Histochemistry and Cell Biology (2018) 150:187–205
https://doi.org/10.1007/s00418-018-1684-7

ORIGINAL PAPER

Localization of cannabinoid receptors CB1, CB2, GPR55, and PPARα in the canine gastrointestinal tract

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Accepted: 2 June 2018 / Published online: 7 June 2018
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Abstract

The endocannabinoid system (ECS) is composed of cannabinoid receptors, their endogenous ligands, and the enzymes involved in endocannabinoid turnover. Modulating the activity of the ECS may influence a variety of physiological and pathophysiological processes. A growing body of evidence indicates that activation of cannabinoid receptors by endogenous, plant-derived, or synthetic cannabinoids may exert beneficial effects on gastrointestinal inflammation and visceral pain. The present ex vivo study aimed to investigate immunohistochemically the distribution of cannabinoid receptors CB1, CB2, G protein-coupled receptor 55 (GPR55), and peroxisome proliferation activation receptor alpha (PPARα) in the canine gastrointestinal tract. CB1 receptor immunoreactivity was observed in the lamina propria and epithelial cells. CB2 receptor immunoreactivity was expressed by lamina propria mast cells and immunocytes, blood vessels, and smooth muscle cells. Faint CB2 receptor immunoreactivity was also observed in neurons and glial cells of the submucosal plexus. GPR55 receptor immunoreactivity was expressed by lamina propria macrophages and smooth muscle cells. PPARα receptor immunoreactivity was expressed by blood vessels, smooth muscle cells, and glial cells of the myenteric plexus. Cannabinoid receptors showed a wide distribution in the gastrointestinal tract of the dog. Since cannabinoid receptors have a protective role in inflammatory bowel disease, the present research provides an anatomical basis supporting the therapeutic use of cannabinoid receptor agonists in relieving motility disorders and visceral hypersensitivity in canine acute or chronic enteropathies.

Keywords Enteric nervous system · Immunohistochemistry · Inflammatory bowel disease · Mast cells · Palmitoylethanolamide

Introduction

The endocannabinoid system (ECS) is composed of cannabinoid receptors, endocannabinoids, and the enzymes for their production and degradation (Stella 2004; Ligresti et al. 2016; Lu and Anderson 2017). It classically comprises the cannabinoid receptors types 1 and 2 (CB1 and CB2), the endocannabinoids N-arachidonylethanolamine (anandamide; AEA) and 2-arachidonylglycerol (2-AG), and the enzymes responsible for endocannabinoid biosynthesis and degradation (Iannotti et al. 2016). Currently, the definition of the ECS is expanding to include, besides the classical cannabinoid receptors and endocannabinoids, several fatty acid derivatives—the so-called endocannabinoid-like mediators, such as palmitoylethanolamide (PEA)—as well as other non-CB receptors (Kreitzer and Stella 2009; Iannotti et al. 2016). This is the case, for example, for the G protein-coupled receptor 55 (GPR55), nuclear peroxisome proliferator-activated receptor alpha (PPARα), and transient receptor potential vanilloid type 1 (TRPV1), all of which are currently considered as possible cannabinoid receptors (Brown et al. 2005; Di Marzo et al. 2002; Izzo and Sharkey 2010; Lauchner et al. 2008; Lin et al. 2011; Petrosino and Di Marzo 2017; Tuduri et al. 2017). Notably, the ligands of this

Marco Pietra and Roberto Chiocchetti shared the senior authorship of this study.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00418-018-1684-7) contains supplementary material, which is available to authorized users.

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manifold receptor system often activate more than one target site in conjunction to exert their effect (O'Sullivan 2016). Thus, the evolving idea of the 'endocannabinoidome'—a more complex system including endocannabinoids and endocannabinoid-like mediators, their redundant metabolic enzymes and ''promiscuous'' molecular targets (i.e., receptors)—is increasingly gaining ground (Maione et al. 2013). The ECS is widely expressed in the central nervous system (CNS), cardiovascular, gastrointestinal, immune, and reproductive system (Maccarrone et al. 2015; Cabral et al. 2015).

The CB1 receptor is expressed mostly in the CNS and peripheral nerves (Hu and Mackie 2015; Freundt-Revilla et al. 2017), and the CB2 receptor is mainly expressed in immune cells (Di Marzo and Izzo 2006). Many neuronal cell types, distributed in several CNS areas, express the CB1 receptor; however, the wide distribution of the CB1 receptor limits its potential as a pharmacological target for CNS diseases, due to the psychoactive side effects associated with CB1 receptor agonists and antagonists (Moreira et al. 2009). A recent study highlighted the expression of the CB2 receptor in neurons and inflammatory non-neuronal cells of the CNS, e.g., microglia (Malfitano et al. 2014). The evidence that the CB2 receptor is upregulated in a variety of CNS diseases characterized by microglia and/or astroglia activation suggests that the CB2 receptor might represent a promising pharmacological target to be considered in diseases characterized by neuroinflammation (Skaper et al. 2013; Navarro et al. 2016; Cassano et al. 2017; Chen et al. 2017; Freundt-Revilla et al. 2018).

In the gastrointestinal tract (GIT), cannabinoid receptors regulate motility, secretion, sensation, emesis, satiety, and inflammation (Izzo 2004; Duncan et al. 2005a, b, 2008; Storr and Sharkey 2007; Wright et al. 2008; Sharkey and Wiley 2016; Lee et al. 2016; Di Patrizio 2016). The CB1 receptor is expressed by neurons of the enteric nervous system (ENS) of rodents (Duncan et al. 2005a, b), guinea-pig (Coutts et al. 2002), pig (Kulkarni-Narla et al. 2000), and ferret (Van Sickle et al. 2001). The CB2 receptor may be expressed by macrophages, plasma cells, mast cells, dendritic cells, lymphocytes, smooth muscle cells, epithelial cells, and enteric neurons (Facci et al. 1995; Wright et al. 2005, 2008; Duncan et al. 2005b, 2008; Svensson et al. 2010; Ke et al. 2016). Several investigations suggest that CB1 or CB2 receptors might have a protective role in inflammatory bowel disease (IBD) in humans, and support the potential therapeutic effects of targeting these pathways using pharmacological agents (Izzo 2004; Di Marzo and Izzo 2006; Duncan et al. 2008; Di Marzo and Piscitelli 2011; Di Patrizio 2016; Gyires and Zádori 2016; Fabisiak and Fichna 2017). The activation of CB1 receptor reduces emesis, intestinal motility and secretion, and inhibits gastric acid secretion and relaxation of the lower esophageal sphincter (Izzo and Coutts 2005). Activation of the CB2 receptor in pathological conditions such as inflammatory bowel disease (IBD) or endotoxic inflammation reduces intestinal motility (Izzo 2004); thus, activation of CB2 receptor seems to represent a novel mechanism for the re-establishment of normal gastrointestinal transit after an inflammatory stimulus.

Palmitoylethanolamide (PEA), a lipid mediator structurally related to AEA, is used in human and veterinary medicine for its neuroprotective, anti-neuroinflammatory, analgesic, and antipruritic properties (Re et al. 2007; Gabrielssson et al. 2016; Petrosino and Di Marzo 2017; Cremon et al. 2017). It was isolated for the first time from lipid fractions of soybeans, egg yolk, and peanut meal and was then found in a wide variety of food sources (data reviewed in Petrosino and Di Marzo 2017). Several investigators have identified different mode of action of PEA (Iannotti et al. 2016; Petrosino and Di Marzo 2017), which seems to have a direct effect upon PPARα (Lo Verme et al. 2005a; Lo Verme et al. 2005b; Gabrielsson et al. 2016), GPR55 (Ryberg et al. 2007; Cantarella et al. 2011), and CB2 (Facci et al. 1995). The latter receptor may also be activated through an indirect mechanism (De Petrocellis et al. 2001; Guida et al. 2017). Finally, PEA directly and indirectly acts on TRPV1 (Ho et al. 2008; De Petrocellis and Di Marzo 2010), a receptor usually expressed by nociceptive dorsal root ganglia sensory neurons (Caterina et al. 1997) that undergoes desensitization by endocannabinoids (Zygmunt et al. 1999; Ambrosino et al. 2013).

PEA, which also seems to act favourably on visceral pain (Jaggar et al. 1998; Farquhar-Smith et al. 2002; Gabrielsson et al. 2016), represents a promising natural approach for hypersensitivity management in dogs with intestinal inflammation. A prerequisite for the therapeutic potential of PEA in pathological GIT conditions such as acute or chronic enteropathies is the cellular distribution of the receptors PEA is known to act upon, i.e., the cannabinoid receptors, in different tracts of the canine digestive system. Therefore, the present study aimed to immunohistochemically characterize the cellular expression of CB1, CB2, GPR55, and PPARα receptors in *ex-vivo* GIT tissues of dogs.

Materials and methods

Animals

Gastrointestinal tissues were collected from three dogs (#1 female, 8-month-old Chihuahua; #2 spayed female, 11-year-old Labrador Retriever, and #3 male, 17-year-old West Highland White Terrier), that did not have a history of gastrointestinal disorders and did not show gross alteration of the gastrointestinal wall.

Animals died spontaneously or were euthanized for humane reasons due to different diseases and tissues were collected following consent from the owners.

According to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, the Italian legislation (D. Lgs. n. 26/2014) did not require any approval by competent authorities or ethics committees, because this research did not influence any therapeutic decisions.

Tissue collection

GIT samples (pylorus, descending duodenum, ileum, and distal colon) were collected within 1 h of death and were longitudinally opened along the pyloric small curvature and intestinal mesenteric border. Tissues were then washed in phosphate-buffered saline (PBS), fixed, and processed to obtain cryosections (2.0 cm × 0.5 cm), which were later processed for immunohistochemistry, as previously described (Chiocchetti et al. 2015; Giancola et al. 2016, 2017).

Immunofluorescence

Cryosections were hydrated in phosphate-buffered saline (PBS), and processed for immunostaining. To block non-specific binding, the sections were incubated in a solution containing 20% normal goat or donkey serum (Colorado Serum Co. Denver, CO, USA), 0.5% Triton X-100 (Sigma-Aldrich, Milan, Italy, Europe), and bovine serum albumin (1%) in PBS for 1 h at room temperature (RT). The cryosections were incubated overnight in a humid chamber at RT with a cocktail of primary antibodies (Table 1) diluted in 1.8% NaCl in 0.01M PBS containing 0.1% sodium azide. After washing in PBS (3 × 10 min), the sections were incubated for 1 h at RT in a humid chamber with the secondary antibodies (Table 2) diluted in PBS. Cryosections were then washed in PBS (3 × 10 min) and mounted in buffered glycerol at pH 8.6 with 4′,6-diamidino-2-phenylindole—DAPI (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Since classic (CB1 and CB2) and "new" cannabinoid receptors (GPR55 and PPAR-α) might be located on different cellular types, we employed a panel of specific antibodies with the aim of co-localizing the cannabinoid receptors' immunoreactivity on enteric neurons, enteric glial cells (EGCs), mast cells (MCs), macrophages, and plasma cells. In particular, to identify enteric neurons and glial cells, anti-human neuronal protein (HuC/HuD) and anti-GFAP antibodies were used, respectively. To identify MCs, different antibodies against MC tryptase were used. To identify macrophages, we utilized the anti-ionized calcium binding adapter molecule 1 antibody (IBA1). Plasma cells were identified using the anti-IgA antibodies. Endothelial cells were identified using two different endothelial markers, i.e., the mouse anti-CD31 antibody, and the rabbit anti-Factor VIII-related antigen/von Willebrand factor. To identify enterochromaffin cells, we utilized the anti-serotonin antibody.

