Targeting the endocannabinoid system for the treatment of abdominal pain in irritable bowel syndrome

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Abstract | The management of visceral pain in patients with disorders of gut–brain interaction, notably irritable bowel syndrome, presents a considerable clinical challenge, with few available treatment options. Patients are increasingly using cannabis and cannabinoids to control abdominal pain. Cannabis acts on receptors of the endocannabinoid system, an endogenous system of lipid mediators that regulates gastrointestinal function and pain processing pathways in health and disease. The endocannabinoid system represents a logical molecular therapeutic target for the treatment of pain in irritable bowel syndrome. Here, we review the physiological and pathophysiological functions of the endocannabinoid system with a focus on the peripheral and central regulation of gastrointestinal function and visceral nociception. We address the use of cannabinoids in pain management, comparing them to other treatment modalities, including opioids and neuromodulators. Finally, we discuss emerging therapeutic candidates targeting the endocannabinoid system for the treatment of pain in irritable bowel syndrome.

Disorders of gut–brain interaction (DGBIs) are a group of diseases characterized by chronic or recurrent gastrointestinal symptoms in the absence of underlying organic abnormalities[1]. DGBIs are highly prevalent on a global scale, have a detrimental effect on the quality of life of patients, are among the principal causes of health-care seeking and lead to considerable health-care costs[1,2]. DGBIs are defined by and classified according to the Rome consensus, based on the presumed anatomical site of origin of the symptoms and the symptom characteristics[1]. The best known DGBI is irritable bowel syndrome (IBS), characterized by chronic abdominal pain associated with altered bowel habits[3,4]. Pain is a key symptom in several other DGBIs, such as functional chest pain, epigastric pain syndrome, biliary pain, anorectal pain and centrally mediated abdominal pain[1]. In many of these conditions, visceral hypersensitivity resulting from disordered gut–brain interaction has been implicated in the pathogenesis of pain[5], and peripherally and centrally acting pharmacotherapeutic agents are used in managing pain in DGBIs[4,6]. Abdominal pain is commonly considered the most bothersome symptom for patients, is a key predictor of disease effect and health-care utilization, and is often the most difficult symptom to control[4–6] (Supplementary Box 1). Owing to the challenges of managing abdominal pain, many patients have turned to cannabis as an alternative therapeutic approach given its perceived beneficial properties[7–9]. Cannabis and cannabinoids, such as cannabidiol (CBD), are increasingly available, generally accepted, and widely used recreationally and medicinally, especially in countries and/or territories where it is legal or decriminalized. Cannabis acts at receptors of the endocannabinoid system (ECS), an endogenous lipid mediator signalling system involved in the control of diverse gastrointestinal functions such as motility, barrier function, inflammation and gut–brain signalling[10–12]. The ECS has been considered a potentially relevant target for the treatment of DGBIs[12–14]. This Review summarizes the current knowledge on the ECS and how it can potentially serve as a molecular therapeutic target for the treatment of abdominal pain in IBS and other DGBIs.

The endocannabinoid system

The fundamental components of the ECS are the G protein-coupled receptors termed cannabinoid receptor 1 (CB_1) and 2 (CB_2), two endogenous ligands for these receptors, the endocannabinoids, anandamide (*N*-arachidonoylethanolamine) and 2-arachidonoylglycerol (2-AG), and the biosynthetic and degradative enzymes for these endocannabinoids[15] (Supplementary Box 2). Particular attention has been paid to the degradative enzymes for anandamide and 2-AG, fatty acid amide hydrolase (FAAH) and

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Key points

- The management of abdominal pain in disorders of gut–brain interaction, including irritable bowel syndrome (IBS), is challenging.
- Patients are increasingly using cannabis and cannabinoids as an alternative therapy to treat pain and altered bowel habits in IBS.
- Cannabis acts on the cannabinoid receptors (CB_1 and CB_2) of the endocannabinoid system (ECS), which consists of ligands for these receptors, anandamide and 2-arachidonoylglycerol as well as the biosynthetic and degradative enzymes for these ligands.
- The ECS is a logical molecular target for the treatment of IBS as it regulates gastrointestinal motility, secretion, barrier function, inflammation, gut microbiota and visceral sensitivity.
- Cannabinoid therapeutics have been developed but are of limited use in managing pain in IBS; the effects of cannabis have not been rigorously examined in much-needed large clinical trials.
- Evidence supports the putative analgesic properties of a peripherally restricted CB_2 receptor agonist in IBS. Future therapies could target additional ECS components for the treatment of IBS and other disorders of gut–brain interaction.

monoacylglycerol lipase (MAGL), respectively[16,17]. Inhibiting these enzymes raises endocannabinoid levels and augments their actions locally. These tools have provided experimental evidence derived from in vitro and animal studies supporting physiological and pathophysiological roles of endocannabinoids and have clinical therapeutic potential for various conditions, for example, in stress-related anxiety disorders where clinical trials have been undertaken[16,18–20]. Endocannabinoids are not stored in vesicles or granules but are made 'on demand' from membrane lipids following elevations in intracellular calcium or in response to extracellular receptor activation[21]. Endocannabinoids require a carrier protein to overcome the energetic barrier of the hydrophilic environment of the extracellular space and synapse to activate their receptors rapidly. Fatty acid-binding proteins were discovered in biochemical studies to mediate the intracellular transport of anandamide from the plasma membrane for degradation by FAAH[22]. In addition, fatty acid-binding protein 5 was found, in vitro, to regulate 2-AG signalling at excitatory glutamatergic synapses in the brain[23]. What makes this finding particularly interesting is in vitro evidence that astrocytes were the source of fatty acid-binding protein 5 (REF.[23]). It remains to be determined whether this is the mechanism in the central nervous system (CNS) in vivo and whether this mechanism is used by enteric or primary afferent neurons innervating the gut.

Cannabinoid receptor signalling. Over the past 30 years, some remarkable features of the ECS, or what has been termed the endocannabinoidome, have emerged[12,24] (BOX 1). The ECS is capable of exquisite local regulatory control in physiological and pathophysiological conditions. Phytocannabinoids (cannabinoids derived from the cannabis plant, as opposed to endogenous 'endocannabinoids'), such as CBD, act on the receptors or enzymes of the ECS as well as on other receptor systems or ion channels and, in many cases, the exact mechanisms of action remain uncertain[25]. Later in this Review, we outline some of the ECS features that apply to controlling pain and inflammation in the gastrointestinal tract.

