Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Pharmacology

A potential role for GPR55 in gastrointestinal functions

Rudolf Schicho[1] and Martin Storr[2]

Despite sharing little homology (10–15%) with cannabinoid-1 (CB_1) and cannabinoid-2 (CB_2) receptors, the G protein-coupled receptor 55 (GPR55) was initially thought to be a new member of the cannabinoid receptor family. Apart from being activated by various exogenous cannabinoids, GPR55 is also activated by endocannabinoids like anandamide, which is found in organs with high GPR55 expression such as the brain and the gastrointestinal (GI) tract. The phylogenetic distance to the classical CB receptors and its pharmacological responsiveness to certain cannabinoids suggests that GPR55 may constitute a novel class of cannabinoid receptors. GPR55 influences mechanisms in the nervous system, vasculature, kidney and bone. Recent research revealed that GPR55 is also involved in cancer development and inflammatory pain. Because of its presence in the GI tract, several studies have

discuss the potential role of GPR55 in GI functions.

Addresses
[1] Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Austria
[2] Department of Medicine II, Klinikum Großhadern, Ludwig-Maximilians University, 81377 Munich, Germany

Corresponding author: Storr, Martin (gidoc@gmx.com, mstorr@ucalgary.ca)

Current Opinion in Pharmacology 2012, **12**:653–658

This review comes from a themed issue on **Gastrointestinal**

Edited by **Gareth J Sanger**

For a complete overview see the Issue and the Editorial

Available online 10th October 2012

http://dx.doi.org/10.1016/j.coph.2012.09.009

The GPR55 receptor — a G protein-coupled receptor activated by lysophosphatidylinositol (LPI) and cannabinoids

Since the discovery that effects of cannabinoids are due to actual ligand–receptor interaction and not to some kind of non-specific membrane disturbance, the cannabinoid (CB) receptors, a family of seven-transmembrane spanning (7TM) G protein-coupled receptors (GPCRs), have become the focus of intense research. Over the years it has become obvious that the diverse biological effects of cannabinoids (natural cannabinoids, synthetic cannabinoids and endocannabinoids) cannot be explained only by the actions of the two established CB receptors (CB_1 and CB_2), but that cannabinoid activity is mediated also by various ionotropic receptors and uncharacterized GPCRs (reviewed in [1••]).

Structure of GPR55

Several years ago, a GeneBank search for transmembrane regions characteristic of the GPCR family (High Throughput Genome; HTG) revealed several new 7TM/GPCRs, among them the GPR55 gene [2]. GPR55 was subsequently cloned and soon localized by Northern blot analysis in human brain and liver as well as in rat intestine and spleen [2]. The ability of GPR55 to respond to cannabinoids was later documented by two independent patents [3] indicating that GPR55 may represent a new cannabinoid receptor. Although ... ow ... es ... ed cannabinoid receptor family, despite higher sequence homologies within the conserved regions [3]. Amino acid residues at the binding and activity sites of human CB_1 and CB_2 align poorly with human GPR55 implicating that GPR55 may not share the same binding pockets with the classical CB receptors [4]. However, the fact that many cannabinoid compounds are able to activate GPR55 may suggest that it belongs to a novel group of cannabinoid receptors that, like CB_1 and CB_2, could play a potential role in the physiology and pathophysiology of the GI tract [5]. Phylogenetically, GPR55 is part of the purine receptor cluster of the δ group of rhodopsin receptors [6] together with GPR18, another cannabinoid-responsive GPCR [7•,8]. From other GPCRs closely related to GPR55 (GPR35, GPR92 and GPR23), no reports have been published yet as to whether they are activated or inhibited by cannabinoids [1••]. Recent data indicate that lysophosphatidic acid (LPA) may act as their natural ligand [9–11]. Rodent GPR55 shows up to 78% homology with the human GPR55 [12•].

