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A role for O-1602 and G protein-coupled receptor GPR55 in the control of colonic motility in mice

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ABSTRACT

Objective: The G protein-coupled receptor 55 (GPR55) is a novel cannabinoid (CB) receptor, whose role in the gastrointestinal (GI) tract remains unknown. Here we studied the significance of GPR55 in the regulation of GI motility.
Design: GPR55 mRNA and protein expression were measured by RT-PCR and immunohistochemistry. The effects of the GPR55 agonist O-1602 and a selective antagonist cannabidiol (CBD) were studied in vitro and in vivo and compared to a non-selective cannabinoid receptor agonist WIN55,212-2. $CB_{1/2}^{-/-}$ and $GPR55^{-/-}$ mice were employed to identify the receptors involved.
Results: GPR55 was localized on myenteric neurons in mouse and human colon. O-1602 concentration-dependently reduced evoked contractions in muscle strips from the colon (~60%) and weakly (~25%) from the ileum. These effects were reversed by CBD, but not by CB_1 or CB_2 receptor antagonists. I.p. and i.c.v. injections of O-1602 slowed whole gut transit and colonic bead expulsion; these effects were absent in $GPR55^{-/-}$ mice. WIN55,212-2 slowed whole gut transit effects, which were counteracted in the presence of a CB1 antagonist AM251. WIN55,212-2, but not O-1602 delayed gastric emptying and small intestinal transit. Locomotion, as a marker for central sedation, was reduced following WIN55,212-2, but not O-1602 treatment.
Conclusion: GPR55 is strongly expressed on myenteric neurons of the colon and it is selectively involved in the regulation of colonic motility. Since activation of GPR55 receptors is not associated with central sedation, the GPR55 receptor may serve as a future target for the treatment of colonic motility disorders.

1. Introduction

The prevalence of functional gastrointestinal disorders (FGID), such as irritable bowel syndrome (IBS), is currently estimated at 10–20%. It has a tendency to increase, in particular in the societies adopting Western style of living (Philpott et al., 2011). Symptoms manifested by FGID patients – predominantly altered motility patterns, stool inconsistency and bloating are not life-threatening, but are often associated with abdominal pain and have a negative impact on life quality. Thus, such disturbances have become a heavy economic burden due to increased work absenteeism, as well as increased use of health care services (Drossman, 2006). Current

Abbreviations: CBD, cannabidiol; CB_1, cannabinoid-1; CB_2, cannabinoid-2; cDNA, complementary DNA; COX-2, cyclooxygenase-2; GE, gastric emptying; GPR55, G protein-coupled receptor 55; EFS, electrical field stimulation; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; GI, gastrointestinal; i.c.v., intracerebroventricular administration; i.p., intraperitoneal; KRS, Krebs–Ringer solution; LMMP, longitudinal muscle-myenteric plexus layer; MAGL, monoacylglycerol lipase; PPARα, peroxisome proliferator-activated receptor-alpha; RT-PCR, reverse transcription polymerase chain reaction; TRPV1, transient receptor potential vanilloid 1.

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understanding of the pathogenesis of FGID and their clinical resolution are unsatisfactory. So far, hypotheses suggest low grade inflammation (Mayer and Collins, 2002; Philpott et al., 2011), food allergy (Atkinson et al., 2004) or disturbances in the bi-directional communication between the gut and the central nervous system (CNS) (Fichna and Storr, 2012). New therapeutic strategies, alleviating motility disturbances and pain without adverse, mainly related to the central nervous system side effects are therefore urgently needed.