Specificity of the primary antibodies

The specificity of the mouse anti-HuC/HuD antibody was recently demonstrated in dog tissues by Western blot analysis (Giancola et al. 2016). The supplier of the anti-CB1

Table 1 Primary antibodies used in the study

Primary antibody	Host	Code	Dilution	Source
CB1	Rabbit	Orb10430	1:200	Biorbyt
CB2	Rabbit	AB45942	1:200	Abcam
CD31	Mouse	M0823 Clone JC70A	1:30	Dako
GFAP	Chicken	AB4674	1:800	Abcam
GPR55	Rabbit	NLS6817	1:200	Novus Biol.
Factor VIII	Rabbit	A0082	1:1000	Dako
HuC/HuD	Mouse	A21271	1:200	Life Technologies
IBA1	Goat	NB100-1028	1:80	Novus Biol.
IgA	Rabbit	A80-103A	1:1000	Bethyl Lab.
IgA	Goat	NB724	1:1000	Novus Biol.
PPARα	Rabbit	NB600-636	1:200	Novus Biol.
Serotonin	Mouse	Ab16007; # 5HT-H209	1:500	Abcam
Tryptase	Rabbit	PAB070Ca01	1:80	Cloude-Clone Corp.
Tryptase	Mouse	NBP1-40202	1:200	Novus Biol.
Tryptase	Mouse	M7052 Clone AA1	1:200	Dako

Primary antibodies Suppliers Abcam, Cambridge, UK; Bethyl Laboratories, Montgomery, TX, USA; Biorbyt Ltd., Cambridge, UK; Agilent, Santa Clara, CA, USA; Cloude-Clone Corp., Huston, TX, USA; Dako Cytomation, Golstrup, Denmark; Fitzgerald Industries Int., Inc. Concord, MA, USA; Life Technologies, Carlsbad, CA, USA; Novus Biologicals, Littleton, CO, USA

Table 2 Secondary antibodies used in the study

Secondary antibody	Host	Code	Dilution	Source
Anti-mouse IgG Alexa 594	Goat	A11005	1:200	Life Technologies
Anti-mouse IgG Alexa 488	Donkey	20,010	1:100	Biotium
Anti-mouse IgG Alexa 594	Donkey	AB150132	1:1000	Abcam
Anti-rabbit IgG FITC	Goat	401,314	1:200	Calbiochem-Novabiochem
Anti-rabbit 488	Donkey	AB150073	1:800	Abcam
Anti-goat IgG 594	Donkey	AB150132	1:600	Abcam
Anti-chicken TRITC	Donkey	703-025-155	1:200	Jackson

Secondary antibodies Suppliers Abcam, Cambridge, UK; Biotium, Inc. Hayward, CA, USA; Calbiochem-Novabiochem, San Diego, CA, USA; Jackson Immuno Research Laboratories, Inc. Baltimore Pike, PA, USA; Life technologies, Carlsbad, CA, USA;

receptor antibody, raised in rabbit against the human CB1 receptor, predicts cross-reactivity with the dog, mouse, and rat antigen. The sequence of canine CB1 protein is homologous (98.3%) to the sequence of human CB1 protein (https://www.uniprot.org/) (Anday and Mercier 2005). The anti-CB2 receptor antibody was raised in rabbit and directed against residues 200–300 of the rat CB2 receptor. The sequence of the canine CB2 protein is homologous (98.3%) to the sequence of the rat CB2 protein (https://www.uniprot.org/). The anti-CB2 receptor antibody utilized in the present study has already been tested on dog tissues (Campora et al. 2012). Based on the sequence identities, the antibodies against CB1 and CB2 receptors should cross-react with the same antigens in the dog.

The antibody anti-GPR55 receptor was raised against a 17 amino acid synthetic peptide of human GPR55 receptor. The sequence of canine GPR55 protein is homologous (83.5%) to the sequence of human GPR55 protein (https://www.uniprot.org/); furthermore, the antibody supplier indicated greater (94%) cross-reactivity of the antibody with the canine GPR55 protein. Taken together, this suggests that this antibody should cross-react with the same antigen in the dog.

The anti-PPARα receptor antibody was raised in rabbit against the synthetic peptide of mouse PPAR-α. The sequence of canine PPARα protein is homologous (90%) to the sequence of mouse PPARα protein (https://www.uniprot.org/). Furthermore, the supplier of the anti-PPARα receptor antibody predicted cross-reactivity with the same antigen in the dog.

Despite the presumed or already demonstrated specificity of the anti-cannabinoid receptor (CB1, CB2, GPR55, PPARα) antibodies utilized in the present research on canine tissues, we tested their specificity by Western blot (WB) analysis (Fig. 1).

In addition, to confirm that CB1 antibody staining was specific for CB1 protein, the rabbit anti-CB1 antibody was tested on brain cryosections obtained from wild-type mice and mice with congenital deficiency of CB1 (Marsicano et al. 2002). In the cryosections of CB1 null mice, the specific CB1 staining of axons was absent (Supplementary Fig. 1a–f). Furthermore, the rabbit anti-CB1 antibody was tested on wholemount preparations of rat MP and SMP, in which neurons of both the plexuses showed CB1 receptor immunolabelling (Supplementary Fig. 1g–l).

To identify MCs, we utilized the only commercially available anti-dog tryptase antibody (Cloude-Clone, PA B070Ca01, Huston, TX, USA). Since this antibody was raised in rabbit, as were all the anti-cannabinoid receptor antibodies utilized in this study, we co-localized the dog-specific anti-tryptase antibody with two commercially available anti-human tryptase antibodies raised in mouse: Dako, M 7052 (Clone AA1) and Novus Biol (NBP1-40202). The first antibody (clone AA1) has already been used in canine intestinal tissues (Kleinschmidt et al. 2007). The supplier of the second antibody (NBP1-40202) indicated cross-reactivity with canine tryptase (canine tryptase shows 76% homology with human tryptase; http://www.uniprot.org/). The dog-specific rabbit anti-tryptase antibody labelled a greater number of MCs in all GIT layers (data not shown) than those labelled by the human-specific anti-tryptase antibodies, which were not immunolabelled with the other two antibodies (data not shown). Nevertheless, since both the mouse anti-tryptase antibodies labelled a large number of lamina propria MCs (Novus Biol. > Dako), which were also immunolabelled by the dog-specific anti-tryptase antibody, we utilized these two mouse anti-human tryptase antibodies in co-localization studies to identify lamina propria MCs bearing cannabinoid receptors.

The goat anti-IgA antibody (Novus Biol., NB100-1028) is directed against porcine IgA; although the specificity of this antibody has not been tested on dog tissues, in the present research we co-localized the goat anti-IgA antibody with the rabbit anti-IgA antibody (Bethyl Lab., A80-103A). The two antibodies co-localized perfectly in the same immunocytes/plasma cells (data not shown). Thus, in the present study, the goat anti-IgA antibody was utilized to identify immunocytes bearing cannabinoid receptors.

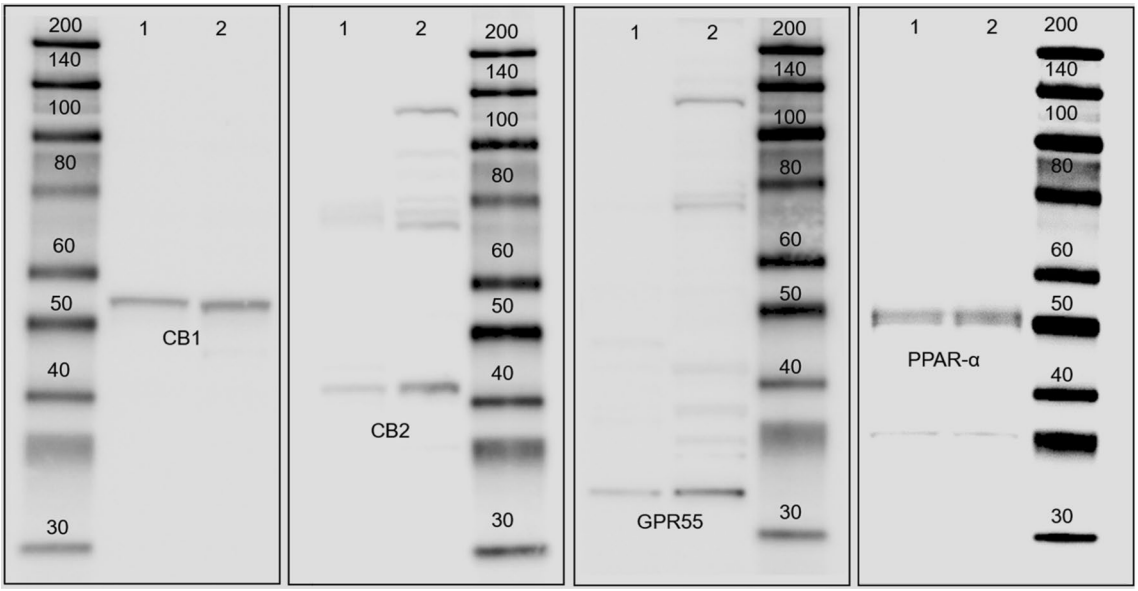


Fig. 1 Representative image of Western blots (WB) analysis showing the specificity of the primary antibodies utilized: rabbit anti-cannabinoid receptor 1, rabbit anti-cannabinoid receptor 2, rabbit anti-G protein-coupled receptor 55 (GPR55), and rabbit anti nuclear peroxisome proliferator-activated receptor alpha (PPARα). Each antibody showed a major band close to the theoretical molecular weight. Lane 1 = dog small intestine, lane 2 = mouse small intestine. The images of the different immunoblots were slightly adjusted in brightness and contrast to match their backgrounds

The anti-IBA1 antibody should recognize CNS microglia (Pierezan et al. 2014) and macrophages; in the present study the antibody recognized canine gut macrophages (and canine CNS microglia; data not shown). The anti-IBA1 antibody used (Novus Biol. NB100-1028) was raised in goat and is directed against porcine IBA1. Since the dog IBA1 molecule shows 91.2% identity with the porcine one (https://www.uniprot.org/), it is plausible that this antibody may also recognize canine IBA1. Nevertheless, the specificity of this antibody has not been tested on dog tissues.

The antibody anti-CD31, that has been already used in dog tissues (Kader et al. 2001), unequivocally identified the endothelial cells of blood vessels. The specificity of this antibody was tested in a co-localization study with the other endothelial marker, i.e., the anti-Factor VIII-related antigen/von Willebrand factor antibody (Preziosi et al. 2004). The two antibodies co-localized in the same endothelial cells (data not shown).

Specificity of the secondary antibodies

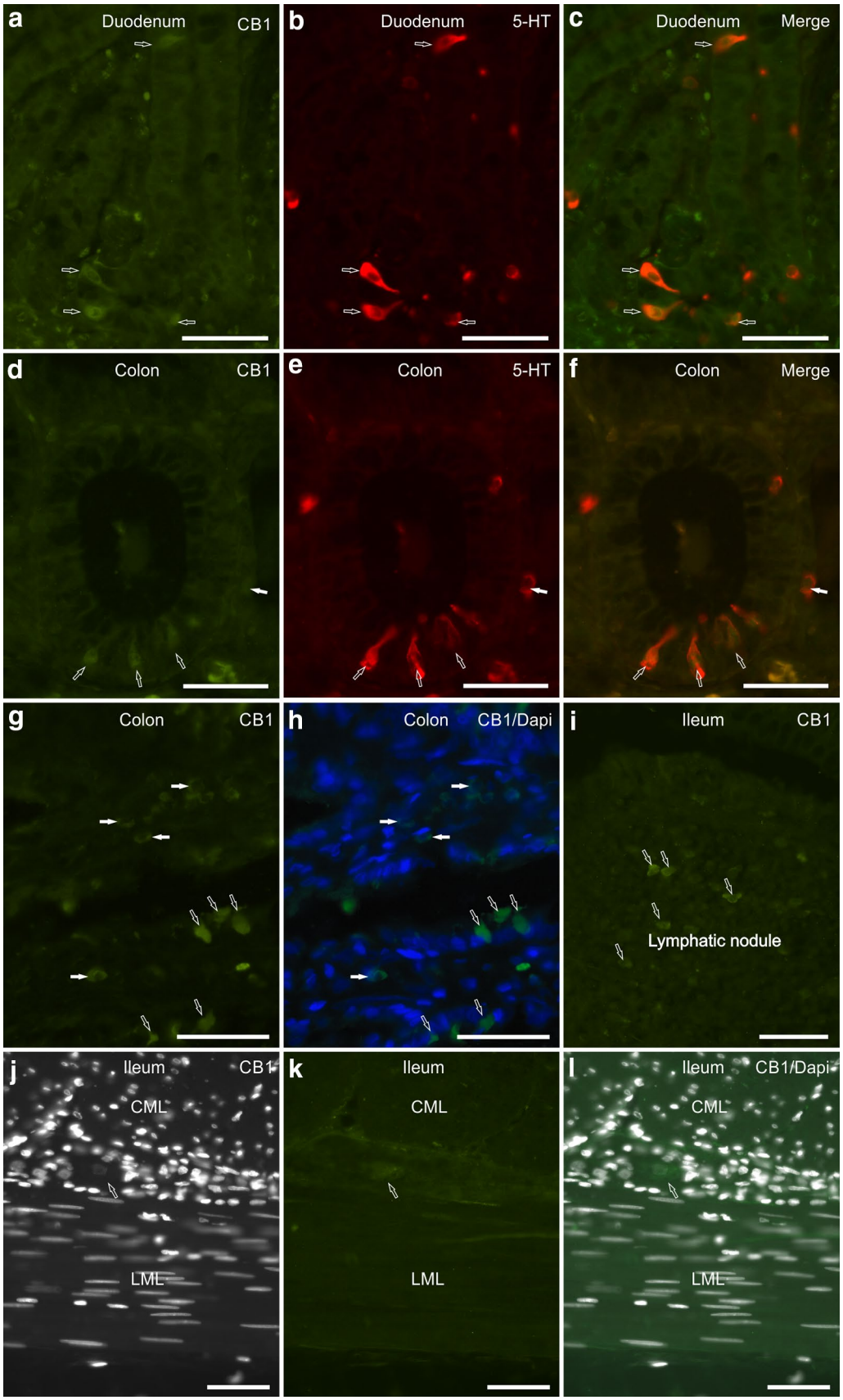
The specificity of the secondary antibodies was tested by applying them after omission of the primary antibodies. Neither stained cells could be detected after omitting the primary antibodies. In double-immunostaining protocols, control experiments were also carried out to check for non-specific binding of secondary antibodies to the inappropriate primary antibodies by omission of one or other of the first stage reagents. Furthermore, incubation with two primary antibodies followed by only one secondary antibody was carried out to check for the existence of any cross-reactivity between primary and secondary antibodies. Finally, incubation with any single primary antibody followed by the appropriate secondary antibody was also performed to ensure that the labeling pattern for each marker in the double-stained sections was in agreement with that observed in the single-labeled sections. No evidence of nonspecific binding was found.