The crystal structures, activation and signalling mechanisms of CB_1 and CB_2 were identified[26–28] in the past few years. In particular, the structure of human CB_2 revealed how small molecules affected CB_2 differently from CB_1 (REF.[28]); CB_1 is the dominant receptor expressed on neurons in the nervous system[12,29]. Additionally, CB_1 was found to localize intracellularly on mitochondria in neurons and astrocytes, enabling subcellular-specific CB_1 receptor regulation of neural circuits[30,31]. CB_1 is expressed at a higher abundance than CB_2 in dorsal root ganglia and the enteric nervous system (ENS) in mice and rats[32–34]. CB_2 is mainly expressed in immune cells and peripheral tissues, including the colonic mucosa[34,35], particularly in epithelial cells, where the CB_{2A} isoform predominates[36]. Based on animal studies, CB_2 is also expressed on central and peripheral neurons[37], including neurons within the ENS[32] and visceral primary sensory neurons innervating the gut[36] (FIG. 1).

The cannabinoid receptors are coupled to $G_{i/o}$ proteins and, when activated, cause the inhibition of adenylyl cyclase and decreased production of cyclic AMP (cAMP) (FIG. 2). In nerves, the release of β–γ dimers led to inhibition of N-type and P/Q-type Ca^{2+} channels and activation of inwardly rectifying and A-type potassium channels as well as to the activation of mitogen-activated protein kinases and focal adhesion kinases[38,39]. In general, activation of cannabinoid receptors is inhibitory, serving as a brake to limit excitation. The binding of cannabinoid receptors to β-arrestin is a critical step in the internalization of the receptor. However, β-arrestins are also signal transducers for intracellular signalling pathways, such as extracellular signal-regulated kinase and JUN N-terminal kinase, which were found to mediate some of the actions of endocannabinoids[38,39]. CB_1 signalling is regulated by the cannabinoid receptor-interacting protein 1a, which interacts with G protein-coupled receptors and β-arrestin to alter function[40].

ECS function in the gastrointestinal tract

Gastrointestinal motility. By inhibiting acetylcholine release from the myenteric plexus, endocannabinoids and phytocannabinoids act via CB_1 to reduce

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Box 1 | **Features of the endocannabinoid system**

Feature	Significance
Endocannabinoids are retrograde neurotransmitters[259]	An elegant mechanism to finely tune the nervous system in an activity-dependent manner[260]
Anandamide is an endogenous intracellular ligand of the TRPV1 ion channel: a dual endocannabinoid/ endovanilloid[261]	Activation of TRPV1 is algesic; thus, local anandamide levels and the levels of expression and distribution of CB_1 and TRPV1 regulate the balance of activation of nociceptive and anti-nociceptive systems in sensory neurons
Anandamide and 2-AG are members of classes of lipid mediators — NAEs and 2-AG — that share biosynthetic and degradative enzymes but not the cannabinoid receptors[16]	Many of these molecules have potent biological actions in the gastrointestinal tract and elsewhere, notably the NAE *N*-palmitoylethanolamide, which is anti-inflammatory and anti-nociceptive, acting via PPARα, as well as other receptors (for example, GPR55, TRPV1)[262–264]
Cannabinoid receptors can exist in multiple forms as heterodimers or homodimers, are widely distributed in essentially every organ and tissue in the body, and are subject to regulation in pathophysiological conditions[38,264]	Dimerization provides a mechanism for signal integration upon simultaneous receptor activation by two ligands; the full importance of cannabinoid receptor dimers remains to be determined, but this feature potentially allows for cell type and tissue localization tuning of intracellular signalling
The ECS in the gastrointestinal tract is regulated by the gut microbiota and vice versa; gut endocannabinoids regulate the luminal microbial communities of the gut[16,167,265]	The ECS is a nexus of signalling between the host and microbiota and has a pivotal role in mediating the effects of dietary and other environmental perturbations in the gut on health and disease

2-AG, 2-arachidonoylglycerol; ECS, endocannabinoid system; GPR55, G protein-coupled receptor 55; NAEs, *N*-acylethanolamines; PPARα, peroxisome proliferator-activated receptor-α; TRPV1, transient receptor potential cation channel subfamily V member 1.

contractility and propulsive motility throughout the gastrointestinal tract[41,42]. Hons et al. provided evidence that CB_1 inhibited neurotransmitter release at enteric synapses and depressed synaptic strength under basal conditions and in an activity-dependent manner in the mouse intestine[43]. These findings extend observations made in cell culture showing that CB_1 regulated the activity state of enteric neurons[44]. Activation of CB_1 dampened spontaneous network activity, whereas inhibiting the receptor had the opposite effect. Interestingly, in light of the finding that CB_1 is localized on mitochondria in the brain[30,31], are observations that CB_1 agonists inhibited mitochondrial transport in cultured enteric nerves from Guinea pigs[44]. However, it is not yet known whether mitochondria in enteric neurons or enteric glia express CB_1, but, if they do, this opens up many new avenues to understand the control of synaptic transmission in the ENS.

In the ENS, it has not been conclusively demonstrated that endocannabinoids are retrograde transmitters as they are in the CNS, but Hons et al. revealed a novel form of metaplasticity in the ENS in mice. This ENS metaplasticity consists of a balance between endocannabinoid and purinergic signalling at the enteric synapses that maintain network homeostasis[43]. These actions help explain accelerated intestinal transit observed in mice in the absence of CB_1 receptors and following administration of some CB_1 antagonists[45,46], which are consistent with the physiological control of gastrointestinal motility by the ECS and the ability of CB_1 antagonists to enhance transit. The CB_1 gene (*CNR1*) is highly expressed in neurons of the human colonic myenteric plexus[47], and specific genetic variations of *CNR1* were shown to confer an increased risk for developing IBS[48] or were associated with an increased dronabinol-induced reduction in fasting proximal colonic motility in patients with IBS[49].

CB_2 appears to have little or no role in the control of motility under physiological conditions. However, in mouse and rat models of IBS (administration of endotoxin, chronic water avoidance stress or intestinal inflammation evoked by oil of mustard), CB_2 activation slowed the enhanced motility[50–52]. Whether this directly affects motility or an epiphenomenon associated with CB_2-dependent reduced inflammation remains to be determined. The expression of *Cnr2* (encoding CB_2) is low in the mouse ENS[47], but *CNR2* is higher in the human ENS[47] and might also be inducible[37].

Further evidence to support a role of the ECS in regulating gastrointestinal motility comes from mouse studies using pharmacological inhibitors of endocannabinoid metabolism. FAAH or MAGL inhibitors inhibited propulsive motility[50,53–55], whereas inhibiting DAGL enhanced it[53]. Interestingly, *Mgll*-knockout mice demonstrated markedly elevated intestinal levels of 2-AG that led to internalization, desensitization and functional antagonism of the CB_1 receptor[56]. These mice did not show altered whole-gut transit but demonstrated accelerated colonic propulsion, consistent with the loss of an endogenous inhibitory tone. Additionally, palmitoylethanolamide (PEA), the naturally occurring acylethanolamide related to anandamide whose levels are also regulated by FAAH, was shown to normalize propulsive motility in oil of mustard-induced intestinal inflammation in mice[57]. Extending these preclinical findings are observations that the expression and activity of FAAH were lower in 32 patients with slow transit constipation than in 24 healthy control individuals[58] and that polymorphisms of *FAAH* were associated with faster colonic transit in patients with diarrhoea-predominant IBS[59]. These observations support the concept that endocannabinoid mechanisms have a role in the control of colonic motility in humans and that genetic variations leading to functional alterations are associated with disease.