The endocannabinoid system (ECS) consists of cannabinoid $(CB)_1$ and CB_2 receptors, their endogenous ligands anandamide and 2-arachidonylglycerol, and the synthesizing and degrading enzymes for these ligands. Cannabinoids and the ECS are involved in the regulation of GI motility in physiological and pathophysiological conditions (Izzo et al., 2001; Massa et al., 2005; Storr et al., 2008; Pertwee et al., 2010). In particular, the involvement of the cannabinoid receptors CB_1 and CB_2 and the endocannabinoid degrading enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) has been reported (Pinto et al., 2002; Duncan et al., 2008; Storr et al., 2009). In the last two decades, in vitro and in vivo studies have revealed roles for both central and peripheral CB receptors in the control of GI motility (Coutts and Izzo, 2004; Izzo et al., 2001; Storr and Sharkey, 2007). Interestingly, not all cannabinoid effects on GI motility can be explained by actions on CB_1 and CB_2 receptors and numerous additional sites of actions have been suggested. These include transient receptor potential vanilloid 1 (TRPV1) receptors, peroxisome proliferator-activated receptor-alpha (PPARα) and cyclooxygenase-2 (COX-2) inhibition (Pertwee et al., 2010; Piomelli, 2003).

Recently, an orphan G protein-coupled receptor 55 (GPR55) was shown to be a binding site for cannabinoids and became a likely candidate for mediating some of the previously unexplained non-CB_1, non-CB_2 effects induced by certain cannabinoids (Moriconi et al., 2010; Pertwee, 2007; Ross, 2009). Radioligand binding studies demonstrated GPR55 binding for both endocannabinoids and synthetic cannabinoids that may also be agonists or antagonists at CB_1 or CB_2 receptors, or both. The atypical synthetic cannabinoid O-1602 was found to activate GPR55 with negligible binding to CB_1 and CB_2 and is considered as a selective GPR55 agonist (Ryberg et al., 2007; Whyte et al., 2009; Johns et al., 2007; Schicho et al., 2010). Recent data from a transgenic $GPR55^{-/-}$ mouse model further supports O-1602 as a GPR55 agonist (Whyte et al., 2009). Little information is available on antagonists at the GPR55 receptor, although cannabidiol (CBD) appears to be a selective GPR55 receptor antagonist (Pertwee, 2007; Ryberg et al., 2007; Whyte et al., 2009; Thomas et al., 2007).

Localization of GPR55 is rarely studied. However, convincing data shows that GPR55 mRNA is expressed in the brain (Sawzdargo et al., 1999). The GPR55 activation was reported to have effects on osteoclast function, bone density and cancer cell proliferation, but surprisingly there is no conclusive information yet available shedding light on the localization and function of GPR55 in the gastrointestinal (GI) tract (Whyte et al., 2009; Sawzdargo et al., 1999; Pineiro et al., 2010; Andradas et al., 2010; Lin et al., 2011).

The present study aimed at identifying whether GPR55 is expressed in the GI tract and where it is localized. We also investigated the role of the GPR55 receptor in the regulation of mouse GI motility in vitro and in vivo, utilizing a selective GPR55 agonist, O-1602 and the antagonist, CBD. The effects were further analysed employing a well-characterized cannabinoid receptor agonist WIN55,212-2 and selective CB_1 and CB_2 antagonists. The involvement of respective receptors in the action of O-1602 and CBD was also studied in the $CB_{1/2}^{-/-}$ and $GPR55^{-/-}$ mice. Finally, the action of GRP55-selective compounds in the central nervous effects was characterized.

2. Material and methods

2.1. Animals

Male CD1, $GPR55^{-/-}$ and $CB_{1/2}^{-/-}$ mice and their littermates on a C57Bl/6 background were used throughout the study (22–26 g). The CD1 mice were purchased from Charles River (Sherbrooke, QC, Canada). The $GPR55^{-/-}$ mice were acquired from the Texas Institute of Genomic Medicine (TIGM, Houston, TX) and bred at the animal facilities of the Department of Psychological and Brain Sciences, Indiana University, Bloomington, USA (Wu et al., 2010). The $CB_{1/2}^{-/-}$ mice were bred at the mouse facility at the University of Calgary, Canada from a pair of animals provided by A. Zimmer (University of Bonn, Germany). CD1 mice for the PCR experiments were housed in the animal facility of the Technical University of Munich, Germany.