Fluorescence microscopy

Preparations were examined on a Nikon Eclipse Ni microscope equipped with the appropriate filter cubes to distinguish the fluorochromes employed. The images were recorded with a Nikon DS-Qi1Nc digital camera and NIS Elements software BR 4.20.01 (Nikon Instruments Europe BV, Amsterdam, Netherlands). Slight adjustments to contrast and brightness were made using Corel Photo Paint, whereas the figure panels were prepared using Corel Draw (Corel Photo Paint and Corel Draw, Ottawa, ON, Canada).

Western blot: specificity of the primary antibodies

Tissue samples (canine small intestine) were collected, frozen in liquid nitrogen, and stored at − 80 °C until sample processing. In addition, the mouse small intestine was


a
Duodenum
CB1
b
Duodenum
5-HT
c
Duodenum
Merge
d
Colon
CB1
e
Colon
5-HT
f
Colon
Merge
g
Colon
CB1
h
Colon
CB1/Dapi
i
Ileum
CB1
Lymphatic nodule
j
Ileum
CB1
CML
LML
k
Ileum
CML
LML
l
Ileum
CB1/Dapi
CML
LML

◂**Fig. 2** Photomicrograph showing CB1 receptor immunoreactivity in the mucosa (**a–i**) and myenteric plexus (**j–l**) of the dog small and large intestine. **a–f** Open arrows indicate enterochromaffin cells of the duodenum (**a–c**) and colon (**d–f**) co-expressing moderate CB1- and bright 5-HT-immunoreactivity. The white arrow **d–f** indicates a 5-HT immunoreactive cell of the lamina propria. **g–h** White arrows and open arrows indicate some lamina propria and epithelial cells, respectively, which showed CB1 receptor immunoreactivity. **i** Arrows indicate immunocytes of the ileal lymphatic nodule showing CB1 receptor immunoreactivity. **j–l** The arrow indicates a myenteric plexus neuron showing weak and punctate CB1 receptor immunoreactivity. Abbreviations: LML, longitudinal muscle layer; CML, circular muscle layer, Scale bar: **a–l** 50 µm

utilized as positive control. 50 mg of tissue was homogenized in 500 µl of SDS buffer (Tris–HCl, 62.5 mM; pH 6.8; SDS, 2%; and glycerol, 20%) supplemented with a protease inhibitor cocktail (Sigma-Aldrich, Co, St. Louis, MO, USA). Total protein content was determined by Peterson's Modification of Lowry Method using a Protein Assay Kit. Aliquots containing 20 µg of total proteins were separated on Bolt 4–12% bis-Tris Plus (Life Technologies Ltd, Paisley, UK) for 45 min at 165 V. The proteins were then electrophoretically transferred onto a nitrocellulose membrane by a semi-dry system (Trans Turbo Blot Bio -Rad). Non-specific binding on nitrocellulose membranes was blocked with 5% milk powder in PBS-T20 (Phosphate Buffer Saline-0.1% Tween-20) for 1 h at RT. After blocking treatment, the membranes were incubated overnight at 4 °C with the primary antibodies (Table 1) (CB1 1:500, CB2 1:1000; GPR55 1:500; PPARα 1:2000) diluted in Tris-buffered saline-T20 (TBS-T20 20 mM Tris–HCl, pH 7.4, 500 mM NaCl, 0.1% T-20). After washes, the blots were incubated with a goat anti rabbit biotin-conjugate antibody (1:50,000 dilution in TBS-T20, 1 h at RT) and then with a 1:1000 dilution of an anti-biotin horseradish peroxidase (HRP)-linked antibody (40 min at RT). Immunoreactive bands were visualized using chemiluminescent substrate (Clarity Western ECL Substrate Bio Rad), according to the manufacturer's instructions. The intensity of the luminescent signal was acquired by Chemidoc Instrument and the apparent molecular weight of the resultant bands was analyzed by Quantity One Software (Bio-Rad). Western blot analysis of CB1 revealed a band of ~ 52 kDa (theoretical molecular weight of canine CB1 52,782 kDa; Fig. 1). Western blot analysis of CB2 revealed a band of ~ 40 kDa (theoretical molecular weight of canine CB2 40,107 kDa; Fig. 1). Western blot analysis of GPR55 revealed a band of ~ 35 kDa (theoretical molecular weight of canine GPR55 36,85 kDa; Fig. 1). Western blot of PPARα revealed a band of ~ 52 kDa (theoretical molecular weight of canine PPARα 52,123 kDa; Fig. 1). Overall Wb analysis confirmed the specificity of the primary antibodies utilized in the present study.

Results

CB1 immunoreactivity

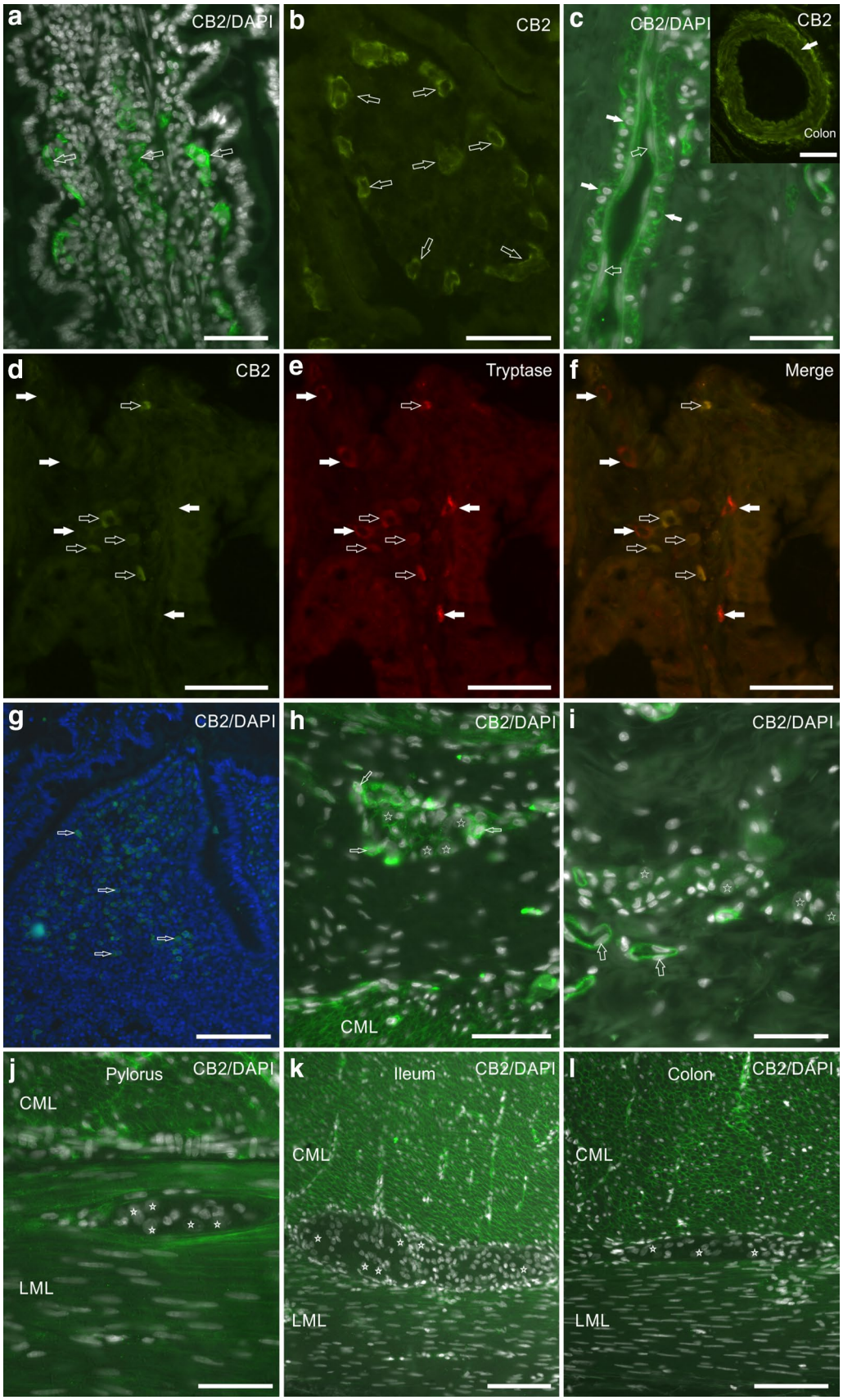
In the pylorus, small and large intestine, CB1 receptor immunoreactivity was expressed by serotonin-immunoreactive enterochromaffin cells (Fig. 2a–f). In the small and large intestine, CB1 immunoreactivity was detected in the cell membrane and cytoplasm of some lamina propria and epithelial cells (Fig. 2g, h). In the ileum of the youngest dog, in which submucosal and mucosal lymphatic nodules were evident, bright CB1 receptor immunoreactivity was identified in unidentified immunocytes localized in the portion of the lymphatic nodules within the lamina propria (Fig. 2i). In the enteric plexuses, faint and punctate CB1 receptor immunoreactivity was observed in some unidentified MP neurons (Fig. 2j–l). CB1 receptor immunoreactivity was undetectable in blood vessels and muscular layers.

CB2 immunoreactivity

CB2 immunoreactivity was widely distributed in all the digestive tracts considered.

Mucosa

The cell membrane of the endothelial and smooth muscle cells of mucosal (Fig. 3a, b) and submucosal (Fig. 3c) blood vessels showed bright CB2 immunoreactivity, above all in the small intestine, which allowed visualization of the blood vascular pattern along the major axis of the villi. When the villi were cut orthogonally, it was possible to observe CB2-immunolabelled vessels arranged like clock numbers around the circumference of the villus (Fig. 3b). Co-localization experiments indicated that CB2 immunolabelled endothelial cells showed CD31 immunoreactivity; however, there were small areas in which the two different immunolabelling were non-overlapped, indicating that other cellular elements (most likely pericytes) expressed CB2 immunoreactivity (Supplementary Fig. 2). It is of note that some lamina propria tryptase-immunoreactive MCs showed cytoplasmic CB2 immunoreactivity (Fig. 3d–f). CB2 immunoreactivity was also observed in the cytoplasm of some unidentified immunocytes within intestinal lymphatic nodules (Fig. 3g). The cell membrane of smooth muscle cells of the *muscularis mucosae* (*mm*) showed bright CB2 immunoreactivity in all the digestive tracts considered (data not shown).


a
CB2/DAPI
b
CB2
c
CB2/DAPI
CB2
Colon
d
CB2
e
Tryptase
f
Merge
g
CB2/DAPI
h
CB2/DAPI
CML
i
CB2/DAPI
j
Pylorus
CB2/DAPI
CML
LML
k
Ileum
CB2/DAPI
CML
LML
l
Colon
CB2/DAPI
CML
LML

◂**Fig. 3** Photomicrograph showing longitudinal cryosections of dog gastrointestinal tract immunolabelled with the anti-cannabinoid receptor 2 antibody (CB2). In **a, c, g–i** the cellular nuclei were labelled with the nuclear stain DAPI. Arrows indicate bright CB2-immunolabelled endothelial cells of blood capillaries running along the major axis of the duodenal villus (longitudinal section, **a**; when the villus was cut orthogonally (**b**), it was possible to observe CB2-immunolabelled vessels arranged like clock numbers around the circumference of the villus. **c** White and open arrows indicate, respectively, the nuclei of smooth muscle and endothelial cells expressing strong CB2 immunoreactivity. In the insert, the white arrow indicates a thick submucosal artery of the colon showing CB2 immunoreactivity. **d–e** Open arrows indicate mast cells of the lamina propria of the colon which were immunoreactive for CB2 (**d**) and tryptase (**e**); white arrows indicate mast cells which were tryptase-immunoreactive and CB2-negative (**f**, merged image). **g** Arrows indicate some CB2-immunoreactive immunocytes within a duodenal mucosa lymphatic nodule. **h** Stars indicate the nuclei of small neurons of the submucosal plexus showing moderate CB2 immunoreactivity on cell membrane. Arrows indicate nuclei of smaller dimension belonging to enteric glial cells showing bright CB2 immunoreactivity. **i** Stars indicate the nuclei of submucosal neurons showing faint and diffuse cytoplasmic CB2 immunoreactivity. The arrows indicate the nuclei of endothelial cells, which showed strong CB2-immunolabelling. **j–l** Stars indicate the nuclei of myenteric plexus neurons of pylorus (**j**), ileum (**k**) and colon (**l**), which were CB2-negative. On the contrary, the smooth muscle cells of the longitudinal (LML) and circular muscle layers (CML) showed intense CB2 immunoreactivity. Scale bar: **a–j** 50 µm; **k, l**, 100 µm

Muscular layers

In the pylorus, CB2 immunoreactivity was observed on the cell membrane of the smooth muscle cells of both layers of the *tunica muscularis* (Fig. 3j). In the circular muscle layer (CML), there were some patchy distributed clusters of smooth muscle cells that showed stronger immunolabelling. The CB2 immunoreactivity of the muscular layers of the small intestine showed strong immunolabelling, which was attenuated in the colon (small intestine > colon > pylorus) (Fig. 3k–l). In both the intestinal tracts, CB2 immunoreactivity decreased towards the outermost part of the longitudinal muscle layer (LML).