In 12 healthy subjects, the CB_1 antagonist rimonabant was shown to increase postprandial pressure in the lower oesophageal sphincter and inhibit transient lower oesophageal sphincter relaxations and meal-induced gastric accommodation reflex[60,61]. The latter effect might contribute to decreased nutrient intake associated with rimonabant therapy[62]. However, further studies that address the specific role of the ECS in the control of gastrointestinal motility in humans in health and disease are required.

Epithelial function. The location of CB_1 and CB_2 receptors in the ENS and epithelial cells in the gut and of CB_2 receptors on immune cells suggests that the ECS might influence intestinal epithelial transport and barrier function. In support of this suggestion, *Cannabis* spp.

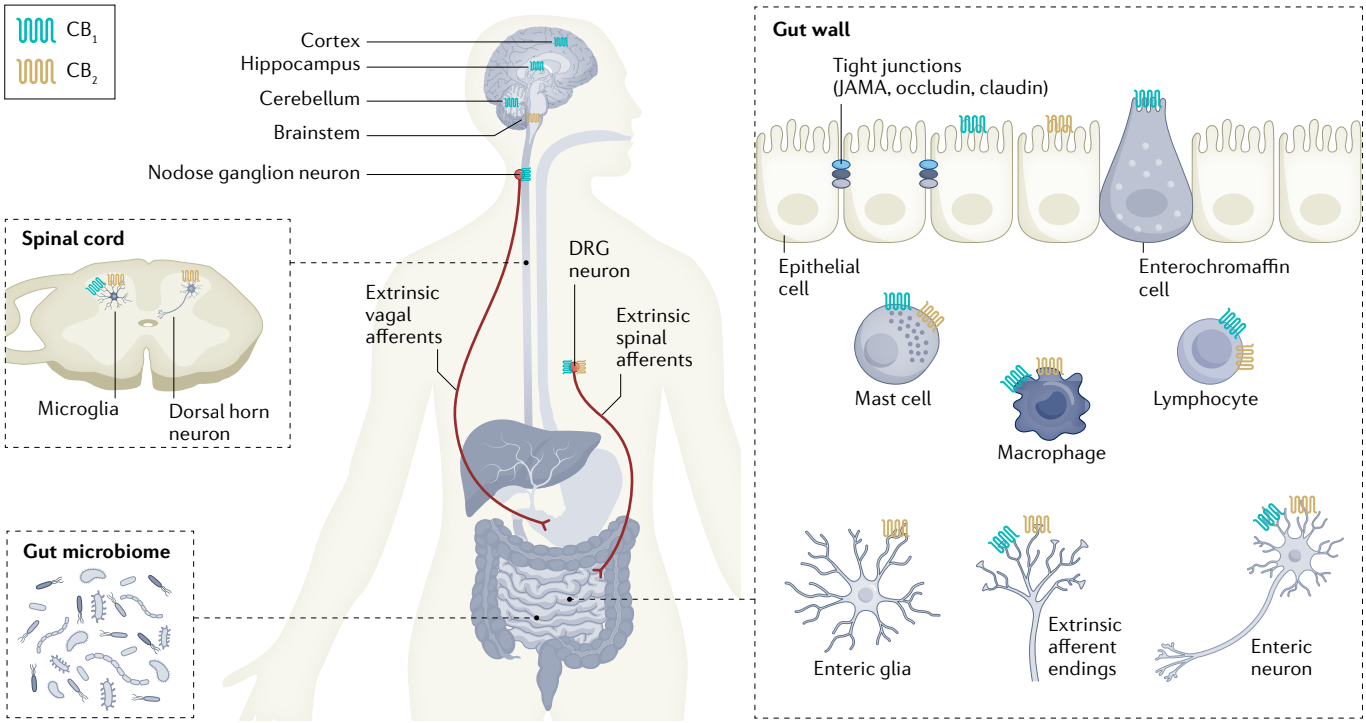

Fig. 1 | **CB_1 and CB_2 expression in cell types throughout the gut–brain–microbiota axis.** Cannabinoid receptors are widely distributed throughout the organs and cells of the gut–brain–microbiota axis. Cannabinoid receptor 1 (CB_1) and CB_2 expression occurs within various regions of the brain, brainstem, spinal cord, nodose or dorsal root ganglia (DRG), extrinsic sensory afferent endings and enteric neurons, as well as in non-neuronal structures, including microglia and enteric glia, mast cells, epithelial cells, enterochromaffin cells and immune cells.

has been used extensively for the treatment of diarrhoea owing, in part, to its anti-secretory activity. The first experimental evidence to support the inhibition of intestinal secretion by the ECS came from Tyler et al., who showed that a CB_1 agonist produced anti-secretory effects in vitro through a neuronal mechanism involving the inhibition of acetylcholine release from neurons of the submucosal plexus[63]. Subsequent in vitro studies confirmed the importance of CB_1 in the anti-secretory effects of the ECS[64]. In a mouse model, administration of cholera toxin increased intestinal secretion and, in addition, elevated the levels of the endocannabinoid anandamide and the expression of *CNR1* mRNA, suggesting a compensatory response to this pathogen[65]. Although CB_2 is expressed in the gut, there is no experimental evidence supporting its role in intestinal secretion.

The ECS has been shown to have a role in intestinal permeability. In Caco-2 cells, a cell line originally derived from a colon carcinoma, intestinal permeability induced by either ethylenediaminetetraacetic acid or cytokines was decreased by Δ^9-tetrahydrocannabinol (THC) or CBD through a CB_1-mediated mechanism[66,67]. Furthermore, anandamide and 2-AG production and CB_1 activation were shown to modulate intestinal mucosal permeability under normal, inflammatory and hypoxic conditions in Caco-2 cells and human intestinal mucosa[68].

The ECS modulates epithelial barrier integrity through CB_1-mediated mechanisms, in part through interactions with the gut microbiota. Specifically, the addition of lipopolysaccharide, a cell wall product of gram-negative bacteria, to Caco-2 cell monolayers led to enhanced epithelial barrier permeability and reduced expression of mRNA for occludin and zonula occludens 1 (REF.[69]). CB_1 antagonism with rimonabant but not CB_2 antagonism with SR144528 inhibited the lipopolysaccharide-induced changes in permeability and tight junction mRNA expression[69]. Together, these preclinical results imply that the ECS can affect intestinal secretion and permeability through CB_1-mediated mechanisms. However, the relative importance of ECS in the regulation of epithelial permeability in people with IBS remains poorly understood despite the knowledge that defects in gut barrier function have a fundamental role in the pathogenesis of DGBIs. However, a study in 31 patients with IBS found increased *CNR2* mRNA expression compared with 32 asymptomatic controls[70]. CB_2 was localized in immune cells in the mucosa of patients with IBS, suggesting that the ECS, through CB_2, might have an immunomodulatory effect, which in turn can affect intestinal secretion or barrier function[70]. This finding points to the necessity of additional studies to enhance our understanding of the ECS in health and disease. Future research is required to determine the translational relevance of data from experimental models in the regulation of intestinal secretion and gut barrier integrity in patients with IBS.

