations have reported the presence of a novel cannabinoid-sensitive vascular receptor [45–48]. Abnormal cannabidiol (abn-CBD) was shown to induce mesenteric vascular relaxation in CB₁ and CB₂ knockout mice [45] and the effects were antagonized by a drug lacking CB₁/CB₂ activity (O-1918) [46]. This became a classic example of a novel cannabinoid-sensitive target requiring delineation. Excitement grew when GPR55 was shown to be sensitive to abn-CBD [3], however, Johns et al. [49] found that abn-CBD-induced decreases in mean arterial pressure were similar in both wild-type and GPR55 knockout mice, suggesting the presence of a distinct abn-CBD-sensitive target in endothelium. Recent data suggests that this alternative abn-CBD target may be another orphan receptor, GPR18 [50].

Using a fluorescent ligand binding approach, Daly et al. [51] suggest that GPR55 may be expressed in mouse mesenteric arteries. T1117 is a derivative of the GPR55 agonist AM251 with a fluorescent tetramethylrhodamine group added. The addition of the fluorescent group does not influence GPR55 agonist properties, but renders the compound unable to bind CB₁ [51]. T1117 labelled all three vascular layers of the MMA, and pre-incubation of excess unlabelled AM251 inhibited T1117 binding.

Interestingly, anandamide can be released by vascular endothelial cells [52] and in principle may bind both CB₁ and GPR55 on the surface of the endothelium. In support of this hypothesis, one study in human umbilical vein endothelial cells reported that anandamide induced an increase in intracellular Ca²⁺, which was blocked by the CB₁ antagonist rimonabant and the CB₁-insensitive (and putative GPR55 antagonist) ligand O-1918 [53]. Furthermore, the authors also show that the putative GPR55 agonist O-1602 (CB₁-insensitive) could evoke GPR55-mediated Ca²⁺ elevations, thus raising the possibility that both CB₁ and GPR55 may contribute to anandamide-induced

Ca²⁺ release in the endothelium. However, given the discrepancies surrounding the GPR55-mediated effects of O-1602 and the debate as to whether O-1918 is indeed an antagonist of GPR55, studies in knockout animals or exploiting the new specific GPR55 ligands would be useful to confirm or deny these findings.

Thus it appears CB₁ and GPR55 are both found in endothelial cells, are both cannabinoid-sensitive and intriguingly, evidence suggests their downstream signalling interacts in a very graceful manner to influence the other's outcome. When integrins are unclustered, anandamide activates the CB₁-Gα_i-Syk pathway, which inhibits the GPR55-PI3K-Bmx-PLC-Ca²⁺ cascade at the level of Syk [53]. However, when integrins cluster, this uncouples CB₁ from β₁-integrin and unleashes the uninhibited GPR55-PI3K-Bmx-PLC-Ca²⁺ cascade following anandamide stimulation.

Interestingly, a similar signalling crosstalk has recently been described in human blood cells. Cannabinoids induce diverse responses in blood cells, such as migration, proliferation, cytokine production, apoptosis, reactive oxygen species production and chemotaxis, with certain cannabinoid ligands wielding potentially therapeutic immunosuppressant actions [54, 55]. However, increasing evidence in the immune system suggests the presence of novel cannabinoid targets [50, 54, 56] and recent studies have shown GPR55 expression in a number of human blood cell types [34, 57]. LPI and AM251 have recently been reported as GPR55 agonists in human neutrophils, promoting RhoA-dependent chemotaxis [57]. Furthermore, the authors describe an elegant downstream signalling interaction between GPR55 and CB₂ receptors. When activated together, GPR55 and CB₂ signalling pathways were significantly enhanced with clear synergistic potentiation of RhoA mediated chemotaxis, a common outcome of GPR55 and CB₂ signalling [57]. However, a negative signalling interaction was observed during neutrophil 'respiratory burst', at the level of reactive oxygen species generation. Furthermore, LPI reduced 2AG-induced activation of the GTPase Rac2. Thus, following initial functional synergism to induce chemotaxis, GPR55 and CB₂ disengage, and GPR55 acts to restrict excessive CB₂-mediated oxidative damage by blocking CB₂'s downstream signalling. Interestingly, a previous study has highlighted a novel cannabinoid-sensitive target in neutrophils, exhibiting negative co-operativity with CB₂ receptors [56], although based on pharmacological evidence this is unlikely to be GPR55.

Thus it appears that in the vasculature and blood, GPR55 may be expressed on the same cells and be acti-

vated by the same cannabinoid ligands to induce not only GPR55-derived effects, but also to influence the downstream signalling and functional output of the traditional cannabinoid receptors. Thus in future studies it will be important to assess GPR55-CB_{1/2} receptor crosstalk in other cells and tissues and any functional relevance of such interaction.