Enteric neurons and glia

In the intestinal submucosal plexus (SMP), there were a few ganglia in which neurons and glial cells showed weak-to-moderate CB2 immunoreactivity (Fig. 3h–i), either on the cell membrane (Fig. 3h) or within the cytoplasm (Fig. 3i). On the contrary, the neurons and glial cells of the myenteric plexus (MP) did not show any CB2 immunoreactivity (Fig. 3j–l).

GPR55 immunoreactivity

GPR55 immunoreactivity was mainly distributed in the mucosa (Fig. 4a–i) and muscular layers (Fig. 4j–l).

Mucosa

A large number of lamina propria and epithelial cells expressed bright GPR55 immunoreactivity (Fig. 4a–c). In the pylorus, there were some thin and elongated enterochromaffin cells, which showed bright nuclear and weaker cytoplasmic GPR55 immunostaining (Fig. 4a). GPR55 immunolabelled enterochromaffin cells were also visible in the intestine, in particular in the colon. Furthermore, epithelial cells of the inner portion of the mucosa also showed diffuse cytoplasmic GPR55-immunolabelling in this tract (Fig. 4b). Co-localization experiments indicated that a large number of lamina propria cells showing cytoplasmic GPR55 immunoreactivity were IBA1-immunoreactive macrophages (Fig. 4d–f), IgA-immunoreactive plasma cells (Fig. 4g–i), and tryptase-immunoreactive MCs (data not shown).

Muscular layers

The GPR55 receptor distribution showed regional and local differences. In the stomach, GPR55 immunoreactivity was not homogenously distributed and the immunolabelling was more evident in the CML than in the LML (data not shown). In the duodenum, the CML showed faint GPR55-immunolabelling, as did the inner portion of the LML; on the contrary, the outer third of the LML showed very strong GPR55 immunoreactivity (Fig. 4j). In addition, in the ileum, the smooth muscle cells of the outer portion of the LML showed a higher density of the GPR55 receptor, whereas in the colon all the LML expressed bright GPR55 immunoreactivity, with a tendency for increased immunolabelling in its outer portion (Fig. 4k–l). It is noteworthy that the inner portion of the CML (ICML), i.e., the small portion of the CML composed by 6–12 smooth muscle cells facing towards the submucosa (Zelcer and Daniel 1979; Eddinger 2009), showed intense GPR55-immunolabelling (data not shown).

Enteric neurons and glia

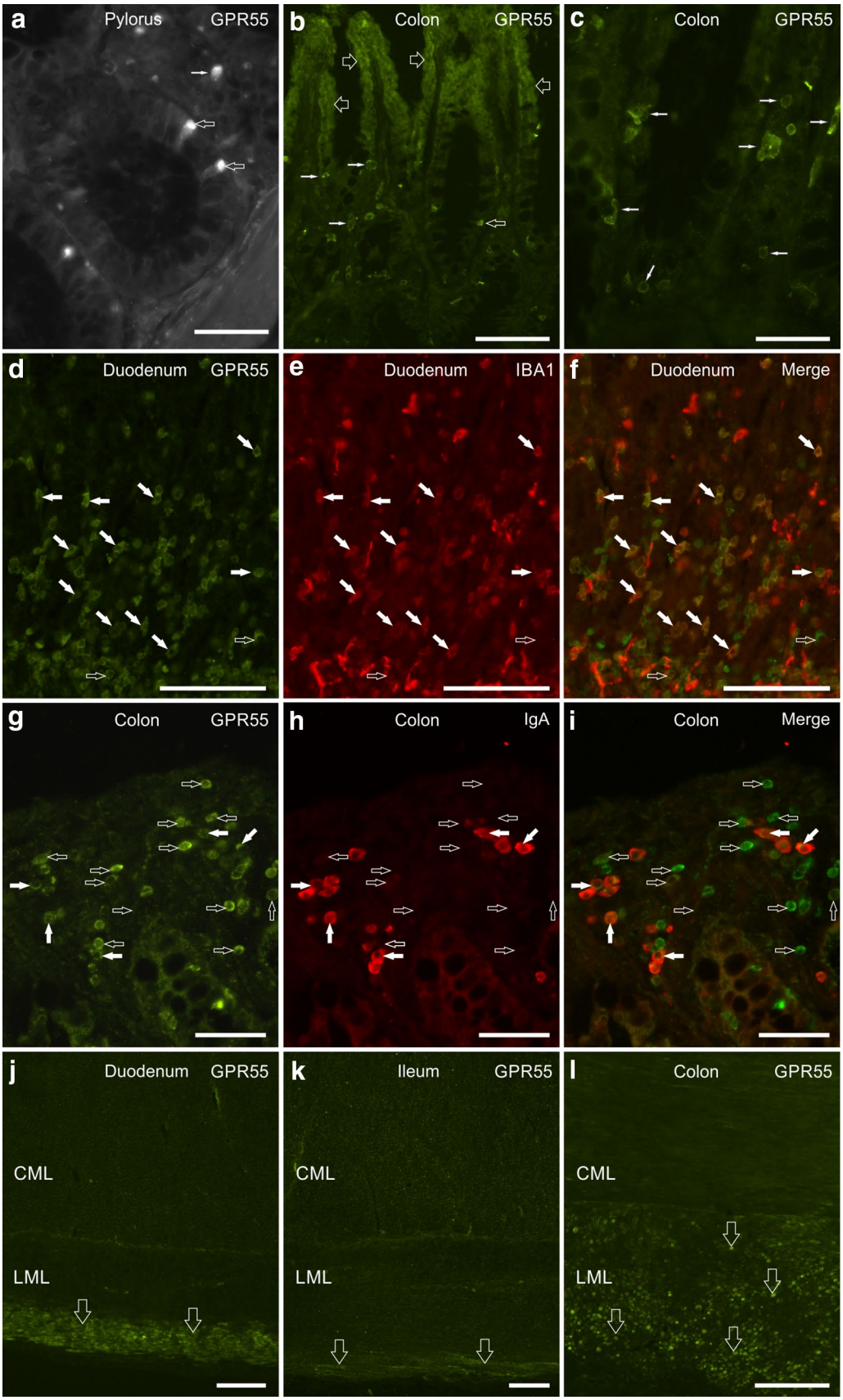
No GPR55 immunoreactivity was displayed by enteric neurons or glial cells in the tracts considered.

PPARα immunoreactivity

Bright PPARα immunoreactivity was expressed by lamina propria cells, epithelial cells, blood vessels, smooth muscle cells of the *mm* and *tunica muscularis* (Fig. 5a–h), and EGCs (Fig. 6a–i).

Mucosa

In the pylorus, PPARα immunoreactivity was evident in the cytoplasm and nucleus of serotonin-immunoreactive


a Pylorus GPR55
b Colon GPR55
c Colon GPR55
d Duodenum GPR55
e Duodenum IBA1
f Duodenum Merge
g Colon GPR55
h Colon IgA
i Colon Merge
j Duodenum GPR55
CML
LML
k Ileum GPR55
CML
LML
l Colon GPR55
CML
LML

◂**Fig. 4** Photomicrograph showing cryosections of dog gastrointestinal tract immunolabelled with the anti-GPR55 antibody. **a–c** Small white arrows indicate lamina propria cells of pylorus (**a**) and colon (**b, c**) showing bright GPR55 immunoreactivity. The small open arrows **a, b** indicate GPR55-immunolabelled enterochromaffin cells of the pylorus (**a**) and colon (**b**). Large open arrows indicate epithelial cells of the inner portion of the mucosa of the colon, which showed diffuse GPR55 immunoreactivity. **d–f** The white arrows indicate that GPR55-immunoreactive cells (**d**) of the duodenal mucosa co-expressed IBA1 immunoreactivity (**e**), which indicates that these cells were predominantly macrophages. The open arrow indicates GPR55-immunoreactive cells, which were not IBA1-positive. **g–i** White arrows indicate lamina propria cells of the colon which co-expressed GPR55- and IgA immunoreactivity, indicating that these cells were plasma cells. Open arrows, on the contrary, indicate GPR55-immunoreactive cells, which were not IgA-positive plasma cells. **j–l** Distribution of GPR55-immunolabelling within the circular (CML) and longitudinal muscle layer (LML) of the duodenum (**j**), ileum (**k**) (cut in longitudinal sections) and colon (**l**) (cut in transverse section). Arrows indicate bright GPR55-immunolabelling in the external portions of the small intestine LML (**j–k**) and in the whole LML of the colon. Scale bar: **a–c, g–i** 50 µm; **d–f, j–l** 100 µm

enterochromaffin cells of the deeper portions of the gastric glands (data not shown). In the intestine, the strongest PPARα immunoreactivity was expressed by the *mm*, from which bundles of smooth muscle cells reached the tips of the villi (Fig. 5a); of note, the muscular cells showed their strongest immunolabelling on the apex of the villi. PPARα immunoreactivity was also expressed by blood vessel endothelial cells (Fig. 5a, b). In the lamina propria of the villi, and in particular in their apex, PPARα immunoreactivity was observed in a network of thin and elongated cellular processes arising from small cells of unknown nature (Fig. 5c); these cells were easily observed when the villi were cut orthogonally to their major axis. Furthermore, some small lamina propria cells with an irregular outline and short cellular processes, which resembled dendritic cells (Junginger et al. 2014), showed brilliant PPARα-immunolabelling within the cytoplasm (Fig. 5d). In addition, MCs showed cytoplasmic PPARα immunoreactivity (data not shown).

Muscular layers

PPARα receptor, as seen for GPR55 receptor, showed a different distribution in the *tunica muscularis* and was well represented in both the muscular layers, but more concentrated in the LML, and in particular in its external portion, in the pylorus, duodenum and colon (Fig. 5e, g). In the LML of the ileum, PPARα-immunolabelling was mainly observed concentrated within its outer portion, although there were some ileal tracts in which PPARα receptor were seen in smooth muscle cells scattered in the LML (Fig. 5f). It should be noted that, in contrast to what was observed for GPR55 immunoreactivity, the ICML was PPARα-negative (Fig. 5h).

Enteric neurons and glia

PPARα immunoreactivity was strongly expressed by GFAP-immunoreactive glial cells of the SMP and MP (MP > SMP), whereas the HuC/HuD-immunoreactive neurons were always PPARα-negative (Fig. 6a–i).

Discussion

CB1 receptor

The observation of CB1 receptor immunolabelling of enteric neurons is consistent with data observed in pig, guinea-pig, rat, mouse, and ferret ENS (Kulkarni-Narla and Brown 2000; Van Sickle et al. 2001; Coutts et al. 2002; Storr et al. 2004; Duncan et al. 2005a), in which the CB1 receptor was mainly expressed by cholinergic excitatory motor neurons. In humans, the activity of the CB1 receptor was functionally demonstrated in the ileum by Croci et al. (1998) and CB1 receptor immunoreactivity has been described in enteric neurons and nerve fibres (Wright et al. 2005; Marquez et al. 2009).