Inflammation and immune function. Although there is abundant evidence for CB_1 regulation of immune function and for many of the other receptors of the ECS, the CB_2 receptor is found primarily on immune cells.

The highest expression levels under physiological conditions are observed in B cells, natural killer cells, monocytes, macrophages, microglia and T cells[71]. CB_2 expression levels are upregulated in patients with IBS and found in various immune-cell populations, including $CD4^+$ T cells and $EMR1^+$ eosinophils in the lamina propria of the gastrointestinal tract[70]. Immune cells not only respond to cannabinoids but they also synthesize and release endocannabinoids, which can then act locally[71]. Therefore, the ECS regulates immune signalling in ways analogous to other lipid (eicosanoids) and protein (cytokine) mediators. What is important about endocannabinoids is that they seem exclusively inhibitory and anti-inflammatory, serving as a brake to excessive immune activation[71–73]. The effects of endocannabinoids on various immune-cell populations are described in excellent reviews[71–73]. Here, we focus on studies that illustrate immune mechanisms regulated by the ECS specifically relevant to visceral pain.

Inflammation of the gastrointestinal tract is generally associated with abdominal pain[74]. The ECS is an important endogenous regulator of intestinal inflammation. Notably, increased levels of CB_2 expression have been demonstrated in preclinical mouse models of gastrointestinal inflammation (mouse trinitrobenzene sulfonic acid (TNBS)-induced colitis and $Tnf^{\Delta ARE/+}$ mice)[75,76] and gut tissue from humans with inflammatory bowel disease[34] or IBS[70,77]. CB_1 and CB_2 have a role in mediating the actions of endocannabinoids and peroxisome proliferator-activated receptor-α (PPARα) mediates the effects of PEA[12,78,79]. Fichna et al. made the surprising observation that activation of central CB_1 is sufficient to reduce inflammation in a mouse model of colitis (TNBS-induced colitis)[80] but the underlying mechanisms remain to be determined.

The cellular and intracellular mechanisms of action of endocannabinoids in the gastrointestinal tract that underly their anti-inflammatory mechanism of action remain to be fully elucidated. However, there is increasing awareness of the role the ECS has at the level of the intestinal epithelium not only to regulate barrier function, as discussed earlier, but also in local immune regulation. This role is exemplified by the findings that the secretion of *N*-acylethanolamine endocannabinoids via the epithelial P-glycoprotein efflux pump attenuated inflammation at the intestinal mucosal surface in mice via CB_2 on neutrophils[81]. This anti-inflammatory pathway counteracts the epithelial release of the pro-inflammatory eicosanoid hepoxilin A_3 and serves as a homeostatic mechanism to limit the potential for damage caused by unregulated neutrophil migration across the epithelium[81]. These findings were extended to show that core components of the gut microbiota, genera within the Clostridia and Bacilli classes, regulated the expression of epithelial P-glycoprotein[82] in mice, thereby illustrating how the ECS modulates a microbial–epithelial–immune axis that, if unbalanced, leads to a pro-inflammatory milieu that triggers visceral hypersensitivity.

Visceral hypersensitivity

Emerging evidence from mouse and rat models of colonic hypersensitivity (post-inflammatory models or water avoidance stress) points to an important role for the ECS in visceral pain behaviours (FIG. 3). CB_1 and CB_2 agonists attenuate visceral hypersensitivity

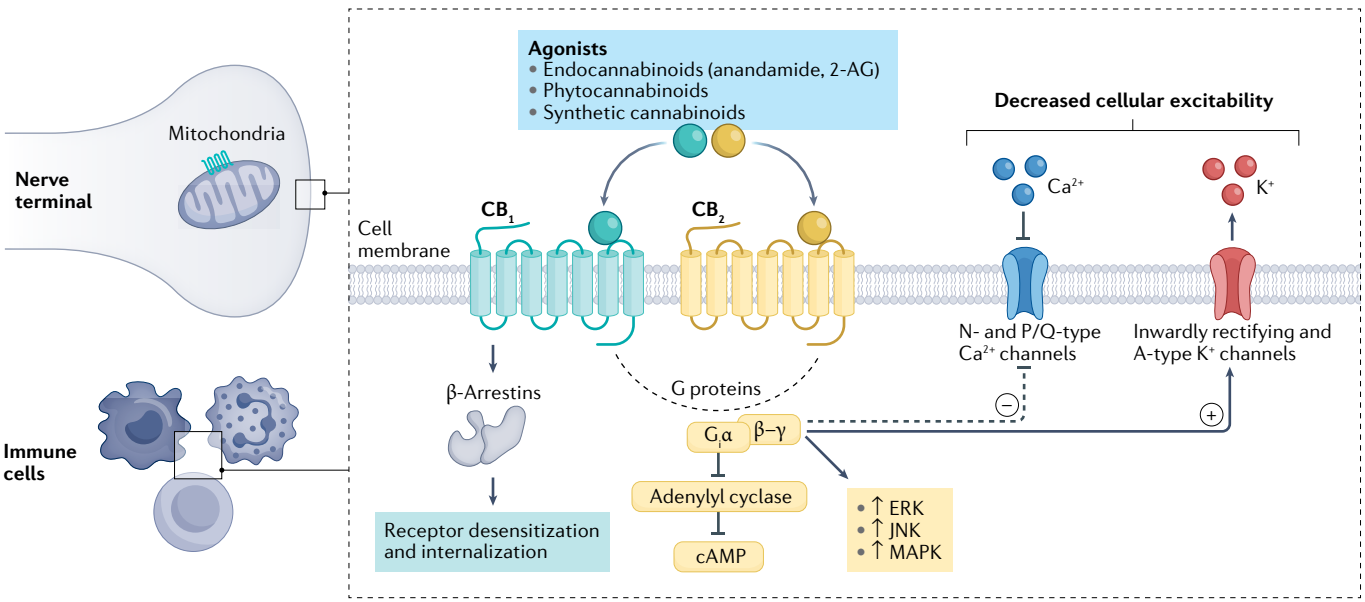

Fig. 2 | **CB_1 and CB_2 structure, second messenger signalling mechanisms, and downstream targets within cells.** Cannabinoid receptor 1 (CB_1) and CB_2 are expressed in neurons and immune cells. Activation of CB_1 or CB_2 by agonists, such as endocannabinoids, phytocannabinoids and synthetic cannabinoids, results in G protein activation and downstream inhibition of voltage-gated calcium channels and activation of potassium channels, mechanisms that result in reduced cellular excitability. Activation of CB_1 and CB_2 also results in β-arrestin activation and subsequent receptor desensitization and internalization. G protein activation also triggers downstream signalling cascades, resulting in reduced adenylyl cyclase and cyclic AMP (cAMP) production with reciprocal increases in extracellular signal-regulated kinase (ERK), JUN N-terminal kinase (JNK) and mitogen-activated protein kinase (MAPK) levels, which alter a variety of cellular functions. 2-AG, 2-arachidonoylglycerol.