All experiments were performed in CD1 mice unless otherwise stated. Animals were matched by age and body weight. Mice were housed at a 12:12-h light–dark cycle in sawdust coated plastic cages with access to standard laboratory chow and tap water ad libitum. Mice were allowed 1 week of acclimatization prior to use. For in vitro experiments mice were sacrificed by cervical dislocation and their GI tract was isolated and washed with normal solution. All experiments were approved by the University of Calgary Animal Care Committee and the experiments were performed in accordance with institutional animal ethics committee guidelines following the guidelines established by the Canadian Council of Animal Care. The $GPR55^{-/-}$ mouse experiments were approved by the Indiana University (Bloomington, IN, USA) Institutional Animal Care and Use Committee.

All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. RNA isolation and RT-PCR

To determine GPR55 mRNA expression in mouse GI tract, total RNA was extracted from the longitudinal muscle-myenteric plexus (LMMP) and the mucosal layer of the mouse colon and ileum. Tissue preparation: Adult CD1 mice were killed by cervical dislocation. The small and large intestine was removed and cleaned in ice-cold PBS. Attached mesenterial fat was removed and the longitudinal muscle layer with attached myentric plexus (LMMP) was removed from the circular muscle layer by peeling. Subsequently, the mucosa was removed from the circular muscle layer by scraping. Tissues were cut into small pieces, immediately frozen in liquid nitrogen and stored at −80 °C until use. RNA isolation, reverse transcription, PCR amplification, and agarose gel electrophoresis are described in detail in the Appendix.

2.3. Immunohistochemistry

Whole mount preparations of the ileum and the distal colon were obtained for the immunohistochemical detection of GPR55 using standard approaches. The procedures were as follows: after incubating in a solution of 0.1 M PBS, 4% donkey serum, and 0.1% Triton-X-100 for 1.5 h, the whole mounts were exposed to rabbit anti-GPR55 (K. Mackie), 1:800, overnight at 4 °C. To visualize immunoreactivity, a fluorophore-conjugated secondary antibody (anti-rabbit Cy3; 1:800; Jackson ImmunoResearch, West Grove, PA, USA) was used. The specimens were examined under a Zeiss Axioplan brightfield/fluorescence microscope and photographed with a digital camera (Sensys, Photometrics, Tucson, AZ, USA). Brightness and contrast of the images were adjusted using Adobe Photoshop®. Negative controls in which the primary antibody was omitted and specificity controls with antigen–antibody preabsorption were performed.

Paraffin-embedded sections of human colon were obtained from the tissue bank of the University of Graz, Austria. Sections were deparaffinized, microwaved for 2 × 5 min cycles in 10 mM citrate buffer and then processed by the ABC method (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's protocol. Sections were incubated with rabbit anti-GPR55 (1:800; Cayman Chemical Company, Ann Arbor, Michigan, USA), visualized with 3-3′-diaminobenzidine and counterstained with haematoxylin. The specificity of the antibody was tested by omitting the primary antibody and by incubating the GPR55 antibody with blocking peptide provided by the manufacturer (Cayman Chemical Company, Ann Arbor, Michigan, USA).

2.4. Isolated intestinal segments

The experiments on isolated intestinal segments were performed using a setup described previously. At the beginning of each experiment, 0.5 g tension was applied, the tissue was incubated for 30 min under standard conditions and then stimulated with bethanechol (10^{-5} M) in order to obtain a maximal contraction (100% contraction). The tissue was then washed 3 times and the experiment was started. In a first set of experiments, tissues were exposed to either the GPR55 agonist O-1602 (10^{-10}–10^{-6} M), the cannabinoid receptor agonist WIN55,212-2 (10^{-10}–10^{-6} M), the CB_1 antagonists AM251 and SR141716A (both 10^{-7} M), the CB_2 antagonist AM630 (10^{-7} M), and the GPR55 antagonist CBD (10^{-7} M). Changes in tension or basal activity were then recorded for 60 min.