3. GPR55 in Bone

Cannabinoid receptors are expressed in human bone cells and are involved in numerous metabolic processes, ultimately regulating bone mass [58]. Recently, GPR55 has been described in cells that both generate (osteoblasts) and resorb (osteoclasts) bone. GPR55 activation regulates osteoclastogenesis, cell polarization and bone resorption, however, the function of GPR55 in osteoblasts is unclear [59]. GPR55 signalling in osteoclasts involves prominent activation of RhoA and ERK1/2, together with effects on the actin cytoskeleton and the effects are absent in GPR55 knockout mice [59]. Intriguingly, male GPR55 knockout mice exhibit a significant increase in bone mass, which manifests as an increased osteoclast number, yet impaired osteoclast function, which is absent in female knockout mice. Furthermore, the authors show that cartilaginous tissues (chondrocytes) are also increased in the male knockout mice trabecular bone [59]. Therefore, it appears GPR55 may act to induce bone resorption and a GPR55 antagonist may represent a novel therapeutic in treating bone pathologies such as osteoporosis. Indeed, in male mice, an 8-week in vivo treatment paradigm with the putative GPR55 antagonist cannabidiol significantly reduced serum type 1 collagen C-terminal telopeptide fragments, a biochemical marker of bone resorption [59]. However, GPR55 may represent a limited target due to the lack of effect observed in female mice and further work is required to ascertain the reason for this sexual difference.

4. GPR55 in Nociception

Endocannabinoids and their cannabinoid receptor targets are present at supraspinal, spinal and peripheral sites, where they regulate the signalling of pain pathways [60]. The first study to exploit GPR55 knockout mice generated excitement in the field of pain research as the animals were resistant to mechanical hyperalgesia associated with Freund's complete adjuvant (FCA)-induced inflammatory pain and partial nerve ligation, a model of neuropathic hypersensitivity [61]. Mechanical hyperalgesia was completely absent for up to 2 weeks after FCA injection and up to 28 days after ligation in GPR55 knockout mice. Intriguingly, the levels of anti-inflammatory

IL-4, IL-10, IFN- γ and GM-CSF in paws of FCA-injected GPR55 knockout mice were significantly elevated, suggesting that an altered immune response may explain the analgesic phenotype [61]. Indeed, GPR55 mRNA has been found in a variety of cells and tissues involved in the immune response [34]. Data describes the existence of a novel anandamide-sensitive receptor in sensory neurons [62], therefore it is intriguing to note that GPR55 is expressed in sensory neurons and activation of the receptor may induce neuronal excitability [36], all of which may explain the lack of neuropathic hypersensitivity in the GPR55 knockout mice [61]. However, despite GPR55 presence in dorsal root ganglia, which are an integral component of nociceptive neurocircuitry, it appears specifically located in large diameter neurons [36], which typically detect innocuous stimuli.

CB₁ agonists have been shown to be analgesic in certain models of neuropathic pain [60], thus it was surprising when rimonabant was also shown to reduce neuropathic pain in a number of models [63, 64]. This suggests that rimonabant may interact with a novel target to inhibit pain sensation. For example, rimonabant may act by blocking endogenous LPI-induced GPR55 activity and thus act as a functional GPR55 antagonist, leading to a similar form of analgesia observed in the knockout animals [22].

A recent study using noxious rotation of an inflamed rat knee joint has pharmacologically described a novel, non-cannabinoid pain target with similarity to GPR55 [65]. Local administration of O-1602 significantly reduced the mechanosensitivity of unmyelinated C-fibres and the effect was unaltered by co-administration of CB₁ and CB₂ antagonists (AM281 and AM630, respectively). However, the effect was completely blocked following co-administration with the putative GPR55 antagonist O-1918 [65]. However, given the discrepancies surrounding the GPR55-mediated effects of O-1602 and the debate as to whether O-1918 is indeed an antagonist of GPR55, the involvement of GPR55 in these effects is putative at best, thus studies in knockout animals or exploiting the new specific GPR55 ligands would be useful to confirm or deny this data.

Based on current evidence it seems apparent that GPR55 ligands may be therapeutically exploited for the treatment of certain pain pathologies.