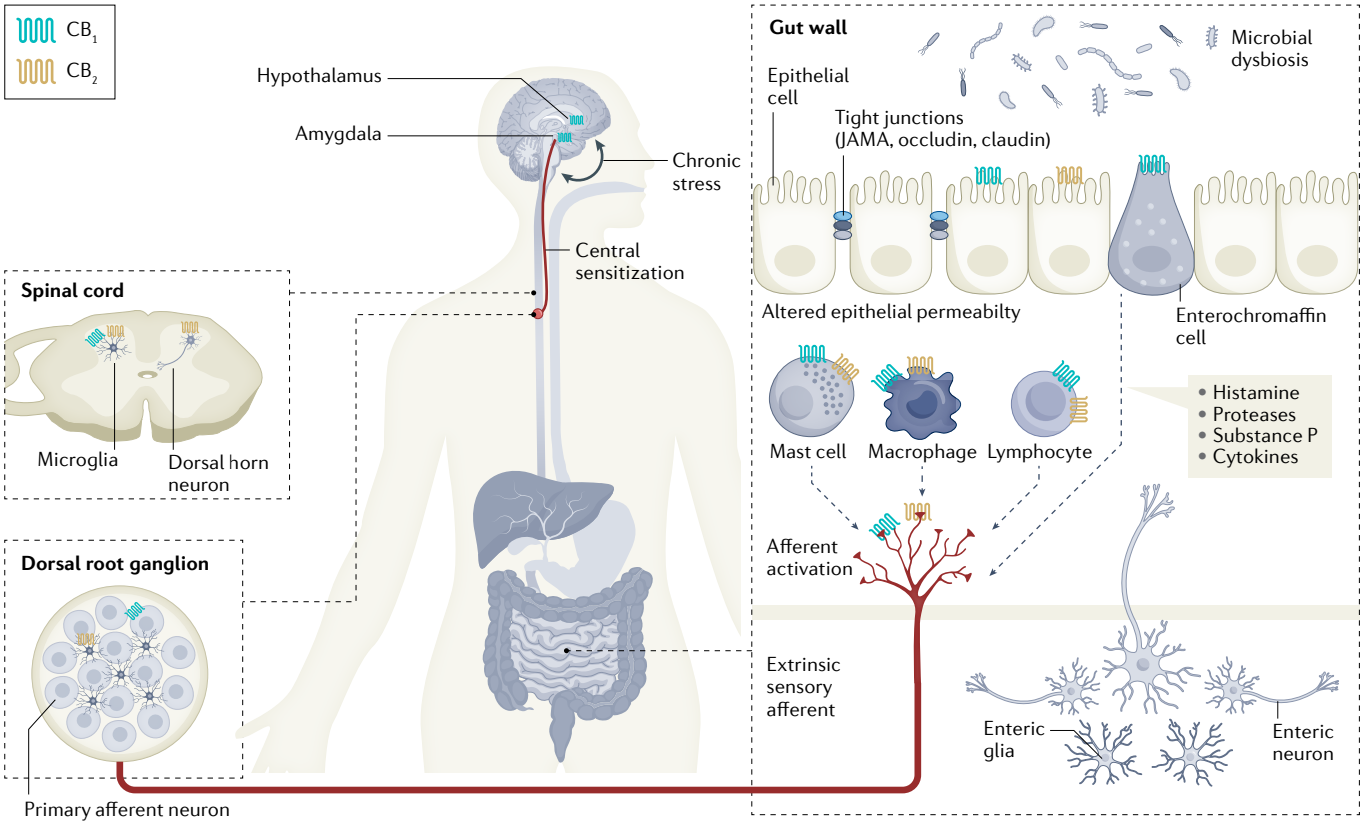

Fig. 3 | **Endocannabinoid system targets of visceral pain.** Key components of the gut–brain–microbiota axis are associated with the initiation of nociceptive signalling and the processing of pain. Alterations in these pathways can drive visceral hypersensitivity, pain transmission and perception. Activation of cannabinoid receptor 1 (CB_1) and CB_2 can reduce visceral hypersensitivity, pain transmission and pain perception via a variety of different mechanisms outlined in this figure, including the composition of the gut microbiota, the regulation of epithelial permeability, activation of visceral primary afferent nerves and visceral sensitivity, and the regulation of spinal and supraspinal processing. Stress can additionally modulate pain processing by dysregulation of the endocannabinoid system.

and abdominal pain-like behaviours in rats and mice[36,83–87]. However, the therapeutic potential of cannabinoid agonists to treat visceral pain in IBS is limited by their central psychoactive effects. Peripherally restricted CB_1 or CB_2 agonists were shown to possess robust analgesia in mice and rat models of somatic, inflammatory and post-inflammatory visceral pain[35,85,86,88]. In patients with IBS, visceral pain is exacerbated during episodes of stress[89] but the importance of the ECS in this process is currently unknown. Interestingly, an endocannabinoid-like dietary supplement, PEA/polydatin, alleviated abdominal pain and discomfort in a phase IIb study in 54 patients with IBS[77]. There is emerging evidence based on a rat model suggesting that the ECS of the gastrointestinal tract can modulate stress-induced visceral hypersensitivity through a CB_1 receptor-mediated mechanism[90], suggesting a therapeutic potential of CB_1 agonists with poor CNS penetrance in patients with visceral pain.

Agonists of both CB_1 and CB_2 receptor-mediated mechanisms were shown in rats to diminish hypersensitivity due to TNBS-induced colitis, with a CB_1 antagonist also enhancing colitis-induced hyperalgesia, suggesting an endogenous inhibitory tone of the ECS[87]. Similar protective effects of CB_2 activation in a preclinical model of TNBS-induced colitis were shown and these effects were blocked in the presence of a CB_2 antagonist and were absent in CB_2-deficient mice[75]. Bradykinin-induced activation of mesenteric afferents in vivo was reversed by the selective CB_2 agonist AM1241, and the CB_2 antagonist AM630 (REF.[91]) was completely abolished by this effect. Other studies in rats and mice show that a highly selective peripherally restricted CB_2 agonist can reverse colitis-induced chronic visceral hypersensitivity ex vivo and in vivo in a concentration- and CB_2-dependent manner[36].