In the present study, we observed CB1 receptor immunoreactivity in the epithelial cells, including enterochromaffin cells. This is in line with what was observed in human GIT mucosa, where the CB1 receptor was identified on gastric parietal cells, epithelial cells, goblet cells, and enteroendocrine cells (Wright et al. 2005; Pazos et al. 2008; Marquez et al. 2009; Ligresti et al. 2016). It is known that under physiological conditions, the activation of the CB1 receptor reduces gastric acidic secretion and regulates the release of enteroendocrine peptides such as cholecystokinin (Sykaras et al. 2012). The presence of CB1 receptor immunoreactivity in canine small intestine is especially important, given the body of evidences that shows that CB1 receptor in the upper small intestine of rodents controls palatable food intake and overeating in diet-induced obesity (DiPatrizio et al. 2011; Argueta and DiPatrizio 2017). In addition, the evidence of CB1 receptor immunoreactivity in serotonin expressing enterochromaffin cells of the dog upper gastrointestinal tract may suggest a peripheral mechanism of action of cannabinoids in the modulation of nausea and vomiting. In fact, it is well known that cannabinoids may inhibit nausea and vomit with a central (and peripheral) action, since CB1 receptor is scattered on neurons of the brainstem nuclei involved in emesis (Ray et al. 2009; Darmani 2010). The activation of CB1 receptor of 5-HT releasing enterochromaffin cells may limit nausea and vomit by reducing 5-HT release and consequently decreasing the excitability of 5-HT3 receptor of the vagal sensory nerve fibers of the upper gastrointestinal tract (Hu et al. 2007). In the present study, we

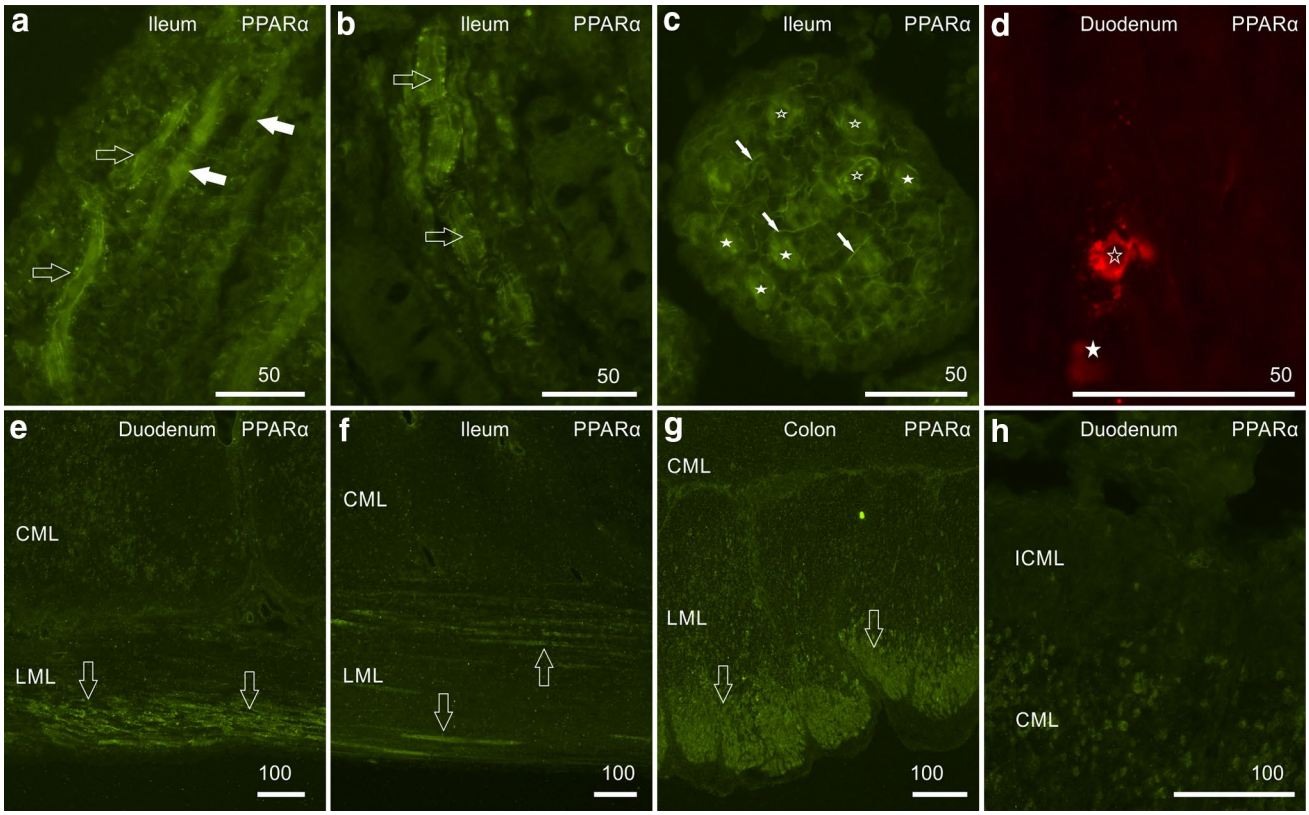


Fig. 5 Photomicrograph showing cryosections of dog small and large intestine immunolabelled with the antibody anti-PPARα. **a, b** Open arrows and white arrows indicate, respectively, blood vessels and fascicles of smooth muscle in a villus of ileal mucosa showing bright PPARα. **c** Arrows indicate thin and elongated PPARα-immunoreactive cellular processes of unknown nature, visible in the apex of a villus cut orthogonally to its major axis. The white stars and open stars indicate, respectively, fascicles of smooth muscle cells and blood vessels showing PPARα immunoreactivity. **d** The open star indicates a strong PPARα-immunoreactive lamina propria dendritic-like cell showing strong PPARα immunoreactivity, close to another less visible blurred cell (white star), because it is out of focus. **e–h** Distribution of PPARα -immunolabelling within the circular (CML) and longitudinal muscle layer (LML) of the duodenum (**e, h**), ileum (**f**) (cut in longitudinal sections) and colon (**g**) (cut in transverse section). Arrows indicate bright PPARα-immunolabelling in the external portions of the small and large intestine longitudinal muscle layer LML, in which the immunostaining was more evident. In the ileum (**f**), PPARα-immunoreactive smooth muscle cells could also be scattered throughout the whole thickness of the LML. In the inner portion of the circular muscle layer (ICML) (**h**), PPARα immunoreactivity was almost undetectable. Scale bar: **a–d** 50 µm; **e–h** 100 µm

observed unidentified lamina propria cells expressing CB1 receptor immunoreactivity, which were more concentrated in the small intestine lymphatic nodules. Regarding the expression of CB1 receptor immunoreactivity on epithelial cells, it is interesting to consider that enteric microbiota may regulate the expression of the CB1 receptor on enterocytes, and this in turn may control gut permeability (Muccioli et al. 2010). Consistently, CB1 activation has recently been suggested to play a key role in intestinal mucosa permeability, both in healthy and disease states (Karwad et al. 2017a).

The presence of the CB1 receptor on lamina propria cells is consistent with the finding by Marquez et al. (2009), who reported CB1 receptor immunoreactivity in mucosal plasma cells. Moreover, enteric microbiota may regulate the expression of the CB1 receptor on enterocytes, and this in turn may control gut permeability (Muccioli et al. 2010). Consistently, CB1 activation has recently been suggested to play a key role in intestinal mucosa permeability, both in healthy and disease states (Karwad et al. 2017a).

CB2 receptor

CB2 receptor immunoreactivity was absent in the MP, whereas it was observed in some neurons and EGCs of the submucosa, in which it showed different degrees of brightness. This is not surprising, since the CB2 receptor—although usually expressed by non-neuronal elements such as immunocytes and inflammatory cells—has been repeatedly demonstrated in the central (Cabral et al. 2008) and peripheral nervous system, including enteric neurons (Duncan et al. 2005b, 2008; Wright et al. 2008). To the best of our knowledge, this is the first time that CB2 immunoreactivity has been reported

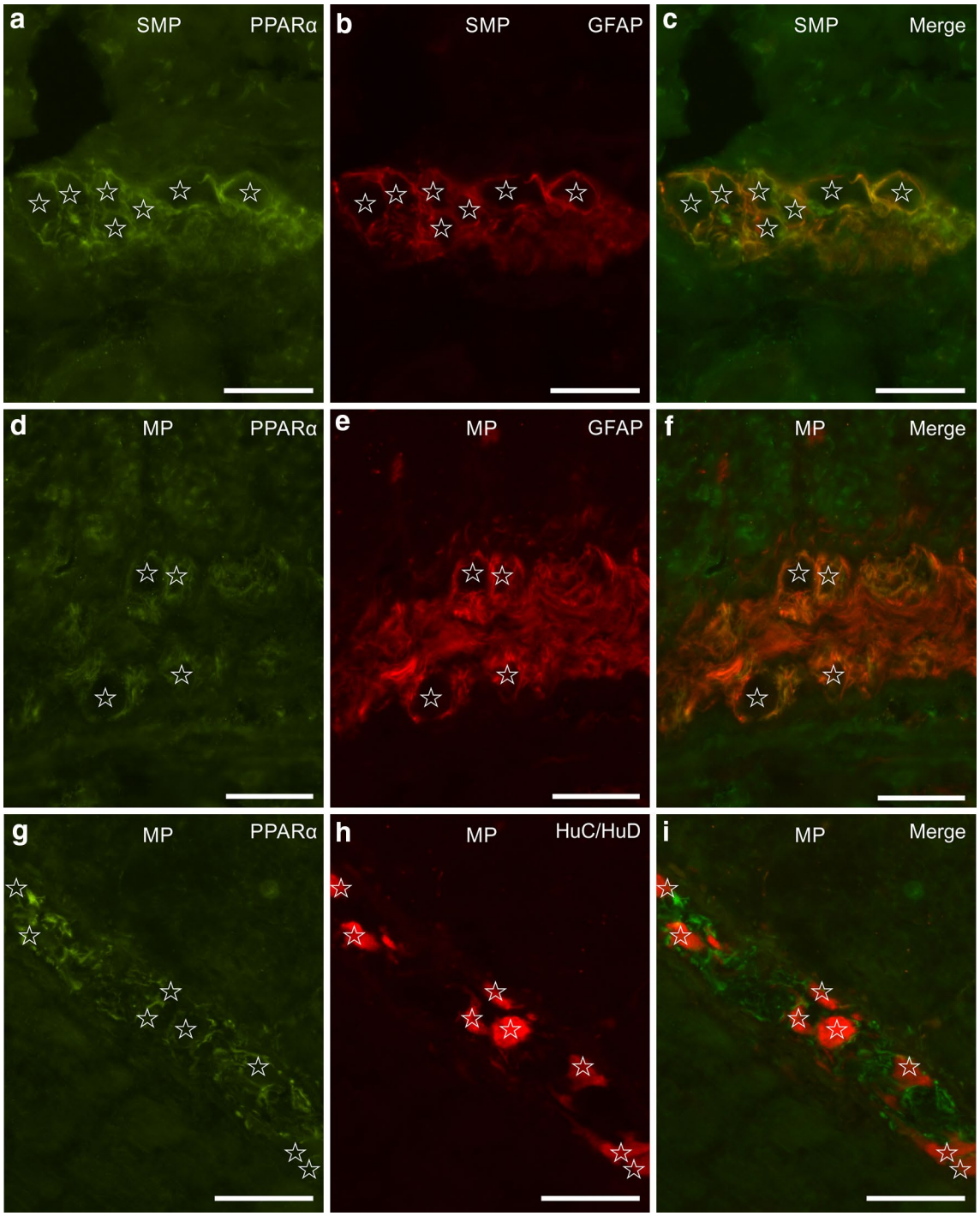


Fig. 6 Photomicrograph showing cryosections of submucosal plexus (SMP) (**a**–**c**) and myenteric plexus (MP) (**d**–**i**) of the dog duodenum, immunolabelled with the anti-PPARα antibody. **a**–**f** Stars indicate SMP (**a**–**c**) and MP (**d**–**f**) neurons (not visible) encircled by enteric glial cells which were immunoreactive for PPARα (**a**) and GFAP (**b**) (**c, f**, merge images). **g**–**i** Stars indicate MP HuC/HuD-immunoreactive neurons (**h**) which were PPARα-negative (**g**). On the contrary, a network of PPARα-positive cellular processes belonging to enteric glial cells is visible around HuC/HuD neurons. Scale bar: **a**–**i** 50 µm

in EGCs. Notably, this could be related to the expression of PPARα on the same cell type (see below). The localization of CB2 receptor in the EGCs is consistent with the expression of CB2 receptor on astrocytes of healthy and inflamed CNS (Sheng et al. 2005; Freundt-Revilla et al. 2018).