5. GPR55 in Cancer

It has been demonstrated in several models of cancer [66–68], and in tumours from diverse origins (brain, breast, pancreas, hematopoietic system, etc.) that canna-

binoids can control cancer cell proliferation [69–71]. However, mounting evidence suggests that GPR55 may also play a prominent role in cancer cell dynamics. Firstly, GPR55 is activated by a diverse range of cannabinoid ligands, secondly GPR55 expression has been documented in a variety of human cancer cell lines [72–75] and thirdly the levels of the GPR55 agonist LPI are significantly increased in the plasma of patients with ovarian cancer compared with healthy patients [76, 77]. Interestingly, in human tumours the expression of GPR55 correlates with aggressiveness. High histological grade breast, pancreas and brain tumour samples contained elevated GPR55 mRNA levels compared to low grade tissue and decreased patient glioma survival correlated with higher GPR55 expression [72]. Furthermore, genetic blockade of GPR55 (using selective siRNAs) in cultured ovarian, prostate [74], breast and brain [72] cancer cells decreased proliferation, whereas overexpression induced proliferation [72]. This effect is intriguing in cancer cells, given that in bone GPR55 appears to naturally decrease cell proliferation, as GPR55 knockout mice exhibited increased bone and cartilage mass [59]. Importantly, a similar effect was observed in vivo in a xenograft model of glioblastoma, where GPR55 silencing reduced the number of proliferating cells within the tumours and stalled tumour growth [72]. Therefore, data suggests that increased GPR55 expression or activity induces a proliferative phenotype in cancer cells leading to increased tumour size and pathology.

The majority of cancer studies describing the proliferative effects of GPR55 have not exogenously applied ligands, suggesting the presence of an endogenous GPR55 ligand tone or constitutive activity. However, this picture could be complicated by the recent finding that commercial fetal bovine serum (used in the culture of most cell lines) contains variable biological levels of endocannabinoid molecules [78]. Nevertheless, evidence suggests that inhibition of cytosolic phospholipase A₂, an enzyme previously shown to generate LPI in ras-transformed cells [79, 80], reduced proliferation in prostate cancer cells [74] and GPR55 overexpressing HEK293 cells [72]. Thus it appears that LPI may be generated and released by oncogenic cells, which activate GPR55 in an autocrine fashion to induce cell proliferation.

This finding raises intriguingly therapeutic relevance given the putative action of some cannabinoid compounds to inhibit LPI-induced GPR55 activation [22]. Low concentrations of AM251 or rimonabant for example may represent a novel therapeutic approach, given their ability to significantly decrease LPI-induced re-

sponses, which would effectively decrease cancer cell proliferation. Further work is required to assess this hypothesis and to examine the potential of GPR55 antagonists as putative cancer treatments.

6. GPR55 in Metabolism

The endocannabinoid system controls the central and peripheral regulation of appetite and body weight. Indeed, cannabinoid agonists have the ability to increase desire for and consumption of excess rich, non-nutritious foodstuffs, whereas cannabinoid antagonists can suppress feeding [81]. Furthermore, the cannabinoid system influences energy storage into fat, glucose homeostasis and insulin sensitivity, making it a potential novel target in the control of diabetes [81]. Evidence is beginning to mount that GPR55 may also play a role in energy homeostasis. For example, a polymorphism in the GPR55 gene has been linked with anorexia nervosa in a cohort of female Japanese patients [82]. The authors show that the single point mutation within TM5 (195; glycine-valine) leads to a receptor with weakened functionality, as evidenced by a reduced signalling readout when overexpressed in a Chinese hamster ovary cell line [82]. Thus, a loss of function mutation is linked to anorexia which may suggest higher functionality or expression of GPR55 leads to weight gain. Intriguingly, this hypothesis has been confirmed in a recent human study showing that higher levels of GPR55 expression in visceral fat correlated with higher weight and percentage body fat [83]. There are a number of intriguing findings in this study. Firstly, obese patients express higher levels of GPR55 mRNA in visceral and subcutaneous fat compared to lean controls [83]. Secondly, the correlation between visceral GPR55 expression and weight is much stronger in females than males, which is opposite to the sexually dimorphic bone phenotype observed in knockout mice [59]. Thirdly, it is particularly interesting to note that in obese female patients, plasma levels of LPI were significantly higher (which is also observed in ovarian cancer patients [76]) and positively correlate with weight and percentage body fat [83]. Finally, in visceral adipose tissue explants and primary differentiated visceral adipocytes, LPI induced the expression of genes involved in fat deposition [83].

Taken together, these studies would suggest that a GPR55 agonist may increase weight gain and fat storage. Indeed, a recent study has shown that the putative GPR55 agonist O-1602 does in fact increase food intake and lipid deposition in adipocytes [24]. However, this study ruled out GPR55 in the O-1602 effect, as GPR55-knockout mice

exhibited the same phenotype as wild-type mice [24]. This reinforces the idea that O-1602 should not be used as evidence of a 'GPR55-effect' and any future studies using this drug must be fully aware of the increasing number of off-target effects and control for them. However, this idea is contested by a recent report suggesting GPR55 agonism may improve glucose tolerance in an effect potentially involving enhanced insulin secretion. The authors report GPR55 mRNA and protein in pancreatic islets and suggest the receptor is involved in regulating glucose homeostasis [85].