Mast cells in visceral hyperalgesia. Two of the cardinal features of visceral pain are allodynia and hyperalgesia. They are mediated by the activation and sensitization of visceral primary sensory afferents[92] and by activity in so-called mechanosensitive silent nociceptors, a population of small-diameter primary afferent neurons that express the TRKA receptor and nicotinic acetylcholine receptor-α3 subunit[93]. These nerves become 'un-silenced' in inflammatory conditions allowing the mechanosensitive PIEZO2 ion channels to gate the influx of sodium and calcium, thereby strongly depolarizing the nerve terminals[93]. One of the key mediators of

this effect is nerve growth factor (NGF)[93,94]. An important source of NGF in the gastrointestinal tract are mast cells, whose numbers are increased in patients with IBS as are the levels of NGF[95]. Mast cell activation was found in duodenal mucosal biopsy samples from patients with functional dyspepsia[96] and IBS[97,98]. Mast cell activation is also linked to food allergy-induced visceral pain in mouse models and patients with IBS[99,100].

There is good evidence that the ECS regulates mast cell activation. In rats, anandamide and PEA reduced NGF-induced visceral hyperalgesia via CB_1 and CB_2 (REF.[100]). Moreover, administration of PEA reduced mast cell degranulation and the release of inflammatory mediators and NGF from mast cells[101–103] in cell cultures and mice. Interestingly, Petrosino et al. showed, in cell systems, that PEA might exert its effects indirectly via the stimulation of 2-AG biosynthesis and the activation of CB_2 receptors by 2-AG[103]. However, it remains to be determined whether this occurs in the gastrointestinal tract in vivo. The effects of PEA on mast cells are mediated by CB_1, TRPV1, GPR55, PPARα and/or PPARγ receptors[96,101,102]; hence, there is still a lot to understand about how endocannabinoids regulate mast cells. Nevertheless, their importance was underscored in a study by Sarnelli et al., who proposed that the impaired release of PEA leads to mast cell activation in functional dyspepsia[96]. They demonstrated that acid exposure induced an increase in mast cell density and mediator release that was substantially greater in 20 patients with functional dyspepsia than in 10 control individuals being screened for gastric cancer. The authors then demonstrated an impaired release of endogenous PEA and that PEA inhibited mast cell mediator and NGF release via PPARα receptors. These data reveal a previously unknown endogenous system for the homeostatic control of mast cell activation and, therefore, visceral sensitivity[96]. It remains to be determined why there is impaired PEA release in patients with functional dyspepsia. However, these findings provide a plausible pathophysiological mechanism to explain the pain experienced in patients with functional dyspepsia and offer a novel therapeutic approach given that PEA is available as a food supplement and has been used to treat IBS, as discussed earlier.

Enteric glia–macrophage regulation. A key immune cell type involved in regulating visceral sensitivity at the level of the gastrointestinal tract is the macrophage. Activated macrophages secrete a variety of inflammatory mediators and proteases that regulate the excitability of visceral primary afferent nerves[104]. An example is the macrophage-derived serine protease cathepsin S that activates protease-activated receptor 2, which causes visceral hyperalgesia in TNBS-induced colitis and *Il10*-knockout mice[105] as well as chronic visceral hypersensitivity in mice[106]. In new work, Grubišić et al. demonstrated an enteric glia–macrophage circuit in chronic colitis related to the development of visceral hypersensitivity in mice[107]. These researchers showed that enteric glia were activated by inflammatory mediators such as IL-1β. Upon activation, enteric glia released colony-stimulating factor 1, which promoted the activation and polarization of resident muscularis macrophages closely associated with both enteric glia and visceral primary afferent nerves at the level of the myenteric plexus. The activated macrophages then released mediators leading to heightened visceral sensitivity[107].

Enteric glia and macrophages, similar to mast cells, are subject to regulation by the ECS. Esposito et al. demonstrated that administration of PEA reduced enteric glial cell activation in dextran sodium sulfate-induced colitis and colonic biopsies from patients with ulcerative colitis through PPARα receptors[108]. Similarly, Duncan et al. showed, in rats, that the CB_2 agonist JWH133 attenuated activation of enteric glia stimulated by lipopolysaccharide[32]. Macrophages in the gastrointestinal tract express CB_2 (REFS.[34,109]) and are regulated by endocannabinoids acting via this receptor[34,109]. Functional evidence also points to a role for endogenously released endocannabinoids acting at CB_1 in regulating cytokine secretion from the human intestinal mucosa[68]. Thus, the ECS has the potential to regulate this novel pain mechanism in the gut and future studies should be aimed at investigating this mechanism.

Spinal and supraspinal immune mechanisms. Immune mechanisms of visceral hypersensitivity are also present in the CNS. Here, microglia, the resident macrophage cells of the CNS, have a critical role. At the level of the spinal cord, in mice with acute colitis, microglial activation by colony-stimulating factor 3 (also known as granulocyte colony-stimulating factor) sensitized visceral afferents through a cathepsin S–CX3CR1 pathway that led to the production of nitric oxide via inducible nitric oxide synthase[110]. This enhanced visceral sensitivity persisted for weeks after colitis had resolved, potentially explaining the chronic abdominal pain experienced in patients after an acute episode of enteritis.

Psychological stress is another trigger for the development of chronic abdominal pain in patients with IBS. Modelling this condition with chronic water avoidance stress in rats, Yuan et al. showed that microglial activation in the central nucleus of the amygdala mediated enhanced visceral sensitivity via a complement C1q–C3–CR3 signalling pathway owing to elevated corticosterone, contributing to synaptic remodelling in this region of the limbic system[111,112]. Similarly, in other rat brain regions, including the hippocampus and hypothalamus, microglial activation involving the stress axis was causally linked to the development of visceral hypersensitivity[113,114].

Microglia express CB_1 and CB_2 constitutively and synthesize endocannabinoids. Upon activation, they increase endocannabinoid synthesis and markedly upregulate the expression of CB_2, which dampens activity in these cells[72]. Suppressing microglial activation with CB_2 agonists is an effective treatment for neuropathic pain in various preclinical animal models of disease[72]. However, this mechanism of pain modulation has not, to date, been assessed in visceral pain models or states of visceral hypersensitivity and remains an exciting unexplored therapeutic opportunity for the treatment of chronic abdominal pain.

The brain, visceral pain and the ECS. Central pain processing, elevated anxiety levels and other features that characterize IBS result from the dynamic interactions of distributed brain regions that operate as a series of networks[115]. There is extensive evidence that, in patients with IBS, there are abnormalities in both task-related and resting-state networks, which provide a possible substrate to explain the symptoms of IBS[115]. The ECS regulates the functional connectivity of brain networks, including those involved in emotional arousal, salience and reward[115–118]. Interestingly, CB_1 and FAAH polymorphisms also regulate the placebo effect[119,120], a common feature of IBS[121]. The role of the ECS in brain mechanisms of visceral hypersensitivity has not been studied sufficiently to know whether it is a potential therapeutic target.