We observed very strong CB2 immunoreactivity in endothelial and muscular components of enteric blood vessels. Our observations are consistent with the findings by Golech et al. (2004), Ashton et al. (2006), Marquez et al. (2009), and Dowie et al. (2014), who found CB2-immunolabelling on endothelial vascular cells of human and rat CNS. More specifically, our data are reinforced by the findings of Campora et al. (2012), who observed CB2-immunolabelling on canine endothelial cells in skin. In co-localization studies aimed to identify the co-expression of the CD31- and CB2-immunoreactivity in endothelial cells, we noted a certain degree of non-overlap between the two markers. This evidence may suggest that endothelial cells, and also pericytes, express CB2 immunoreactivity. This last finding, although not demonstrated by the use of a specific marker for pericytes, is of some importance, because it has been shown that the contraction of pericityes can regulate the vascular flow of the capillaries in the intestine (Wille and Schnorr 2003) as well in the CNS. Hall et al. (2014) demonstrated that ischaemia evokes capillary constriction by pericytes, which are major regulators of cerebral blood flow. Zong et al. (2017) showed that exogenous CB1 agonist promotes the vasorelaxation of pericyte-containing rat retinal capillaries. Benyó et al. (2016) showed that in certain cerebrovascular pathologies, activation of CB2 receptor (and probably yet unidentified non-CB1/non-CB2 receptors) appears to improve the blood perfusion of the brain via attenuating vascular inflammation. Thus, the expression of the CB2 receptor in dog gastrointestinal vessels (in smooth muscle cells, endothelium and, perhaps, pericytes) may have relevant therapeutic importance in the treatment of acute and chronic enteropathies. In fact, alteration of the microvascular perfusion and adhesion of leukocytes to the endothelium are hallmark events in inflammation. As already demonstrated in rodents (Kinian et al. 2013; Sardinha et al. 2014), it is possible that even in dogs the use of CB2 receptor agonists might protect the gut microcirculation during inflammation.

The expression of CB2 immunoreactivity in lamina propria cells was expected, since the presence of this receptor among different classes of immunocytes and inflammatory cells has already been reported (Wright et al. 2008; Izzo and Sharkey 2010; Gyires and Zádori 2016; Lee et al. 2016).

Notably, as already shown in experimental rodents (Facci et al. 1995), we observed CB2 receptor immunoreactivity in canine mucosal MCs. The finding is of particular interest if one considers that MCs are now recognized to be involved in a number of non-allergic diseases including IBD and food intolerance (Shea-Donohue et al. 2010; Wouters et al. 2016; Bednarska et al. 2017). During intestinal inflammation, MCs release proinflammatory mediators (e.g., tryptase, chymase, and histamine), which recruit and stimulate adjacent MCs, thus amplifying the inflammatory signal (He 2004). CB2 receptor immunoreactivity on canine gut MCs renders them a potential target for CB2 agonists.

Although CB2 receptor activation is considered to exert no effect on GIT motility under physiological conditions (Izzo et al. 1999), upregulation during experimental GIT inflammation might be envisaged. Indeed, the CB2 receptor seems to be upregulated in the dog (specifically in the SMP) during chronic enteritis (personal observation of Dr. R. Chiocchetti; Supplementary Fig. 3).

GPR55 receptor

The GPR55 receptor, considered by some as the third cannabinoid receptor (Moriconi et al. 2010), is a G protein-coupled receptor sharing 10–14% homology with CB1 and CB2 receptors (Lauckner et al. 2008). Although a detailed description of tissues and cells expressing GPR55 is still lacking, a growing body of evidence shows that GPR55 is widely distributed in the ENS of humans and rodents, in particular in the two ganglionated plexuses (Lin et al. 2011; Ross et al. 2012; Li et al. 2013; Goyal et al. 2017).

In contrast with these findings, we did not detect the GPR55 receptor in ENS neurons, whereas it was abundantly expressed in smooth muscle cells, possibly playing some role in controlling excitability. The peculiar distribution of GPR55 immunoreactivity in the muscular layers, i.e., the high density of the GPR55 receptor in the ICML (small intestine) and outer portion of the LML (colon), suggests that the GPR55 receptor might be involved in ICML relaxation during inflammatory-induced excessive contraction of the intestinal wall. In fact, as hypothesized by Eddinger (2009), ICML cells seem to be primarily involved in maintaining basal intestinal tone, while the muscle cells of the outer portion of the CML are primarily involved in peristalsis. As specified above, GPR55 receptor immunoreactivity was also well represented in the outer portion of the LML. Although the enteric neurons and interstitial cells of Cajal are determinant for the beginning and coordination of peristalsis, smooth muscle cells of the CML and LML have intrinsic myogenic activity (Huizinga et al. 1998). Due to its role in the regulation (increase) of intracellular calcium levels (Lauckner et al. 2008), the GPR55 receptor may thus play a role in the excitability of these smooth muscle cells.

We also found that a large number of lamina propria macrophages, plasma cells, and MCs showed bright GPR55 immunoreactivity (macrophages > plasma cells > MCs). The presence of the GPR55 receptor on macrophages was recently also shown in rodents and humans (Taylor et al. 2015; Lanuti et al. 2015). As reported above, pro-inflammatory mediators released by MCs during intestinal inflammation may induce macrophage accumulation in the basal portion of the lamina propria (He et al. 1997; He and Walls 1998). One could thus speculate that

endocannabinoid-related compounds acting on CB2 receptor (MCs and immunocytes) and/or GPR55 receptor (macrophages and MCs) may limit the inflammatory cascade during GIT disturbances.

PPARα receptor

The PPARα receptor is a ligand-activated transcription factor belonging to the superfamily of nuclear hormone receptors. By modulating gene expression, it plays key roles in maintaining glucose and lipid homeostasis and inhibiting inflammation (Naidenow et al. 2016). Activation of the PPARα receptor is known to exert anti-nociceptive and anti-inflammatory effects, even at the gastrointestinal level (Escher et al. 2001; Azuma et al. 2010; Petrosino and Di Marzo 2017). The strong PPARα immunoreactivity observed in the *mm* and its mucosal emanations, as well as the peculiar localization of this receptor in the smooth muscle cells of CML and LML, suggests a unique role for this receptor (as seen for GPR55 receptor) in GIT motility. Interestingly, whereas in the LML the distribution of the PPARα receptor overlapped that of the GPR55 receptor, in the ICML the former seemed to be missing, whereas the latter was widely distributed. At present, we are not able to speculate on the physiological meaning of this different receptor distribution.

Although it was not possible to precisely identify the strong PPARα-immunoreactive cells within the lamina propria of the villi, their shape, cytoplasmic projections and distribution are suggestive of mucosal dendritic cells (DCs) (Junginger et al. 2014). Notably, DCs are widely distributed in the digestive system and play a relevant role in innate and adaptive immunity and in the maintenance of tolerance (Svensson et al. 2010).

Finally, the expression of PPARα immunoreactivity on cells particularly involved in gut pathophysiology, i.e., the intestinal MCs (Lee et al. 2016; Bischoff 2016; Wouters et al. 2016), suggests a potential role of PPARα agonists in GIT inflammation.

The most intriguing localization of PPARα receptor revealed by our study was at the level of EGCs, i.e., cells that are functionally comparable to CNS astrocytes (Liu et al. 2013; Sharkey 2015) and able to multifunctionally interact with the epithelium, immune system, nerve fibres, lymphatic and blood vessels (Liu et al. 2013). It has been reported that EGC activation may amplify intestinal inflammation (Cirillo et al. 2011; Ochoa-Cortes et al. 2016) and PPARα agonists mitigate it by reducing the glial expression of S100B protein (Esposito et al. 2014).

The robust expression of the PPARα receptor on the muscular and endothelial components of blood vessels suggests a possible role of this receptor in the control of canine GIT blood flow. The hypothesis is sustained by previous observations on the beneficial effects of PPARα agonists on inflammatory responses in vascular smooth muscle cells (Zahradka et al. 2003; Ji et al. 2010) and endothelial cells (Naidenow et al. 2016).

Conclusion

Taken together, the data of the present study show the wide distribution of the cannabinoid receptor ensemble in several cellular types of all layers of the canine GIT. These morphological findings, although not yet supported by physiological or pharmacological evidence, suggest that cannabinoid receptor agonists have a therapeutic potential for controlling gastrointestinal inflammatory conditions and visceral hypersensitivity in this species. The hypothesis is supported by a great deal of evidence on the intestinal protective effects of one of the most studied naturally occurring cannabinoid receptor ligands, PEA (Borrelli et al. 2015). PEA was originally considered to activate the CB2 receptor (Facci et al. 1995; Re et al. 2007; Petrosino et al. 2016), resulting in MCs down-modulation through the so-called ALIA mechanism (Autacoid Local Injury Antagonism) (Aloe et al. 1993; De Filippis et al. 2013; Petrosino and Di Marzo 2017). Currently, PEA has been shown not only to have a strong affinity for other cannabinoid receptors, like GPR55 (reviewed by Petrosino et al. 2016), but also to reduce intestinal radiation injury in a mast cell-dependent manner (Wang et al. 2014), and to normalize intestinal motility through a mechanism involving CB1 receptor (Capasso et al. 2014). Furthermore, using both in vitro and in vivo preclinical models of enteropathies, it has been demonstrated that the activation of PPAR-α by PEA results in inhibition of colitis-associated angiogenesis (Sarnelli et al. 2016), modulation of intestinal permeability (Karwad et al. 2017b), improvement of colon inflammation (Esposito et al. 2014), and protection against ischemia/reperfusion-induced intestinal injury (Di Paola et al. 2012).

In conclusion, the findings of the present research support the potential therapeutic use of non-psychotropic and safe cannabinoid agonists such as PEA (Nestmann 2016) in canine intestinal inflammation and may constitute a starting point for future comparative studies on the possible changes in the cannabinoid receptor ensemble during GIT inflammatory conditions in the dog.

Acknowledgements We are thankful to Dr. Giovanni Marsicano (INSERM U1215, NeuroCentre Magendie, Team 'Endocannabinoids and Neuroadaptation', Bordeaux, France) and to Prof. Giovanna Zoccoli (Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum - University of Bologna) who kindly provided us with brain sample of wild-type and mice with congenital deficiency of CB1. We are thankful to Dr. Catia Barboni (Department of Veterinary Medical Sciences, Alma Mater Studiorum—University of Bologna), who kindly provided us with samples of mouse intestine for the Wb analysis, and with Dr. Marco Luppi (Department of Biomedical and Neuromotor

Sciences, Alma Mater Studiorum—University of Bologna) who kindly provided with sample of rat intestine for wholemount preparations.

Funding This research received a grant from Innovet Italia S.r.l. (2017).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical Approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

References

Aloe L, Leon A, Levi-Montalcini R (1993) A proposed autacoid mechanism controlling mastocyte behaviour. Agents Actions 39(Spec No):C145–C147

Ambrosino P, Soldovieri MV, Russo C et al (2013) Activation and desensitization of TRPV1 channels in sensory neurons by the PPARα agonist palmitoylethanolamide. Br J Pharmacol 168:1430–1434. https://doi.org/10.1111/bph.12029

Anday JK, Mercier RW (2005) Gene ancestry of the cannabinoid receptor family. Pharmacol Res 52:463–466

Argueta DA, DiPatrizio NV. (2017) Peripheral endocannabinoid signaling controls hyperphagia in western diet-induced obesity. Physiol Behav 171:32–39. https://doi.org/10.1016/j.physbeh.2016.12.044

Ashton JC, Friberg D, Darlington CL et al (2006) Expression of the cannabinoid CB2 receptor in the rat cerebellum: an immunohistochemical study. Neurosci Lett 396:113–116

Azuma YT, Nishiyama K, Matsuo Y et al (2010) PPARα contributes to colonic protection in mice with DSS-induced colitis. Int Immunopharmacol 10:1261–1267. https://doi.org/10.1016/j.intimp.2010.07.007

Bednarska O, Walter SA, Casado-Bedmar M et al (2017) Vasoactive intestinal polypeptide and mast cells regulate increased passage of colonic bacteria in patients with irritable bowel syndrome. Gastroenterology S0016-5085:948–960. https://doi.org/10.1053/j.gastro.2017.06.051

Benyó Z, Ruisanchez É, Leszl-Ishiguro M et al (2016) Endocannabinoids in cerebrovascular regulation. Am J Physiol Heart Circ Physiol 310:H785–H801. https://doi.org/10.1152/ajpheart.00571.2015

Bischoff SC (2016) Mast cells in gastrointestinal disorders. Eur J Pharmacol 778:139–145

Borrelli F, Romano B, Petrosino S et al (2015) Palmitoylethanolamide, a naturally occurring lipid, is an orally effective intestinal anti-inflammatory agent. Br J Pharmacol 172:142–158

Brown AJ, Ueno S, Suen K et al (2005) Molecular identification of GPR55 as a third G protein-coupled receptor responsive to cannabinoid ligands. In: Brian T (ed) Symposium on the cannabinoids, pp 24–27

Cabral GA, Raborn ES, Griffin L et al (2008) CB2 receptors in the brain: role in central immune function. Br J Pharmacol 153:240–251