Taken together, current data suggest that the LPI-GPR55 system may represent a novel therapeutic target for treating weight gain and certain metabolic syndromes.

Concluding Remarks

Given the cannabinoid sensitivity of GPR55 and its expression in similar cells and tissues as the traditional cannabinoid receptors, it is likely that GPR55 can explain some of the off-target effects of cannabinoid ligands. However, GPR55 activity cannot complete the picture as a number of discrepancies remain. For example, the GPR55 ligands LPI and O-1602 have non-GPR55 effects in a number of paradigms [23, 24, 84] and pharmacological studies describe unidentified cannabinoid-sensitive targets in the brain and endothelium that have distinct ligand profiles from GPR55 [29]. However, the generation

of new selective ligands for GPR55 will aid the elucidation of its physiological roles and may lead to novel therapeutics in the numerous pathologies in which GPR55 is involved. For example, specific GPR55 antagonists may prove beneficial in slowing tumour proliferation, angiogenesis and cancer pain. GPR55 also has the potential to influence immune responses, bone resorption and pain, thus may represent a useful tool in the treatment of arthritis and arthritic pain. Furthermore, the therapeutic potential of targeting GPR55 in adipocytes may represent an exciting novel approach to the treatment of obesity. However, the therapeutic outcome of GPR55 ligands may be hindered by the apparent crosstalk between LPI-induced GPR55 activity and the cannabinoid system. GPR55 agonists may indirectly block cannabinoid receptor signalling in some tissues and alternatively cannabinoid-based compounds may allosterically block endogenous GPR55 activity. These potential issues may explain some early pharmacological discrepancies and will need to be considered in the production of future potential therapeutics.

In summary, GPR55 has emerged as a lipid-sensitive modulator of numerous cellular processes and pathologies which may be therapeutically exploited in the future.

Disclosure Statement

None.

References

- Mackie K, Stella N: Cannabinoid receptors and endocannabinoids: evidence for new players. *AAPS J* 2006;8:E298–E306.
- Baker D, Pryce G, Davies WL, Hiley CR: In silico patent searching reveals a new cannabinoid receptor. *Trends Pharmacol Sci* 2006; 27:1–4.
- Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson NO, Leonova J, Elebring T, Nilsson K, Drmota T, Greasley PJ: The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007;152:1092–1101.
- Nevalainen T, Irving AJ: GPR55, a lysophosphatidylinositol receptor with cannabinoid sensitivity? *Curr Top Med Chem* 2010;10: 799–813.
- Balenga NA, Henstridge CM, Kargl J, Walchoer M: Pharmacology, signaling and physiological relevance of the G protein-coupled receptor 55. *Adv Pharmacol* 2011;62:251–277.
- Ross RA: The enigmatic pharmacology of GPR55. *Trends Pharmacol Sci* 2009;30:156–163.
- Sharir H, Abood ME: Pharmacological characterization of GPR55, a putative cannabinoid receptor. *Pharmacol Ther* 2010;126: 301–313.
- Sawzdargo M, Nguyen T, Lee DK, Lynch KR, Cheng R, Heng HH, George SR, O'Dowd BF: Identification and cloning of three novel human G protein-coupled receptor genes GPR52, GPR53 and GPR55: GPR55 is extensively expressed in human brain. *Brain Res Mol Brain Res* 1999;64:193–198.
- Petit F, Donlan M, Michel A: GPR55 as a new cannabinoid receptor: still a long way to prove it. *Chem Biol Drug Des* 2006;67:252–253.
- Kotsikourou E, Madrigal KE, Hurst DP, Sharir H, Lynch DL, Heynen-Genel S, Milan LB, Chung TD, Seltzman HH, Bai Y, Caron MG, Barak LS, Abood ME, Reggio PH: Identification of the GPR55 agonist binding site using a novel set of high-potency GPR55 selective ligands. *Biochemistry* 2011;50:5633–5647.
- Kotsikourou E, Lynch DL, Abood ME, Reggio PH: Lipid bilayer molecular dynamics study of lipid-derived agonists of the putative cannabinoid receptor, GPR55. *Chem Phys Lipids* 2011;164:131–143.
- Hurst DP, Grossfield A, Lynch DL, Feller S, Romo TD, Gawrisch K, Pitman MC, Reggio PH: A lipid pathway for ligand binding is necessary for a cannabinoid G protein-coupled receptor. *J Biol Chem* 2010;285:17954–17964.
- Pei Y, Mercier RW, Anday JK, Thakur GA, Zvonok AM, Hurst D, Reggio PH, Janero DR, Makriyannis A: Ligand-binding architecture of human CB2 cannabinoid receptor: evidence for receptor subtype-specific binding motif and modeling GPCR activation. *Chem Biol* 2008;15:1207–1219.