Pharmacology and physiology of GPR55

The pharmacology of GPR55 has not yet been resolved and data are too contradictory as to whether GPR55 can be unambiguously coined as the ‘third cannabinoid receptor’ [1••]. In GTPγS binding assays of overexpressed cell systems (mainly HEK293 cells), GPR55 activation was observed following application of many natural and synthetic cannabinoids as well as following application of endocannabinoids like anandamide but these results were not confirmed in other assays of receptor activation (e.g. β-arrestin activation [13]). Concerning the endogenous ligand(s) for GPR55, the only consistent data converge on a lysophospholipid, namely lysophosphatidylinositol (LPI). Especially, LPI carrying an arachidonic acid

moiety is supposed to have the greatest impact on GPR55 activation [14]. Our knowledge on the physiological role of GPR55 and whether it could be part of the endocannabinoid system is in its infancy. Because of the lack of studies with specific antagonists, not much is known yet about the role of endogenous GPR55 to get a clear picture of the receptor's functions. Some data have been reported from cell types endogenously expressing GPR55 such as from human neutrophils [15••], DRG neurons [16••], PC12 cells [17•], endothelial cells [18••] and also from cells genetically lacking GPR55 expression [19,20]. In addition, GPR55 expression has been found in certain cancer cells [21••,22] and in the endocrine pancreas, where it has been suggested to play a contributory role in insulin secretion [19]. In human neutrophils, GPR55 augments migratory responses of CB_2 to 2-arachidonoyl glycerol (2-AG) at the level of small GTPases, such as Rac2 and Cdc42 [15••] whereas in endothelial cells, activation of GPR55 causes Ca^{++} mobilization, which is, depending on the status of integrin clustering, inhibited by CB_1 activity [18••]. Both findings direct to the intriguing possibility that an interaction may exist between GPR55 and CB receptors in physiological and pathophysiological mechanisms triggered by endocannabinoids. In this context, it is noteworthy that the CB_1 antagonists SR141716A and AM251 also act as GPR55 agonists [23•]. The main differences between GPR55 and CB receptors not only lie in their low homology but also in the activation of different downstream G proteins and downstream signals. In neurons, for instance, CB_1 signals through $G_{i/o}$ proteins, inhibits voltage gated Ca^{++} channels and activates A-type and inwardly rectifying K^+ currents [24]. In contrast to CB receptors, GPR55 signals through $G_{\alpha 12}$ and G_q proteins, activates downstream small G proteins like RhoA and increases Ca^{++} release and K^+ type M-currents [16••]. In osteoclasts, LPI was also shown to stimulate Rho, an effect that was absent in $GPR55^{-/-}$ mice [20]. It seems that GPR55 initiates mostly excitatory and not inhibitory effects, which is in contrast to the role of the classical CB receptors, which mostly initiate inhibitory effects. In line with this concept, GPR55 has been implicated in the development of neuropathic and inflammatory pain [25•]. The reader is referred to several recently published excellent reviews on GPR55 pharmacology and physiology [26••,27••,28••,29•,30].

Is there a functional role for GPR55 in the GI tract?

CB_1 receptors are known to be involved in several motor functions of the GI tract like esophageal sphincter relaxation and gastric emptying (reviewed in [31]) while CB_2 receptors show no involvement in motility, at least not in physiological conditions. However, data have accumulated showing that CB_2 may come into power in situations during intestinal inflammation [32,33,34•,35•]. Since GPR55 can be activated by endogenous and exogenous cannabinoids we discuss in the following paragraphs the possibility that GPR55 may have a functional role in GI physiology and pathophysiology.