Cabral GA, Rogers TJ, Lichtman AH (2015) Turning over a new leaf: cannabinoid and endocannabinoid modulation of immune function. J Neuroimmune Pharmacol 10:193–203. https://doi.org/10.1007/s11481-015-9615-z

Campora L, Miragliotta V, Ricci E et al (2012) Cannabinoid receptor type 1 and 2 expression in the skin of healthy dogs and dogs with atopic dermatitis. Am J Vet Res 73:988–995. https://doi.org/10.2460/ajvr.73.7.988

Cantarella G, Scollo M, Lempereur L et al (2011) Endocannabinoids inhibit release of nerve growth factor by inflammation-activated mast cells. Biochem Pharmacol 82:380–388. https://doi.org/10.1016/j.bcp.2011.05.004

Capasso R, Orlando P, Pagano E et al (2014) Palmitoylethanolamide normalizes intestinal motility in a model of post-inflammatory accelerated transit: involvement of CB_1 receptors and TRPV1 channels. Br J Pharmacol 171:4026–4037. https://doi.org/10.1111/bph.12759

Cassano T, Calcagnini S, Pace L et al (2017) Cannabinoid receptor 2 signaling in neurodegenerative disorders: from pathogenesis to a promising therapeutic target. Front Neurosci 11:30. https://doi.org/10.3389/fnins.2017.00030

Caterina MJ, Schumacher MA, Tominaga M et al (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824

Chen DJ, Gao M, Gao FF et al (2017) Brain cannabinoid receptor 2: expression, function and modulation. Acta Pharmacol Sin 38:312–316. https://doi.org/10.1038/aps.2016.149

Chiocchetti R, Giancola F, Mazzoni M et al (2015) Excitatory and inhibitory enteric innervation of horse lower esophageal sphincter. Histochem Cell Biol 143:625–635. https://doi.org/10.1007/s00418-014-1306-y

Cirillo C, Sarnelli G, Esposito G et al (2011) S100B protein in the gut: the evidence for enteroglial-sustained intestinal inflammation. World J Gastroenterol 17:1261

Coutts AA, Irving AJ, Mackie K et al (2002) Localisation of cannabinoid CB(1) receptor immunoreactivity in the guinea pig and rat myenteric plexus. J Comp Neurol 448:410–422

Cremon C, Stanghellini V, Barbaro MR et al (2017) Randomised clinical trial: the analgesic properties of dietary supplementation with palmitoylethanolamide and polydatin in irritable bowel syndrome. Aliment Pharmacol Ther 45:909–922. https://doi.org/10.1111/apt.13958

Croci T, Manara L, Aureggi G et al (1998) In vitro functional evidence of neuronal cannabinoid CB1 receptors in human ileum. Br J Pharmacol 125:1393–1395

Darmani NA (2010) Mechanisms of broad-spectrum antiemetic efficacy of cannabinoids against chemotherapy-induced acute and delayed vomiting. Pharmaceuticals (Basel) 3:2930–2955. https://doi.org/10.3390/ph3092930

De Petrocellis L, Di Marzo V (2010) Non-CB1, non-CB2 receptors for endocannabinoids, plant cannabinoids, and synthetic cannabimimetics: focus on G-protein-coupled receptors and transient receptor potential channels. J Neuroimmune Pharmacol 5:103–121. https://doi.org/10.1007/s11481-009-9177-z

De Petrocellis L, Davis JB, Di Marzo V (2001) Palmitoylethanolamide enhances anandamide stimulation of human vanilloid VR1 receptors. FEBS Lett 506:253–256

De Filippis D, Negro L, Vaia M et al (2013) New insights in mast cell modulation by palmitoylethanolamide. CNS Neurol Disor Drug Targets 12:78–83

Di Patrizio N (2016) Endocannabinoids in the Gut. Cannabis Cannabinoid Res 1:67–77. https://doi.org/10.1089/can.2016.0001

Di Marzo V, Izzo AA (2006) Endocannabinoid overactivity and intestinal inflammation. Gut 55:1373–1376

Di Marzo V, Piscitelli F (2011) Gut feelings about the endocannabinoid system. Neurogastroenterol Motil 23:391–398. https://doi.org/10.1111/j.1365-2982.2011

Di Marzo V, De Petrocellis L, Fezza F et al (2002) Anandamide receptors. Prostaglandins Leukot Essent FattyAcids 66:377–391

Di Paola R, Impellizzeri D, Torre A et al (2012) Effects of palmitoylethanolamide on intestinal injury and inflammation caused by ischemia-reperfusion in mice. J Leukoc Biol 91:911–920

DiPatrizio NV, Astarita G, Schwartz G et al (2011) Endocannabinoid signal in the gut controls dietary fat intake. Proc Natl Acad Sci USA 108:12904–12908. https://doi.org/10.1073/pnas.1104675108

Dowie MJ, Grimsey NL, Hoffman T et al (2014) Cannabinoid receptor CB2 is expressed on vascular cells, but not astroglial cells in the post-mortem human Huntington's disease brain. J Chem Neuroanat 59–60:62–71. https://doi.org/10.1016/j.jchemneu.2014.06.004

Duncan M, Davison JS, Sharkey KA (2005a) Review article: endocannabinoids and their receptors in the enteric nervous system. Aliment Pharmacol Ther 22:667–683

Duncan M, Ho W, Shariat N et al (2005b) Distribution of the CB2 receptor in enteric nerves of the rat ileum. Symposium on the Cannabinoids. Burlington, VT, International Cannabinoid Research Society

Duncan M, Mouihate A, Mackie K et al (2008) Cannabinoid CB2 receptors in the enteric nervous system modulate gastrointestinal contractility in lipopolysaccharide-treated rats. Am J Physiol Gastrointest Liver Physiol 295:G78–G87. https://doi.org/10.1152/ajpgi.90285.2008

Eddinger TJ (2009) Unique contractile and structural protein expression in dog ileal inner circular smooth muscle. J Smooth Muscle Res 45:217–230

Escher P, Braissant O, Basu-Modak S et al (2001) Rat PPARs: quantitative analysis in adult rat tissues and regulation in fasting and refeeding. Endocrinology 142:4195–4202

Esposito G, Capoccia E, Turco F et al (2014) Palmitoylethanolamide improves colon inflammation through an enteric glia/toll like receptor 4-dependent PPAR-α activation. Gut 63:1300–1312. https://doi.org/10.1136/gutjnl-2013-305005

Fabisiak A, Fichna J (2017) Cannabinoids as gastrointestinal anti-inflammatory drugs. Neurogastroenterol Motil. https://doi.org/10.1111/nmo.13038

Facci L, Dal Toso R, Romanello S (1995) Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. Proc Natl Acad Sci USA 92:3376–3380

Farquhar-Smith W, Jaggar S, Rice A (2002) Attenuation of nerve growth factor-induced visceral hyperalgesia via cannabinoid CB1 and CB2-like receptors. Pain 97:11–21

Freundt-Revilla J, Kegler K, Baumgärtner W et al (2017) Spatial distribution of cannabinoid receptor type 1 (CB1) in normal canine central and peripheral nervous system. PLoS One 12:e0181064. https://doi.org/10.1371/journal.pone.0181064

Freundt-Revilla J, Heinrich F, Zoerner A et al (2018) The endocannabinoid system in canine Steroid-Responsive Meningitis-Arteritis and Intraspinal Spirocercosis. PLoS One 13:e0187197. https://doi.org/10.1371/journal.pone.0187197

Gabrielsson L, Mattsson S, Fowler CJ (2016) Palmitoylethanolamide for the treatment of pain: pharmacokinetics, safety and efficacy. Br J Clin Pharmacol 82:932–942. https://doi.org/10.1111/bcp.13020

Giancola F, Fracassi F, Gallucci A et al (2016) Quantification of nitrergic neurons in the myenteric plexus of gastric antrum and ileum of healthy and diabetic dogs. Auton Neurosci 197:25–33. https://doi.org/10.1016/j.autneu.2016.04.004

Giancola F, Rambaldi AM, Bianco F et al (2017) Localization of the 5-hydroxytryptamine 4 receptor in equine enteric neurons and extrinsic sensory fibers. Neurogastroenterol Motil. https://doi.org/10.1111/nmo.13045

Golech SA, McCarron RM, Chen Y et al (2004) Human brain endothelium: coexpression and function of vanilloid and endocannabinoid receptors. Brain Res Mol Brain Res 132:87–92

Goyal H, Singla U, Gupta U et al (2017) Role of cannabis in digestive disorders. Eur J Gastroenterol Hepatol 29:135–143

Guida F, Luongo L, Boccella S et al (2017) Palmitoylethanolamide induces microglia changes associated with increased migration and phagocytic activity: involvement of the CB2 receptor. Sci Rep 7:375. https://doi.org/10.1038/s41598-017-00342-1

Gyires K, Zádori ZS (2016) Role of Cannabinoids in gastrointestinal mucosal defense and inflammation. Curr Neuropharmacol 14:935–951

Hall CN, Reynell C, Gesslein B et al (2014) Capillary pericytes regulate cerebral blood flow in health and disease. Nature 508:55–60. https://doi.org/10.1038/nature13165

He SH (2004) Key role of mast cells and their major secretory products in inflammatory bowel disease. World J Gastroenterol 10:309–318

He SH, Walls AF (1998) Human mast cell chymase induces the accumulation of neutrophils, eosinophils and other inflammatory cells in vivo. Br J Pharmacol 125:1491–1500

He SH, Peng Q, Walls AF (1997) Potent induction of a neutrophil and eosinophil-rich infiltrate in vivo by human mast cell tryptase: selective enhancement of eosinophil recruitment by histamine. J Immunol 159:6216–6225

Ho WS, Barrett DA, Randall MD (2008) 'Entourage'effects of *N*-palmitoylethanolamide and N-oleoylethanolamide on vasorelaxation to anandamide occur through TRPV1 receptors. Br J Pharmacol 155:837–846

Hu SS, Mackie K (2015) Distribution of the endocannabinoid system in the central nervous system. Handb Exp Pharmacol 231:59–93. https://doi.org/10.1007/978-3-319-20825-1_3

Hu DL, Zhu G, Mori F et al (2007) Staphylococcal enterotoxin induces emesis through increasing serotonin release in intestine and it is downregulated by cannabinoid receptor 1. Cell Microbiol 9:2267–2277. https://doi.org/10.1111/j.1462-5822.2007.00957.x

Huizinga JD, Ambrous K, Der-Silaphet T (1998) Co-operation between neural and myogenic mechanisms in the control of distension-induced peristalsis in the mouse small intestine. J Physiol 506:843–856

Iannotti FA, Di Marzo V, Petrosino S (2016) Endocannabinoids and endocannabinoid-related mediators: targets, metabolism and role in neurological disorders. Prog Lipid Res 62:107–128. https://doi.org/10.1016/j.plipres.2016.02.002

Izzo AA (2004) Cannabinoids and intestinal motility: welcome to CB2 receptors. Br J Pharmacol 142:1201–1202

Izzo AA, Coutts AA (2005) Cannabinoids and the digestive tract. Handb Exp Pharmacol 168:573–598

Izzo AA, Sharkey KA (2010) Cannabinoids and the gut: New developments and emerging concepts. Pharmacol Ther 126:21–38

Izzo AA, Mascolo N, Pinto L et al (1999) The role of cannabinoid receptors in intestinal motility, defaecation and diarrhoea in rats. Eur J Pharmacol 384:37–42

Jaggar S, Sellaturay S, Rice A (1998) The endogenous cannabinoid anandamide, but not the CB2 ligand palmitoylethanolamide, prevents the viscero-visceral hyperreflexia associated with inflammation of the rat urinary bladder. Neurosci Lett 253:123–126

Ji H, Wang H, Zhang F et al (2010) PPARγ agonist pioglitazone inhibits microglia inflammation by blocking p38 mitogen-activated protein kinase signaling pathways. Inflamm Res 59:921–929. https://doi.org/10.1007/s00011-010-0203-7

Junginger J, Lemensieck F, Moore PF et al (2014) Canine gut dendritic cells in the steady state and in inflammatory bowel disease. Innate Immun 20:145–160. https://doi.org/10.1177/1753425913485475

Kader KN, Moore LR, Saul JM et al (2001) Isolation and purification of canine adipose microvascular endothelial cells. Microvasc Res 61:220–226. https://doi.org/10.1006/mvre.2001.2296

Karwad MA, Couch DG, Theophilidou, Eb et al (2017a) The role of CB1 in intestinal permeability and inflammation. FASEB J 31:3267–3277. https://doi.org/10.1096/fj.201601346R

Karwad MA, Macpherson T, Wang B et al (2017b) Oleoylethanolamine and palmitoylethanolamine modulate intestinal permeability in vitro via TRPV1 and PPARα. FASEB J 31:469–481. https://doi.org/10.1096/fj.201500132