- 14 Picone RP, Khanolkar AD, Xu W, Ayotte LA, Thakur GA, Hurst DP, Abood ME, Reggio PH, Fournier DJ, Makriyannis A: (-)-7'-Isothiocyano-11-hydroxy-1',1'-dimethylheptylhexahydrocannabinol (AM841), a high-affinity electrophilic ligand, interacts covalently with a cysteine in helix six and activates the CB1 cannabinoid receptor. *Mol Pharmacol* 2005;68:1623–1635.
- 15 Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA: International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB₁ and CB₂. *Pharmacol Rev* 2010;62:588–631.
- 16 Brown AJ, Daniels DA, Kassim M, Brown S, Haslam CP, Terrell VR, Brown J, Nichols PL, Staton PC, Wise A, Dowell SJ: Pharmacology of GPR55 in yeast and identification of GS-K494581A as a mixed-activity glycine transporter subtype 1 inhibitor and GPR55 agonist. *J Pharmacol Exp Ther* 2011;337:236–246.
- 17 Heynen-Genel S, Dahl R, Shi S, Milan L, Hariharan S, Bravo Y, Sergienko E, Hedrick M, Dad S, Stonich D, Su Y, Vicchiarelli M, Mangravita-Novo A, Smith LH, Chung TDY, Sharir H, Barak LS, Abood ME: Screening for Selective Ligands for GPR55 – Agonists. Probe Reports from the NIH Molecular Libraries Program. Bethesda, National Center for Biotechnology Information, 2010.
- 18 Henstridge CM, Balenga NA, Ford LA, Ross RA, Waldhoer M, Irving AJ: The GPR55 ligand L-alpha-lysophosphatidylinositol promotes RhoA-dependent Ca²⁺ signaling and NFAT activation. *FASEB J* 2009;23:183–193.
- 19 Kapur A, Zhao P, Sharir H, Bai Y, Caron MG, Barak LS, Abood ME: Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. *J Biol Chem* 2009;284:29817–29827.
- 20 Henstridge CM, Balenga NA, Schröder R, Kargl JK, Platzer W, Martini L, Arthur S, Penman J, Whistler JL, Kostenis E, Waldhoer M, Irving AJ: GPR55 ligands promote receptor coupling to multiple signalling pathways. *Br J Pharmacol* 2010;160:604–614.
- 21 Yin H, Chu A, Li W, Wang B, Shelton F, Otero F, Nguyen DG, Caldwell JS, Chen YA: Lipid G protein-coupled receptor ligand identification using beta-arrestin PathHunter assay. *J Biol Chem* 2009;284:12328–12338.
- 22 Anavi-Goffer S, Baillie G, Irving AJ, Gertsch J, Greig IR, Pertwee RG, Ross RA: Modulation of L-alpha-lysophosphatidylinositol/GPR55 mitogen-activated protein kinase (MAPK) signaling by cannabinoids. *J Biol Chem* 2012;287:91–104.
- 23 Schicho R, Bashashati M, Bawa M, McHugh D, Saur D, Hu HM, Zimmer A, Lutz B, Mackie K, Bradshaw HB, McCafferty DM, Sharkey KA, Storr M: The atypical cannabinoid O-1602 protects against experimental colitis and inhibits neutrophil recruitment. *Inflamm Bowel Dis* 2011;17:1651–1664.
- 24 Díaz-Arteaga A, Vázquez MJ, Vázquez-Martínez R, Pulido MR, Suarez J, Velásquez DA, López M, Ross RA, de Fonseca FR, Bermudez-Silva FJ, Malagón MM, Diéguez C, Nogueiras R: The atypical cannabinoid O-1602 stimulates food intake and adiposity in rats. *Diabetes Obes Metab* 2011, E-pub ahead of print.
- 25 Katona I, Freund TF: Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat Med* 2008;14:923–930.
- 26 Atwood BK, Mackie K: CB2: a cannabinoid receptor with an identity crisis. *Br J Pharmacol* 2010;160:467–479.
- 27 Stella N: Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia* 2010;58:1017–1030.
- 28 Navarrete M, Araque A: Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. *Neuron* 2010;68:113–126.
- 29 Brown AJ: Novel cannabinoid receptors. *Br J Pharmacol* 2007;152:567–575.
- 30 Breivogel CS, Griffin G, Di Marzo V, Martin BR: Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol Pharmacol* 2001;60:155–163.
- 31 Pistis M, Perra S, Pillolla G, Melis M, Gessa GL, Muntoni AL: Cannabinoids modulate neuronal firing in the rat basolateral amygdala: evidence for CB1- and non-CB1-mediated actions. *Neuropharmacology* 2004;46:115–125.