Motility and inflammation

Similar to CB_1 and CB_2 (reviewed in [36]), expression of GPR55 can be found throughout the GI tract, although a detailed description of tissues and cells expressing GPR55 is still lacking. Real time and semiquantitative PCR show that GPR55 is present in duodenum, jejunum, ileum and the colon of rodents [12•,37,38•]. In particular, GPR55 expression is found in mucosal scrapings and in longitudinal-myenteric plexus preparations of the colon indicating that the receptor is most likely present in both, gastrointestinal epithelial cells and in gastrointestinal enteric neurons [38•]. Additionally, Lin *et al.* were able to detect GPR55 in enteric neurons of the rat ileum by immunohistochemistry [37]. The study showed that the expression of GPR55 was higher in rats treated with lipopolysaccharide (LPS) than in control animals, indicating that GPR55 may be involved in the response of the gut to intestinal inflammation [37]. The authors also suggested that immunohistochemical expression of GPR55 was increased in enteric neurons following LPS treatment, though they did not provide any quantitative data to corroborate this finding. Nevertheless, the detection of GPR55 in enteric neurons raises the possibility that GPR55 has a role in gastrointestinal functions, such as motility and secretion. In support of this hypothesis, the atypical cannabinoid O-1602 (a GPR55 agonist that has shown GTPγS activation in membranes of human recombinant GPR55-expressing cells with an EC_{50} of 1.4 [39•] and 13 nM [12•]), reduced spontaneous contraction in the rat ileum at 0.1 μM while in the colon, only contractions that were induced by LPS were decreased in a dose dependent manner [37]. In contrast, O-1602 had no effect on the membrane potential in the jejunum of untreated mice [37]. This indicates that, depending on the part of the GI tract, GPR55 is probably involved in pathophysiological as well as physiological motor functions. Whether relaxation of gut segments by O-1602 is an entirely GPR55 dependent process is uncertain because of its off-target effects. Thus, O-1602 has been shown to retain vasodilatory [39•], orexigenic [40] and anti-inflammatory activities [38•] in $GPR55^{-/-}$ mice. However, a very recent study demonstrated that O-1602 concentration-dependently reduced electrical field-induced contractions in the colon strips from wild-type and $CB_1{}^{-/-}$/$CB_2{}^{-/-}$ knockout mice, an effect that was significantly inhibited in $GPR55^{-/-}$ knockout mice [41]. Interestingly, under physiological conditions, the inverse CB_1 agonist AM251, identified as a GPR55 agonist [23•], increased upper GI transit and whole gut transit in mice, but had no effect on colonic expulsion [42].

On the basis of the assumption that cannabidiol (CBD) acts as a GPR55 antagonist (CBD was shown to antagonize GPR55 activity to the CB receptor agonist CP55940

with an IC_{50} of 445 nM [12•]) the effect of CBD on gut motility was investigated after LPS treatment [37]. LPS increased GPR55 mRNA expression in rat duodenum and ileum and inhibited intestinal motility in mice while CBD counteracted the slowed GI motility, as measured by the upper GI transit time. CBD also normalized the LPS-induced inhibition of isolated rat smooth muscle strips. However, CBD had no effect on GI transit in normal mice or on the resting membrane potential of jejunum smooth muscle suggesting that an involvement of GPR55 in GI motility is more prevalent during inflammatory than healthy conditions. Upregulation of GPR55 during inflammation may demask the antagonistic effect of CBD on GPR55, though this remains speculative. In this respect, the behavior of GPR55 resembles that of the CB_2 receptor which is also increased in inflammatory conditions, such as IBD [32] and which modulates motor functions only in an inflammatory state [33]. The exact mechanism of GPR55 in regulating GI motility remains elusive until a specific GPR55 antagonist is available. In the meantime, experiments in $GPR55^{-/-}$ mice may be an option to address these questions.

The potential role of GPR55 in intestinal inflammation has been also addressed in experimental colitis [38•]. There, the effects of O-1602 were investigated in $CB_1{}^{-/-}/CB_2{}^{-/-}$ and $GPR55^{-/-}$ mice that had been submitted to dextran sulfate sodium (DSS) which induces inflammation of the colon. O-1602 was still effective in preventing colitis in these knockout mice suggesting that the compound had caused an improvement via unidentified targets (Figure 1). The authors also noted that the severity of the colitis was slightly but significantly lower in the $GPR55^{-/-}$ mice than in the respective wild types. The finding favors the hypothesis that GPR55 may play a pro-inflammatory role in GI inflammation.