Stress, epigenetics and visceral pain. Stress is an important environmental factor in the development and/or exacerbation of IBS symptoms[89]. Stress-induced epigenetic modifications to the neural circuitry involved in the regulation of visceral sensitivity have been reported and extensively reviewed[122,123]. The ECS is critically involved in the regulation of stress circuitry and undergoes epigenetic modification in stress, leading to dysregulated responses that contribute to the pathophysiology of stress, including the development of anxiety and depression[124,125]. For example, in the amygdala, exposure to chronic water avoidance stress in rats increased DNA methylation at the *N3cr1* promoter, which codes for the anti-nociceptive glucocorticoid receptor, and decreased DNA methylation at the pro-nociceptive *Crh* promoter[126]. In the same rat stress model, stress was associated with upregulation of DNA (cytosine-5)-methyltransferase 1-associated methylation of the *Cnr1* (encoding CB_1) promoter and downregulation of glucocorticoid receptor-mediated expression of *Cnr1* in lumbosacral dorsal root ganglia, which contain the cell bodies of the visceral primary afferent nerves that project to the colon but not those that project to somatic structures[127]. Chronic stress concurrently increased gene expression of the histone acetyltransferase EP300 and increased histone acetylation at the *Trpv1* promoter as well as expression of the algesic TRPV1 receptor in the colonic visceral primary afferents of rats. Inhibiting these effects prevented chronic stress-induced increases in visceral pain[127]. Thus, normal visceral sensitivity is regulated by endovanilloid-mediated pro-nociceptive and endocannabinoid-mediated anti-nociceptive signalling from the periphery and, when this homeostatic balance is disrupted by chronic stress, it leads to heightened visceral sensitivity[127].

Gut microbiota and the ECS

The gut microbiota has a pivotal role in gastrointestinal physiology as a key component of the gut–brain axis[128]. Furthermore, it is increasingly recognized as contributing to pathophysiological conditions of the gastrointestinal tract, including IBS and other gut–brain disorders[129,130]. Microbial–host interactions regulate almost every aspect of the digestive and defensive functions of the gut. Microbial mediators include short-chain fatty acids[131,132], single-stranded RNA[133], Toll-like receptor ligands[134–136] and aryl hydrocarbon receptor ligands[137]. Host signalling factors that regulate the composition, growth and gene expression of pathogenic and commensal gut microbes include defensins, secretory IgA and catecholamines (for example, noradrenaline)[138,139]. This section of the Review illustrates how the ECS serves as the nexus of host–microbial signalling.

The lipid mediators and receptors of the ECS serve as both integrators and homeostatic effectors of environmental alterations that affect the gut–brain axis. For example, the ECS in the gastrointestinal tract is directly involved in the regulation of energy balance by modulating neural signalling to the brain, altering behaviours, and regulating gut barrier function and metabolism in response to specific nutrients[140,141], diet[142–144], the state of satiety[145] and the metabolic state[69]. Similarly, in rats and mice, the ECS can regulate gut function and visceral sensitivity in response to stress[52,127,146–149]. Remarkably, the ECS is not only responsive to changes in the gut microbiota but also regulates the composition of the microbiota and the virulence of enteric pathogens. Moreover, microbial–ECS interactions have wide-ranging effects, including in the brain, in which they regulate behaviours observed in IBS[150].

Direct evidence that the microbiota regulates endocannabinoid signalling comes from studies using germ-free mice[151,152]. In these animals, CB_1 (increased) and GPR55 (reduced) gene expression was altered in the ileum and proximal colon and throughout the gastrointestinal tract, respectively. Likewise, in the colon, anandamide was increased, whereas in the jejunum, 2-AG was reduced and other lipid mediators of the ECS were altered along the length of the gut. In concert with these changes, there were similar alterations to the biosynthetic and degradative enzymes throughout the gut. A similar pattern of changes was seen in the brain, where sex-dependent changes were also observed in various components of the ECS[151,152]. A strength of these studies is that the researchers examined the reversibility of these changes by reintroducing a healthy population of mouse gut microbiota using the faecal microbiota transplant (FMT) technique[151,152]. Although not all changes in the ECS were reversible, most were, though it should be noted that the germ-free mice were only recolonized for 1 week, which might not have been sufficient time for a stable microbiota to have developed. As germ-free mice have an immature immune system and developmental alterations to the nervous system, studies in antibiotic-treated mice are often useful adjuncts[153]. The ECS has been evaluated in mice after antibiotic administration to deplete the gut microbiota[154–156]. Some reductions to anandamide levels[156] and elevations in CB_1 and CB_2 expression[154,155] were observed. However, as these studies did not utilize a recolonization group to assess the reversibility of the changes, it is unclear whether they are solely due to the loss of bacteria.

These studies raise the important question of 'do microbial changes to endocannabinoid signalling result in biologically relevant functional effects?' This certainly is the case in experimental mouse models. The probiotic *Lactobacillus acidophilus* upregulated CB_2 in intestinal epithelial cells. Treatment with this probiotic attenuated

visceral hypersensitivity assessed in vivo, an effect that was reversed by a selective CB_2 antagonist[157]. By contrast, Markey et al. used a model in which healthy mice were colonized with the commensal fungus *Candida albicans* for 48 h (REF.[158]). *Candida* colonization caused no changes to the caecal bacterial populations in the gut and no intestinal inflammation. However, there were marked increases in anxiety-like behaviour, accompanied by elevations in plasma corticosterone that were inversely correlated with forebrain anandamide levels. When they treated mice with the FAAH inhibitor URB597, corticosterone levels were reduced to control levels as was anxiety-like behaviour. Whether changes in the gut microbiota would similarly alter the endocannabinoid regulation of visceral sensitivity remains to be determined but, given that corticosterone regulates CB_1 expression in the dorsal root ganglia[127,146], it seems likely. The study from Markey et al. was the first report of microbial manipulation of the ECS that resulted in neuroendocrine changes contributing to anxiety-like behaviour, a very common comorbidity of IBS associated with abdominal pain.