- 32 Hájos N, Ledent C, Freund TF: Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience* 2001;106:1–4.
- 33 Hájos N, Freund TF: Distinct cannabinoid sensitive receptors regulate hippocampal excitation and inhibition. *Chem Phys Lipids* 2002;121:73–82.
- 34 Henstridge CM, Balenga NA, Kargl J, Andradas C, Brown AJ, Irving A, Sanchez C, Waldhoer M: Minireview: recent developments in the physiology and pathology of the lysophosphatidylinositol-sensitive receptor GPR55. *Mol Endocrinol* 2011;25:1835–1848.
- 35 Pietr M, Kozela E, Levy R, Rimmerman N, Lin YH, Stella N, Vogel Z, Juknat A: Differential changes in GPR55 during microglial cell activation. *FEBS Lett* 2009;583:2071–2076.
- 36 Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B, Mackie K: GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc Natl Acad Sci USA* 2008;105:2699–2704.
- 37 Sylantsev S, Jensen TP, Ross RA, Rusakov DA: The enigmatic receptor GPR55 potentiates neurotransmitter release at central synapses. *Society for Neuroscience Conf Proc*, Washington, 2011, Program 653.01, Poster B28.
- 38 Jensen TP, Sylantsev S, Ross RA, Rusakov DA: GPR55 modulates transmitter release and short term plasticity in the hippocampus by initiating store mediated pre-synaptic Ca²⁺ entry. *Soc for Neurosci Conf Proc*, Washington, 2011, Program 448.08, Poster G4.
- 39 Harkany T, Mackie K, Doherty P: Wiring and firing neuronal networks: endocannabinoids take center stage. *Curr Opin Neurobiol* 2008;18:338–345.
- 40 Berghuis P, Rajnicek AM, Morozov YM, Ross RA, Mulder J, Urbán GM, Monory K, Marsicano G, Matteoli M, Canty A, Irving AJ, Katona I, Yanagawa Y, Rakic P, Lutz B, Mackie K, Harkany T: Hardwiring the brain: endocannabinoids shape neuronal connectivity. *Science* 2007;316:1212–1216.
- 41 Obara Y, Ueno S, Yanagihata Y, Nakahata N: Lysophosphatidylinositol causes neurite retraction via GPR55, G13 and RhoA in PC12 cells. *PLoS One* 2011;6:e24284.
- 42 Wu CS, Zhu J, Wager-Miller J, Wang S, O'Leary D, Monory K, Lutz B, Mackie K, Lu HC: Requirement of cannabinoid CB(1) receptors in cortical pyramidal neurons for appropriate development of corticothalamic and thalamocortical projections. *Eur J Neurosci* 2010;32:693–706.
- 43 Ma MT, Yeo JF, Farooqui AA, Zhang J, Chen P, Ong WY: Differential effects of lysophospholipids on exocytosis in rat PC12 cells. *J Neural Transm* 2010;117:301–308.
- 44 Pertwee RG: The pharmacology of cannabinoid receptors and their ligands: an overview. *Int J Obes (Lond)* 2006;30(suppl 1):S13–S18.
- 45 Járαι Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR, Zimmer AM, Bonner TI, Buckley NE, Mezey E, Razdan RK, Zimmer A, Kunos G: Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc Natl Acad Sci USA* 1999;96:14136–14141.
- 46 Offertaler L, Mo FM, Batkai S, Liu J, Begg M, Razdan RK, Martin BR, Bukoski RD, Kunos G: Selective ligands and cellular effectors of a G protein-coupled endothelial cannabinoid receptor. *Mol Pharmacol* 2003;63:699–705.
- 47 Ho WS, Hiley CR: Vasodilator actions of abnormal-cannabidiol in rat isolated small mesenteric artery. *Br J Pharmacol* 2003;138:1320–1332.
- 48 Wagner JA, Varga K, Jarai Z, Kunos G: Mesenteric vasodilation mediated by endothelial anandamide receptors. *Hypertension* 1999;33:429–434.
- 49 Johns DG, Behm DJ, Walker DJ, Ao Z, Shapland EM, Daniels DA, Riddick M, Dowell S, Staton PC, Green P, Shabon U, Bao W, Aiyar N, Yue TL, Brown AJ, Morrison AD, Douglas SA: The novel endocannabinoid receptor GPR55 is activated by atypical cannabinoids but does not mediate their vasodilator effects. *Br J Pharmacol* 2007;152:825–831.