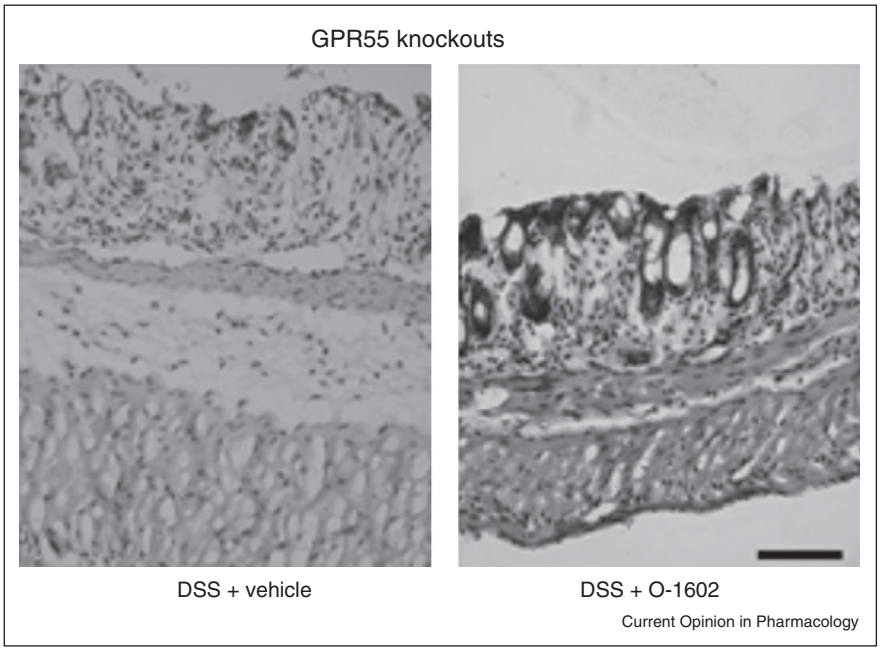
Secretion and mucosal homeostasis

A possible involvement of GPR55 in intestinal secretion remains speculative as this has not been addressed yet. GPR55 is found at sites of the GI tract known to be involved in secretory processes, that is, in the mucosa (mucosal scrapings) of the colon [38•] and in the enteric nervous system [37] which is known to control secretion via intrinsic neurons. A role for GPR55 in secretory processes and mucosal homeostasis is therefore conceivable.

Central regulation of gut motility, food intake and emesis

Since GPR55 is expressed in the brain [2] it could participate in central mechanisms that influence certain functions of the gut. Thus, GPR55 transcripts were demonstrated in the brainstem, which houses the dorsal motor nucleus of the vagal nerve, as well as in the hypothalamus where centers related to energy household are located [2,12•]. This suggests that GPR55 may play a role in food intake. Anandamide and other acylethanolamides such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) activate CBs and GPR55 and induce or inhibit food intake

Figure 1



Hematoxylin/eosin staining of colon wall sections from GPR55 knockout mice exposed to dextran sulfate sodium (DSS) and treated with 5 mg/kg O-1602 (or vehicle) twice daily. The putative GPR55 agonist O-1602 is still effective in decreasing the severity of colitis in GPR55 knockout mice. The mucosa is destructed in the colon of DSS + vehicle-treated mice and there is an increased infiltration of immunocytes in the submucosa. Mucosal damage, cell infiltration and colon thickness decreased noticeably in the O-1602-treated group. Calibration bar: 100 μm.

[43,44]. OEA is widely produced in the intestine and functions as a potent inhibitor of food intake [45], however, unlike PEA, it is a weak GPR55 agonist [12•]. As with CB_1, GPR55 expression could be effected by different levels of anandamide. In the brain, for instance, levels of anandamide were shown to be regulated by fasting [46] and diet composition [47]. The same applies for the gut where anandamide was found to be increased in the small intestine, but not in the stomach, 24 hours after fasting [48]. A recent article describes that a missense polymorphism in the GPR55 gene was associated with *Anorexia nervosa* [49] supporting the idea that GPR55 may play a role in the regulation of food intake.

Emetic reflexes are controlled by CB receptors in the brainstem [50,51]. Vagal afferents express CB_1 receptors which are subject to plastic changes depending on the state of fasting [52]. It would be interesting to determine whether GPR55 is expressed in the nodose ganglion and whether it colocalizes or interacts with CB_1 receptors thereby influencing emesis. Against the idea that GPR55 plays a role in eating disorders it might be argued that $GPR55^{-/-}$ mice do not differ from wild types in their body weights and the amount of their food intake [25•,40]. Likewise, the GPR55 agonist O-1602 induced food intake in mice when given icv [40] and this effect persisted in $GPR55^{-/-}$ mice questioning a major role of GPR55 in food intake.

Is GPR55 involved in GI mechanoreception?