Ke P, Shao BZ, Xu ZQ et al (2016) Activation of cannabinoid receptor 2 ameliorates dss-induced colitis through inhibiting NLRP3 inflammasome in macrophages. PLoS One 11:e0155076. https://doi.org/10.1371/journal.pone.0155076

Kianian M, Al-Banna NA, Kelly ME et al (2013) Inhibition of endocannabinoid degradation in experimental endotoxemia reduces leukocyte adhesion and improves capillary perfusion in the gut. J Basic Clin Physiol Pharmacol 24:27–33. https://doi.org/10.1515/jbcpp-2012-0065

Kleinschmidt S, Meneses F, Nolte I et al (2007) Characterization of mast cell numbers and subtypes in biopsies from the gastrointestinal tract of dogs with lymphocytic-plasmacytic or eosinophilic gastroenterocolitis. Vet Immunol Immunopathol 120:80–92

Kreitzer FR, Stella N (2009) The therapeutic potential of novel cannabinoid receptors. Pharmacol Ther 122:83–96. https://doi.org/10.1016/j.pharmthera.2009.01.005

Kulkarni-Narla A, Brown DR (2000) Localization of CB1-cannabinoid receptor immunoreactivity in the porcine enteric nervous system. Cell Tissue Res 302:73–80

Lanuti M, Talamonti E, Maccarrone M et al (2015) Correction: activation of GPR55 receptors exacerbates oxLDL-induced lipid accumulation and inflammatory responses, while reducing cholesterol efflux from human macrophages. PLoS One 10:e0131850. https://doi.org/10.1371/journal.pone.0131850

Lauckner JE, Jensen JB, Chen HY et al (2008) GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. PNAS 105:2699–2704

Lee Y, Jo J, Chung HY et al (2016) Endocannabinoids in the gastrointestinal tract. Am JPhysiol Gastrointest Liver Physiol 311:G655–G666

Li K, Fichna J, Schicho R et al (2013) A role for O-1602 and G protein-coupled receptor GPR55 in the control of colonic motility in mice. Neuropharma 71:255–263. https://doi.org/10.1016/j.neuropharm.2013.03.029

Ligresti A, De Petrocellis L, Di Marzo V (2016) From phytocannabinoids to cannabinoid receptors and endocannabinoids: pleiotropic physiological and pathological roles through complex pharmacology. Physiol Rev 96:1593–1659. https://doi.org/10.1152/physrev.00002.2016

Lin XH, Yuece B, Li YY et al (2011) A novel CB receptor GPR55 and its ligands are involved in regulation of gut movement in rodents. Neurogastroenterol Motil 23:862-e342. https://doi.org/10.1111/j.1365-2982.2011.01742.x

Liu YA, Chung YC, Pan ST et al (2013) 3-D imaging, illustration, and quantitation of enteric glial network in transparent human colon mucosa. Neurogastroenterol Motil 25:e324-38. https://doi.org/10.1111/nmo.12115

Lo Verme J, Fu J, Astarita G et al (2005a) The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. Mol Pharmacol 67:15–19

Lo Verme J, La Rana G, Russo R et al (2005b) The search for the palmitoylethanolamide receptor. Life Sci 77:1685–1698

Lu Y, Anderson HD (2017) Cannabinoid signaling in health and disease. Can J Physiol Pharmacol 95:311–327. https://doi.org/10.1139/cjpp-2016-0346

Maccarrone M, Bab I, Bíró T et al (2015) Endocannabinoid signaling at the periphery: 50 years after THC. Trends Pharmacol Sci 36:277–296

Maione S, Costa B, Di Marzo V (2013) Endocannabinoids: a unique opportunity to develop multi-target analgesics pain 154(Suppl 1):S87–S93. https://doi.org/10.1016/j.pain.2013.03.023

Malfitano AM, Basu S, Maresz K et al (2014) What we know and do not know about the cannabinoid receptor 2 (CB2). Semin Immunol 26: 369–379. https://doi.org/10.1016/j.smim.2014.04.002

Marquéz L, Suárez J, Iglesias M et al (2009) Ulcerative colitis induces changes on the expression of the endocannabinoid system in the human colonic tissue. PLoS One 4:e6893. https://doi.org/10.1371/journal.pone.0006893

Marsicano G, Wotjak CT, Azad SC et al. (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature 418:530–534. https://doi.org/10.1038/nature00839

Moreira FA, Grieb M, Lutz B (2009) Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. Best Pract Res Clin Endocrinol Metab 23:133–144. https://doi.org/10.1016/j.beem.2008.09.003

Moriconi A, Cerbara I, Maccarrone M et al (2010) GPR55: Current knowledge and future perspectives of a purported "Type-3" cannabinoid receptor. Curr Med Chem 17:1411–1429

Muccioli GG, Naslain D, Bäckhed F et al (2010) The endocannabinoid system links gut microbiota to adipogenesis. Mol Syst Biol 6:392. https://doi.org/10.1038/msb.2010.46

Naidenow J, Hrgovic I, Doll M et al (2016) Peroxisome proliferator-activated receptor (PPAR) α and δ activators induce ICAM-1 expression in quiescent non stimulated endothelial cells. J Inflamm 13:27. https://doi.org/10.1186/s12950-016-0135-2

Navarro G, Morales P, Rodríguez-Cueto C et al (2016) Targeting cannabinoid CB2 receptors in the central nervous system. Medicinal chemistry approaches with focus on neurodegenerative disorders. Front Neurosci 10:406. https://doi.org/10.3389/fnins.2016.00406

Nestmann ER (2016) Safety of micronized palmitoylethanolamide (microPEA): lack of toxicity and genotoxic potential. Food Sci Nutr 5:292–309. https://doi.org/10.1002/fsn3.392

O' Sullivan SE (2016) An update on PPAR activation by cannabinoids. Br JPharmacol 173:1899–1910. https://doi.org/10.1111/bph.13497

Ochoa-Cortes F, Turco F, Linan-Rico A et al (2016) Enteric glial cells: a new frontier in neurogastroenterology and clinical target for inflammatory bowel diseases. Inflamm Bowel Dis 22:433–449. https://doi.org/10.1097/MIB.0000000000000667

Pazos MR, Tolón RM, Benito C et al (2008) Cannabinoid CB1 receptors are expressed by parietal cells of the human gastric mucosa. J Histochem Cytochem 56:511–516. https://doi.org/10.1369/jhc.2008.950741

Petrosino S, Di Marzo V (2017) The pharmacology of palmitoylethanolamide and first data on the therapeutic efficacy of some of its new formulations. Br J Pharmacol 174:1349–1365. https://doi.org/10.1111/bph.13580

Petrosino S, Schiano Moriello A, Cerrato S et al (2016) The anti-inflammatory mediator palmitoylethanolamide enhances the levels of 2-arachidonoyl-glycerol and potentiates its actions at TRPV1 cation channels. Br J Pharmacol 173:1154–1162. https://doi.org/10.1111/bph.13084

Pierezan F, Mansell J, Ambrus A et al (2014) Immunohistochemical expression of ionized calcium binding adapter molecule 1 in cutaneous histiocytic proliferative, neoplastic and inflammatory disorders of dogs and cats. J Comp Pathol 151:347–351. https://doi.org/10.1016/j.jcpa.2014.07.003

Preziosi R, Sarli G, Paltrinieri M (2004) Prognostic value of intratumoral vessel density in cutaneous mast cell tumors of the dog. J Comp Pathol 130:143–151

Ray AP, Griggs L, Darmani NA (2009) Delta 9-tetrahydrocannabinol suppresses vomiting behavior and Fos expression in both acute and delayed phases of cisplatin-induced emesis in the least shrew. Behav Brain Res 196:30–36. https://doi.org/10.1016/j.bbr.2008.07.028

Re G, Barbero R, Miolo A et al (2007) Palmitoylethanolamide, endocannabinoids and related cannabimimetic compounds in

protection against tissue inflammation and pain: potential use in companion animals. Vet J 173:21–30
Ross GR, Lichtman A, Dewey WL et al (2012) Evidence for the putative cannabinoid receptor (GPR55)-mediated inhibitory effects on intestinal contractility in mice. Pharmacology 90:55–65. https://doi.org/10.1159/000339076
Ryberg E, Larsson N, Sjögren S et al (2007) The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol 152:1092–1101
Sardinha J, Kelly ME, Zhou J et al (2014) Experimental cannabinoid 2 receptor-mediated immune modulation in sepsis. Mediat Inflamm 2014:978678. https://doi.org/10.1155/2014/978678
Sarnelli G, D'Alessandro A, Iuvone T et al (2016) Palmitoylethanolamide modulates inflammation-associated vascular endothelial growth factor (VEGF) signaling via the Akt/mTOR pathway in a selective peroxisome proliferator-activated receptor alpha (PPAR-α)-dependent manner. PLoS One 11:e0156198. https://doi.org/10.1371/journal.pone.0156198
Sharkey KA (2015) Emerging roles for enteric glia in gastrointestinal disorders. J Clin Invest 125:918–825. https://doi.org/10.1172/JCI76303
Sharkey KA, Wiley JW (2016) Getting into the weed: the role of the endocannabinoid system in the brain-gut axis. Gastroenterology 151:252–266. https://doi.org/10.1053/j.gastro.2016.04.015
Shea-Donohue T, Stiltz J, Zhao A et al (2010) Mast cells. Curr Gastroenterol Rep 12:349–357. https://doi.org/10.1007/s11894-010-0132-1
Sheng WS, Hu S, Min X et al (2005) Synthetic cannabinoid WIN55, 212-2 inhibits generation of inflammatory mediators by IL-1beta-stimulated human astrocytes. Glia 49:211–219
Skaper SD, Facci L, Giusti P (2013) Glia and mast cells as targets for palmitoylethanolamide, an anti-inflammatory and neuroprotective lipid mediator. Mol Neurobiol 48:340–352. https://doi.org/10.1007/s12035-013-8487-6
Stella N (2004) Cannabinoid signaling in glial cells. Glia 48:267–277
Storr MA, Sharkey KA (2007) The endocannabinoid system and gut-brain signalling. Curr Opin Pharmacol 7:575–582
Storr M, Sibaev A, Marsicano G et al (2004) Cannabinoid receptor type 1 modulates excitatory and inhibitory neurotransmission in mouse colon. Am J Physiol Gastrointest Liver Physiol 286:G110–G117
Svensson M, Chen P, Hammarfjord O (2010) Dendritic cell regulation by cannabinoid-based drugs. Pharmaceuticals 3:2733–2750
Sykaras AG, Demenis C, Case RM et al (2012) Duodenal enteroendocrine I-cells contain mRNA transcripts encoding key endocannabinoid and fatty acid receptors. PLoS One 7:e42373. https://doi.org/10.1371/journal.pone.0042373
Taylor L, Christou I, Kapellos TS et al (2015) Primary macrophage chemotaxis induced by cannabinoid receptor 2 agonists occurs independently of the CB2 receptor. Sci Rep 5:10682. https://doi.org/10.1038/srep10682
Tuduri E, Imbernon M, Bautista R et al (2017) GPR55: a new promising target for metabolism? J Mol Endocrinol 58:R191–R202. https://doi.org/10.1530/JME-16-0253
Van Sickle MD, Oland LD, Ho W et al (2001) Cannabinoids inhibit emesis through CB1 receptors in the brainstem of the ferret. Gastroenterology 121:767–774
Wang J, Zheng J, Kulkarni A et al (2014) Palmitoylethanolamide regulates development of intestinal radiation injury in a mast cell-dependent manner. Dig Dis Sci 59:2693–2703. https://doi.org/10.1007/s10620-014-3212-5
Wille KH1, Schnorr B (2003) The occurrence of hemodynamic effective elements in the intestinal blood vessel system. Anat Histol Embryol 32:94–97
Wouters MM, Vicario M, Santos J (2016) The role of mast cells in functional GI disorders. Gut, 65:155–168. https://doi.org/10.1136/gutjnl-2015-309151
Wright K, Rooney N, Feeney M et al (2005) Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing. Gastroenterology 129:437 – 453
Wright KL, Duncan M, Sharkey KA (2008) Cannabinoid CB2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation. Br J Pharmacol 153:263–270
Zahradka P, Yurkova N, Litchie B et al (2003) Activation of peroxisome proliferator-activated receptors alpha and gamma1 inhibits human smooth muscle cell proliferation. Mol Cell Biochem 246:105–110
Zelcer E, Daniel EE (1979) Electrical coupling in the circular muscles of dog jejunum. Can J Physiol Pharmacol 57:578–580
Zong Y, Zhou X, Cheng J et al (2017) Cannabinoids regulate the diameter of pericyte-containing retinal capillaries in rats. Cell Physiol Biochem 43:2088–2101. https://doi.org/10.1159/000484193
Zygmunt PM, Petersson J, Andersson DA et al (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400:452–457