- 50 McHugh D, Hu SS, Rimmerman N, Juknat A, Vogel Z, Walker JM, Bradshaw HB: N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. *BMC Neurosci* 2010;11:44.
- 51 Daly CJ, Ross RA, Whyte J, Henstridge CM, Irving AJ, McGrath JC: Fluorescent ligand binding reveals heterogeneous distribution of adrenoceptors and 'cannabinoid-like' receptors in small arteries. *Br J Pharmacol* 2010;159:787–796.
- 52 Deutsch DG, Goligorsky MS, Schmid PC, Krebsbach RJ, Schmid HH, Das SK, Dey SK, Arreaza G, Thorup C, Stefano G, Moore LC: Production and physiological actions of anandamide in the vasculature of the rat kidney. *J Clin Invest* 1997;100:1538–1546.
- 53 Waldeck-Weiermair M, Zoratti C, Osibow K, Balenga N, Goessnitzer E, Waldhoer M, Malli R, Graier WF: Integrin clustering enables anandamide-induced Ca²⁺ signaling in endothelial cells via GPR55 by protection against CB1-receptor-triggered repression. *J Cell Sci* 2008;121:1704–1717.
- 54 Pacher P, Mechoulam R: Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog Lipid Res* 2011;50:193–211.
- 55 Tanasescu R, Constantinescu CS: Cannabinoids and the immune system: an overview. *Immunobiology* 2010;215:588–597.
- 56 McHugh D, Ross RA: Endogenous cannabinoids and neutrophil chemotaxis. *Vitam Horm* 2009;81:337–365.
- 57 Balenga NA, Aflaki E, Kargl J, Platzer W, Schröder R, Blättermann S, Kostenis E, Brown AJ, Heinemann A, Waldhoer M: GPR55 regulates cannabinoid 2 receptor-mediated responses in human neutrophils. *Cell Res* 2011;21:1452–1469.
- 58 Whyte LS, Ford L, Ridge SA, Cameron GA, Rogers MJ, Ross RA: Cannabinoids and bone: endocannabinoids modulate human osteoclast function in vitro. *Br J Pharmacol* 2011, E-pub ahead of print.
- 59 Whyte LS, Ryberg E, Sims NA, Ridge SA, Mackie K, Greasley PJ, Ross RA, Rogers MJ: The putative cannabinoid receptor GPR55 affects osteoclast function in vitro and bone mass in vivo. *Proc Natl Acad Sci USA* 2009;106:16511–16516.
- 60 Guindon J, Hohmann AG: The endocannabinoid system and pain. *CNS Neurol Disord Drug Targets* 2009;8:403–421.
- 61 Staton PC, Hatcher JP, Walker DJ, Morrison AD, Shapland EM, Hughes JP, Chong E, Mander PK, Green PJ, Billinton A, Fulleylove M, Lancaster HC, Smith JC, Bailey LT, Wise A, Brown AJ, Richardson JC, Chessell IP: The putative cannabinoid receptor GPR55 plays a role in mechanical hyperalgesia associated with inflammatory and neuropathic pain. *Pain* 2008;139:225–236.
- 62 Evans RM, Scott RH, Ross RA: Multiple actions of anandamide on neonatal rat cultured sensory neurones. *Br J Pharmacol* 2004;141:1223–1233.
- 63 Comelli F, Bettoni I, Colombo A, Fumagalli P, Giagnoni G, Costa B: Rimobabant, a cannabinoid CB1 receptor antagonist, attenuates mechanical allodynia and counteracts oxidative stress and nerve growth factor deficit in diabetic mice. *Eur J Pharmacol* 2010;637:62–69.
- 64 Costa B, Trovato AE, Colleoni M, Giagnoni G, Zarini E, Croci T: Effect of the cannabinoid CB1 receptor antagonist, SR141716, on nociceptive response and nerve demyelination in rodents with chronic constriction injury of the sciatic nerve. *Pain* 2005;116:52–61.
- 65 Schuelert N, McDougall JJ: The abnormal cannabidiol analogue O-1602 reduces nociception in a rat model of acute arthritis via the putative cannabinoid receptor GPR55. *Neurosci Lett* 2011;500:72–76.
- 66 Caffarel MM, Andradas C, Mira E, Perez-Gomez E, Cerutti C, Moreno-Bueno G, Flores JM, Garcia-Real I, Palacios J, Manes S, Guzman M, Sanchez C: Cannabinoids reduce ErbB2-driven breast cancer progression through Akt inhibition. *Mol Cancer* 2010;9:196.
- 67 Qamri Z, Preet A, Nasser MW, Bass CE, Leone G, Barsky SH, Ganju RK: Synthetic cannabinoid receptor agonists inhibit tumor growth and metastasis of breast cancer. *Mol Cancer Ther* 2009;8:3117–3129.