GPR55 was detected in large diameter dorsal root ganglia cells known to represent mechanoreceptors [16••]. This implicates that extrinsic sensory nerve fibers expressing GPR55 very likely innervate the GI tract. GPR55 may be therefore involved in mechanosensation of the GI tract and eventually in the control of GI pain. Splanchnic mechanoreceptors arising from dorsal root ganglia innervate the GI tract at a high percentage [53,54] and have polymodal functions, that is, they can also act as nociceptors [54] or obtain this ability under brief inflammation [55]. A role for GPR55 in mechanoreception and pain is supported by a study of Staton *et al.* who revealed a pro-inflammatory role of GPR55 in mechanical hyperalgesia by demonstrating that $GPR55^{-/-}$ mice failed to develop mechanical hyperalgesia for 2 weeks after intraplantar administration of Freund's complete adjuvants or for 28 days following partial ligation of the sciatic nerve [25•]. On the basis of these observations, GPR55 could play a pro-nociceptive role in the GI tract and contribute to hyperalgesic mechanisms in visceral neurons that underlie the development of irritable bowel syndrome [56]. However, O-1602 did not alter responses to noxious colorectal distension in rats [57] which somewhat argues against an involvement of GPR55 in visceral nociception, but as already mentioned above, the *in vivo* pharmacology of O-1602 may not depend on GPR55. Functional studies addressing the involvement of GPR55 in intestinal mechanoreception have not yet been performed as specific GPR55 antagonists are not yet available. The recent identification of a GPR55 agonist binding site is a promising move to be able to investigate specific GPR55 effects in the near future [58].

Concluding remarks

Despite the paucity of studies on the role of GPR55 in the GI tract, we can positively assume that GPR55 is involved in the regulation of GI functions under physiological and pathophysiological conditions. The expression of GPR55 in brain areas known to govern energy household and bowel functions as well as in enteric epithelial cells and enteric neurons suggests that GPR55 is likely involved in GI processes such as motility and possibly secretion. Its upregulation in gut tissue after systemic inflammation indicates that GPR55 may play a role in pathophysiological mechanisms of intestinal inflammation and this seems to hold true for experimental inflammatory conditions of different kinds. Additionally, the presence of GPR55 in mechanoreceptors raises the intriguing possibility that GPR55 may have a role in the regulation of gut motility via extrinsic nerve fibers, provided that GPR55 is located on mechanoreceptors innervating the GI tract. It will be interesting to find out whether GPR55 interacts with classical CB receptors in response to endocannabinoids in the GI tract and in brain areas affecting GI functions and whether GPR55 may have a role in disorders of food intake. Future studies involving selective agonists, antagonists and possibly $GPR55^{-/-}$ mice are warranted to follow the promising evidence that GPR55 is crucially involved in the regulation of GI function in health and disease and to facilitate possible translation into future treatments of GI diseases.

Disclosures

RS and MS have no conflicts of interest to disclose.

Acknowledgments

RS is supported by grants from the Austrian Science Fund (FWF P 22771), Austrian National Bank (OeNB 14429) and the Franz Lanyar Foundation (351). MS is supported by the Deutsche Forschungsgemeinschaft (DFG).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K *et al.*: **International union of basic and clinical pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB_1 and CB_2.** *Pharmacol Rev* 2010, **62**:588-631.
Very comprehensive review on cannabinoid receptor pharmacology including GPR55

2. 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, ΨGPR53 and GPR55: GPR55 is extensively expressed in human brain.** *Brain Res Mol Brain Res* 1999, **64**:193-198.

3. Baker D, Pryce G, Davies WL, Hiley CR: **In silico patent searching reveals a new cannabinoid receptor**. *Trends Pharmacol Sci* 2006, **27**:1-4.

4. Petitet 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.

5. Schicho R, Storr M: **Alternative targets within the endocannabinoid system for future treatment of gastrointestinal diseases**. *Can J Gastroenterol* 2011, **25**:377-383.

6. Fredriksson R, Lagerström MC, Lundin LG, Schiöth HB: **The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints**. *Mol Pharmacol* 2003, **63**:1256-1272.

7. • 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.
Important study supporting the hypothesis that GPR18 could be the receptor for abnormal cannabidiol (Abn-CBD)

8. McHugh D, Page J, Dunn E, Bradshaw HB: **Δ^9-THC and N-arachidonyl glycine are full agonists at GPR18 and cause migration in the human endometrial cell line, HEC-1B**. *Br J Pharmacol* 2012, **165**:2414-2424.

9. Oka S, Ota R, Shima M, Yamashita A, Sugiura T: **GPR35 is a novel lysophosphatidic acid receptor**. *Biochem Biophys Res Commun* 2010, **395**:232-237.

10. Noguchi K, Ishii S, Shimizu T: **Identification of p2y9/GPR23 as a novel G protein-coupled receptor for lysophosphatidic acid, structurally distant from the Edg family**. *J Biol Chem* 2003, **278**:25600-25606.

11. Kotarsky K, Boketoft A, Bristulf J, Nilsson NE, Norberg A, Hansson S, Owman C, Sillard R, Leeb-Lundberg LM, Olde B: **Lysophosphatidic acid binds to and activates GPR92, a G protein-coupled receptor highly expressed in gastrointestinal lymphocytes**. *J Pharmacol Exp Ther* 2006, **318**:619-628.

12. • 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.
First comprehensive study on GPR55 pharmacology using overexpression in HEK cells

13. 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.

14. Oka S, Toshida T, Maruyama K, Nakajima K, Yamashita A, Sugiura T: **2-Arachidonoyl-sn-glycero-3-phosphoinositol: a possible natural ligand for GPR55**. *J Biochem* 2009, **145**:13-20.

15. •• 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.
This study investigates the interplay of CB_2 and GPR55 signalling pathways and provides evidence that GPR55 modulates inflammatory responses in human neutrophils

16. •• 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 U S A* 2008, **105**:2699-2704.
Excellent study that addresses the role of GPR55 in neurons emphasizing that its cellular functions are distinct to those of cannabinoid receptors

17. • Obara Y, Ueno S, Yanagihata Y, Nakahata N: **Lysophosphatidylinositol causes neurite retraction via GPR55, G_{13} and RhoA in PC12 cells**. *PLoS One* 2011, **6**:e24284.
This study describes the functional role of GPR55 and its endogenous ligand lysophosphatidylinositol in PC12 cells

18. •• Waldeck-Weiermair M, Zoratti C, Osibow K, Balenga N, Goessnitzer E, Waldhoer M, Malli R, Graier WF: **Integrin clustering enables anandamide-induced Ca^{2+} signaling in endothelial cells via GPR55 by protection against CB_1-receptor-triggered repression**. *J Cell Sci* 2008, **121**:1704-1717.
This work provides first evidence that GPR55 and CB_1 may interact in endothelial cells upon stimulation with endocannabinoids

19. Romero-Zerbo SY, Rafacho A, Díaz-Arteaga A, Suárez J, Quesada I, Imbernon M, Ross RA, Dieguez C, Rodríguez de Fonseca F, Nogueiras R, Nadal A, Bermúdez-Silva FJ: **A role for the putative cannabinoid receptor GPR55 in the islets of Langerhans**. *J Endocrinol* 2011, **211**:177-185.

20. 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 U S A* 2009, **106**:16511-16516.

21. •• 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.
This excellent study shows that GPR55 is widely present in human tumor cells and correlates with their aggressiveness

22. 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.

23. • 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.
This work tries to resolve some issues connected with receptor coupling and distinct GPR55 ligands and highlights that GPR55 signaling can be linked to several downstream mechanisms

24. Howlett AC: **Cannabinoid receptor signaling**. *Handb Exp Pharmacol* 2005, **168**:53-79.

25. • Staton PC, Hatcher JP, Walker DJ, Morrison AD, Shapland EM, Hughes JP, Chong E, Mander PK, Green PJ, Billinton A *et al.*: **The putative cannabinoid receptor GPR55 plays a role in mechanical hyperalgesia associated with inflammatory and neuropathic pain**. *Pain* 2008, **139**:225-236.
One of the few *in vivo* studies on GPR55 knockout mice indicating that GPR55 may play a role in the development of mechanical hyperalgesia in pain models

26. •• Ross RA: **The enigmatic pharmacology of GPR55**. *Trends Pharmacol Sci* 2009, **30**:156-163.
This review summarizes the different actions of GPR55 in different cell systems and assays and tries to clear the contentious issues surrounding its pharmacology

27. •• Brown AJ, Robin Hiley C: **Is GPR55 an anandamide receptor?** *Vitam Horm* 2009, **81**:111-137.
This review elegantly discusses the possibility that anandamide is an endogenous ligand of GPR55 and that GPR55 may have several signal modalities