- 68 Wang D, Wang H, Ning W, Backlund MG, Dey SK, DuBois RN: Loss of cannabinoid receptor 1 accelerates intestinal tumor growth. *Cancer Res* 2008;68:6468–6476.
- 69 Guzman M: Cannabinoids: potential anticancer agents. *Nat Rev Cancer* 2003;3:745–755.
- 70 Sarfaraz S, Adhami VM, Syed DN, Afaq F, Mukhtar H: Cannabinoids for cancer treatment: progress and promise. *Cancer Res* 2008;68:339–342.
- 71 Velasco G, Carracedo A, Blazquez C, Lorente M, Aguado T, Haro A, Sanchez C, Galveroerph I, Guzman M: Cannabinoids and gliomas. *Mol Neurobiol* 2007;36:60–67.
- 72 Andradas C, Caffarel MM, Pérez-Gómez E, Salazar M, Lorente M, Velasco G, Guzmán M, Sánchez C: The orphan G protein-coupled receptor GPR55 promotes cancer cell proliferation via ERK. *Oncogene* 2011;30:245–252.
- 73 Ford LA, Roelofs AJ, Anavi-Goffer S, Mowat L, Simpson DG, Irving AJ, Rogers MJ, Rajnicek AM, Ross RA: A role for L-alpha-lysophosphatidylinositol and GPR55 in the modulation of migration, orientation and polarization of human breast cancer cells. *Br J Pharmacol* 2010;160:762–771.
- 74 Piñeiro R, Maffucci T, Falasca M: The putative cannabinoid receptor GPR55 defines a novel autocrine loop in cancer cell proliferation. *Oncogene* 2011;30:142–152.
- 75 Huang L, Ramirez JC, Frampton GA, Golden LE, Quinn MA, Pae HY, Horvat D, Liang LJ, DeMorrow S: Anandamide exerts its antiproliferative actions on cholangiocarcinoma by activation of the GPR55 receptor. *Lab Invest* 2011;91:1007–1017.
- 76 Sutphen R, Xu Y, Wilbanks GD, Fiorica J, Grendys EC, Jr., LaPolla JP, Arango H, Hoffman MS, Martino M, Wakeley K, Griffin D, Blanco RW, Cantor AB, Xiao YJ, Krischer JP: Lysophospholipids are potential biomarkers of ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2004;13:1185–1191.
- 77 Xiao YJ, Schwartz B, Washington M, Kennedy A, Webster K, Belinson J, Xu Y: Electrospray ionization mass spectrometry analysis of lysophospholipids in human ascitic fluids: comparison of the lysophospholipid contents in malignant vs nonmalignant ascitic fluids. *Anal Biochem* 2001;290:302–313.
- 78 Marazzi J, Kleyer J, Paredes JM, Gertsch J: Endocannabinoid content in fetal bovine sera – unexpected effects on mononuclear cells and osteoclastogenesis. *J Immunol Methods* 2011;373:219–228.
- 79 Falasca M, Corda D: Elevated levels and mitogenic activity of lysophosphatidylinositol in k-ras-transformed epithelial cells. *Eur J Biochem* 1994;221:383–389.
- 80 Falasca M, Iurisci C, Carvelli A, Sacchetti A, Corda D: Release of the mitogen lysophosphatidylinositol from H-Ras-transformed fibroblasts; a possible mechanism of autocrine control of cell proliferation. *Oncogene* 1998;16:2357–2365.
- 81 Di Marzo V, Piscitelli F, Mechoulam R: Cannabinoids and endocannabinoids in metabolic disorders with focus on diabetes. *Handb Exp Pharmacol* 2011;203:75–104.
- 82 Ishiguro H, Onaivi ES, Horiuchi Y, Imai K, Komaki G, Ishikawa T, Suzuki M, Watanabe Y, Ando T, Higuchi S, Arinami T: Functional polymorphism in the GPR55 gene is associated with anorexia nervosa. *Synapse* 2011;65:103–108.
- 83 Moreno-Navarrete JM, Catalán V, Whyte L, Diaz-Arteaga A, Vázquez-Martínez R, Rotellar F, Guzmán R, Gómez-Ambrosi J, Pulido MR, Russell WR, Imbernón M, Ross RA, Malagón MM, Dieguez C, Fernández-Real JM, Frühbeck G, Nogueiras R: The L-alpha-lysophosphatidylinositol/GPR55 system and its potential role in human obesity. *Diabetes* 2012;61:281–291.
- 84 Bondarenko A, Waldeck-Weiermair M, Naghdi S, Poteser M, Malli R, Graier WF: GPR55-dependent and -independent ion signalling in response to lysophosphatidylinositol in endothelial cells. *Br J Pharmacol* 2010;161:308–320.
- 85 Romero-Zerbo SY, Rafacho A, Díaz-Arteaga A, Suárez J, Quesada I, Imbernón M, Ross RA, Dieguez C, Rodríguez de Fonseca F, Nogueiras R, Nadal Á, Bermúdez-Silva FJ: A role for the putative cannabinoid receptor GPR55 in the islets of Langerhans. *J Endocrinol* 2011;211:177–185.