28. •• Sharir H, Abood ME: **Pharmacological characterization of GPR55, a putative cannabinoid receptor**. *Pharmacol Ther* 2010, **126**:301-313.
Comprehensive review on GPR55 pharmacology and possible physiological functions of GPR55

29. • 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.
First comprehensive review on physiological and pathophysiological functions of GPR55

30. Nevalainen T, Irving AJ: **GPR55, a lysophosphatidylinositol receptor with cannabinoid sensitivity?** *Curr Top Med Chem* 2010, **10**:799-813.

31. Fioramonti J, Bueno L: **Role of cannabinoid receptors in the control of gastrointestinal motility and perception**. *Expert Rev Gastroenterol Hepatol* 2008, **2**:385-397.

32. Wright KL, Duncan M, Sharkey KA: **Cannabinoid CB_2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation**. *Br J Pharmacol* 2008, **153**:263-270.

33. Duncan M, Mouihate A, Mackie K, Keenan CM, Buckley NE, Davison JS, Patel KD, Pittman QJ, Sharkey KA: **Cannabinoid CB_2 receptors in the enteric nervous system modulate gastrointestinal contractility in lipopolysaccharide-treated rats**. *Am J Physiol Gastrointest Liver Physiol* 2008, **295**:G78-G87.

34. • Storr MA, Keenan CM, Emmerdinger D, Zhang H, Yüce B, Sibaev A, Massa F, Buckley NE, Lutz B, Göke B *et al.*: **Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB_1 and CB_2 receptors**. *J Mol Med (Berl)* 2008, **86**:925-936.
Interesting study describing that manipulation of the endocannabinoid system may have inhibiting effects on the development of colitis

35. • Storr MA, Keenan CM, Zhang H, Patel KD, Makriyannis A, Sharkey KA: **Activation of the cannabinoid 2 receptor (CB_2) protects against experimental colitis**. *Inflamm Bowel Dis* 2009, **15**:1678-1685.
First study demonstrating that activation of CB_2 decreases severity of colitis in experimental mouse models

36. Duncan M, Davison JS, Sharkey KA: **Review article: endocannabinoids and their receptors in the enteric nervous system**. *Aliment Pharmacol Ther* 2005, **22**:667-683.

37. Lin XH, Yuece B, Li YY, Feng YJ, Feng JY, Yu LY, Li K, Li YN, Storr M: **A novel CB receptor GPR55 and its ligands are involved in regulation of gut movement in rodents**. *Neurogastroenterol Motil* 2011, **23** 862-e342.

38. • Schicho R, Bashashati M, Bawa M, McHugh D, Saur D, Hu HM, Zimmer A, Lutz B, Mackie K, Bradshaw HB *et al.*: **The atypical cannabinoid O-1602 protects against experimental colitis and inhibits neutrophil recruitment**. *Inflamm Bowel Dis* 2011, **17**:1651-1664.
This study elegantly demonstrates that O-1602, a synthetic and non-sedative cannabinoid, exerts protective effects on DSS-induced colitis independently of CB and GPR55 receptor activation

39. • Johns DG, Behm DJ, Walker DJ, Ao Z, Shapland EM, Daniels DA, Riddick M, Dowell S, Staton PC, Green P *et al.*: **The novel endocannabinoid receptor GPR55 is activated by atypical cannabinoids but does not mediate their vasodilator effects**. *Br J Pharmacol* 2007, **152**:825-831.
First study performed in GPR55 knockout mice that provides evidence that GPR55 is not responsible for vasodilation by atypical cannabinoids

40. Díaz-Arteaga A, Vázquez MJ, Vazquez-Martínez R, Pulido MR, Suarez J, Velásquez DA, López M, Ross RA, de Fonseca FR, Bermudez-Silva FJ *et al.*: **The atypical cannabinoid O-1602 stimulates food intake and adiposity in rats**. *Diabetes Obes Metab* 2011 http://dx.doi.org/10.1111/j.1463-1326.2011.01515.x.

41. Ross GR, Lichtman A, Dewey WL, Akbarali HI: **Evidence for the Putative cannabinoid receptor (GPR55)-mediated inhibitory effects on intestinal contractility in mice**. *Pharmacology* 2012, **90**:55-65.

42. Storr MA, Bashashati M, Hirota C, Vemuri VK, Keenan CM, Duncan M, Lutz B, Mackie K, Makriyannis A, Macnaughton WK, Sharkey KA: **Differential effects of CB_1 neutral antagonists and inverse agonists on gastrointestinal motility in mice**. *Neurogastroenterol Motil* 2010, **22** 787-e223.

43. Williams CM, Kirkham TC: **Anandamide induces overeating: mediation by central cannabinoid (CB_1) receptors**. *Psychopharmacology (Berl)* 1999, **143**:315-317.

44. Borrelli F, Izzo AA: **Role of acylethanolamides in the gastrointestinal tract with special reference to food intake and energy balance**. *Best Pract Res Clin Endocrinol Metab.* 2009, **23**:33-49.

45. Rodríguez de Fonseca F, Navarro M, Gómez R, Escuredo L, Nava F, Fu J, Murillo-Rodríguez E, Giuffrida A, LoVerme J, Gaetani S *et al.*: **An anorexic lipid mediator regulated by feeding**. *Nature* 2001, **414**:209-212.

46. Kirkham TC, Williams CM, Fezza F, Di Marzo V: **Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol**. *Br J Pharmacol* 2002, **136**:550-557.

47. Watanabe S, Doshi M, Hamazaki T: **n-3 polyunsaturated fatty acid (PUFA) deficiency elevates and n-3 PUFA enrichment reduces brain 2-arachidonoylglycerol level in mice**. *Prostaglandins Leukot Essent Fatty Acids* 2003, **69**:51-59.

48. Matias I, Bisogno T, Di Marzo V: **Endogenous cannabinoids in the brain and peripheral tissues: regulation of their levels and control of food intake**. *Int J Obes (Lond)* 2006, **30**:S7-S12.

49. 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.

50. Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS *et al.*: **Identification and functional characterization of brainstem cannabinoid CB_2 receptors**. *Science* 2005, **310**:329-332.

51. Sharkey KA, Cristino L, Oland LD, Van Sickle MD, Starowicz K, Pittman QJ, Guglielmotti V, Davison JS, Di Marzo V: **Arvanil, anandamide and N-arachidonoyl-dopamine (NADA) inhibit emesis through cannabinoid CB_1 and vanilloid TRPV1 receptors in the ferret**. *Eur. J. Neurosci* 2007, **25**:2773-2782.

52. Burdyga G, Varro A, Dimaline R, Thompson DG, Dockray GJ: **Expression of cannabinoid CB_1 receptors by vagal afferent neurons: kinetics and role in influencing neurochemical phenotype**. *Am J Physiol Gastrointest Liver Physiol* 2010, **299**:G63-G69.

53. Sengupta JN, Gebhart GF: **Characterization of mechanosensitive pelvic nerve afferent fibers innervating the colon of the rat**. *J Neurophysiol* 1994, **71**:2046-2060.

54. Ozaki N, Gebhart GF: **Characterization of mechanosensitive splanchnic nerve afferent fibers innervating the rat stomach**. *Am J Physiol Gastrointest Liver Physiol* 2001, **281**:G1449-G1459.

55. Feng B, Gebhart GF: **Characterization of silent afferents in the pelvic and splanchnic innervations of the mouse colorectum**. *Am J Physiol Gastrointest Liver Physiol* 2011, **300**:G170-G180.

56. Sengupta JN: **Visceral pain: the neurophysiological mechanism**. *Handb Exp Pharmacol* 2009, **194**:31-74.

57. Brusberg M, Arvidsson S, Kang D, Larsson H, Lindström E, Martinez V: **CB_1 receptors mediate the analgesic effects of cannabinoids on colorectal distension-induced visceral pain in rodents**. *J Neurosci* 2009, **29**:1554-1564.

58. Kotsikorou E, Madrigal KE, Hurst DP, Sharir H, Lynch DL, Heynen-Genel S, Milan LB, Chung TD, Seltzman HH, Bai Y *et al.*: **Identification of the GPR55 agonist binding site using a novel set of high-potency GPR55 selective ligands**. *Biochemistry* 2011, **50**:5633-5647.