Another common comorbidity of IBS is depression. Chevalier et al. demonstrated that they could transfer depression-like behaviours observed in chronic unpredictable stress in mice by FMT into naive, recipient germ-free mice or naive mice treated with antibiotics[159]. This model of depression is associated with reduced endocannabinoid signalling, reduced central levels of 2-AG and circulating levels of monoacylglycerols and diacylglycerols. Elevating 2-AG levels pharmacologically with the MAGL inhibitor JZL184 restored central endocannabinoid signalling and completely reversed the depressive-like behaviours in mice that had been given a microbiota transfer from a chronically stressed donor. The researchers also showed that they could reverse the abnormal behaviours and endocannabinoid signalling in the recipient mice with a probiotic, *Lactobacillus plantarum* Lp^{WJL} (REF.[159]). Whether these observations can be translated to patients remains to be determined. The overall composition and diversity of the gut microbiota, rather than specific taxonomic features, are probably involved in the regulation of the gut microbiota–endocannabinoid axis. In a study of 786 adult twins, Minichino et al. showed that increased serum or faecal PEA levels correlated with a reduced or more severe degree of anhedonia and amotivation, respectively, with reduced diversity of gut microbiota composition being the only significantly associated ($P < 0.03$) factor[160]. Nevertheless, microbial modulation of endocannabinoid signalling remains an attractive therapeutic target for future consideration.

The ECS is not only regulated by the gut microbiota but also reciprocally regulates the composition and function of the commensal and pathogenic bacteria in the gut. The phytocannabinoid CB_1 and CB_2 ligand THC, given chronically in the context of a high-fat, high-sugar diet, was found to normalize the increased *Firmicutes* to *Bacteroidetes* ratio and increased the abundance of *Akkermansia muciniphila* in mice fed this diet[161]. This observation was accompanied by beneficial effects on reduced weight gain and a reduction in body fat. Interestingly, blockade of CB_1 also altered the gut microbiota composition, which was associated with reduced weight gain in mice fed a high-fat diet[162]. In this case, 16S rRNA sequencing of mouse faecal samples revealed that the CB_1 antagonist rimonabant markedly increased the relative abundance of *A. muciniphila* and decreased *Lanchnospiraceae* and *Erysipelotrichaceae* in the gut[162]. Changes such as this in the composition of the mouse gut microbiota might be due to alterations in regulatory pathways of lipid metabolism in the gut, leading to alterations in the metabolomic constituents of the gut luminal milieu[163]. Changes in the composition of the gut microbiota are not benign and, as noted earlier, can lead to changes in the host that not only include an altered metabolism[161,162] but can also affect the brain. The latter was demonstrated by Al-Ghezi et al. using a mouse model of multiple sclerosis (experimental autoimmune encephalomyelitis (EAE))[164]. Treating EAE mice with a combination of THC and cannabidiol reduced the inflammatory immune response and clinical symptoms of the disease (weakness and paralysis). The researchers then used the treated mice as FMT donors for separate groups of antibiotic-treated mice who were then given EAE. The mice that received the FMT from animals treated with cannabinoids also had a dramatically reduced extent of disease, illustrating that the microbiota is sufficient to confer cannabinoid-mediated effects[162].

The role of endocannabinoids in regulating the gut microbiota is still an emerging area of investigation. However, Ellerman et al. found that 2-AG can protect mice from enteric bacterial infection by inhibiting the quorum-sensing *Escherichia coli* regulator C pathogen virulence mechanism[165]. To show this, the investigators used *Mgll*-knockout mice, which had elevated 2-AG levels, and demonstrated that these mice had a reduced burden of *Citrobacter rodentium* infection, a mouse model of enteropathogenic *E. coli* infection. The investigators also showed that the pharmacological blockade of MAGL with JZL184 had a similar effect. Interestingly, the effects of 2-AG were not mediated by changes in the commensal microbiota as they could not be transferred by an FMT[165]. However, it should be noted that, in *Mgll*-knockout mice, an altered gut microbiota conferred resistance to diet-induced obesity[166]. These observations on the effects of 2-AG add to an earlier work demonstrating a role for anandamide at the epithelial interface. Szabady et al. showed that anandamide but not 2-AG exported from the mouse intestinal epithelium via the apically restricted multidrug resistance transporter P-glycoprotein regulated luminal neutrophil infiltration and maintained intestinal homeostasis[81]. Together with observations on the endocannabinoid regulation of epithelial tight junctions (reviewed in Cani et al.[167]), these data reveal the powerful role that the ECS serves between the gut microbiota and host mechanisms that are directly or indirectly involved in the control of gut function and visceral sensitivity.

Diet and the ECS

The gut microbiota is markedly influenced by diet, which considerably affects the symptoms of IBS, including visceral pain[168]. The mechanisms of dietary modulation of

Box 2 | **Contributing mechanisms to visceral nociception and visceral pain**

- Neuronal processing along the gut–brain axis occurs via extrinsic sensory afferent neurons that project from the gut to the spinal cord with ascending projections to the brain, which give rise to perceivable sensations.
- Extrinsic gut sensory afferents express pro-nociceptive channels and receptors that can be activated in response to various mediators, leading to acute neuronal hyperexcitability and visceral hypersensitivity.
- This processing can be altered by gut inflammation or infection, altered epithelial permeability, microbiota composition, immune function and host response to perceived pathogens as well as acute and chronic stress.
- Visceral pain can also be modulated centrally at various levels of the neuraxis including the spinal cord, brainstem, midbrain and higher brain centres. Alterations in the brain networks that regulate emotional arousal, central executive function, salience, sensorimotor function and central autonomic function all contribute to the symptoms of irritable bowel syndrome. Pain perception is also modulated by the descending pain modulatory system.

pain are complex and include the regulation of mast cell degranulation[169]. The ECS of the gastrointestinal tract is also regulated by the composition of the diet, notably by fat[170,171]. In mice, high-fat diets increased small intestinal anandamide and/or 2-AG levels[172,173], whereas PEA (and other *N*-acylethanolamine) levels were reduced in a dose-dependent manner in the small intestine, probably owing to changes in biosynthesis[174]. These fat-induced changes in the levels of endocannabinoids occurred rapidly[141]. Changes in diet also regulated the ECS in the mouse brain[175], altering mood and levels of anxiety[176]. A study published in 2021 investigated the effects of a ketogenic diet on CB_1 and CB_2 receptor expression in the gastrointestinal tract in a maternal separation rat model of IBS[177]. This study revealed that a low-carbohydrate, high-fat ketogenic diet upregulated CB_1 and CB_2 gene and protein expression compared with a standard diet, accompanied by a reversal of damage to the crypts seen in this model of early-life stress. These changes point to an additional mechanism whereby the ECS is involved in changes linked to improved intestinal homeostasis via its modulation by diet. This study did not assess the gut microbiota, which would undoubtedly also be involved in the actions of this diet. Untangling the complex web of interactions between diet, the gut microbiota, and the ECS of the brain and gut will be challenging but doing so might be fruitful as it could reveal additional avenues to alleviate the symptoms of IBS. It should also be noted that non-psychoactive phytocannabinoids, including CBD, can be used in food (as nutraceuticals), yet there is currently little evidence for any beneficial effects in IBS[178,179].

The challenge of pain in IBS

IBS is the most extensively studied DGBI. Epidemiological studies show a globally high IBS prevalence, which depends on the population studied and the IBS definition used[2,3,180]. For example, in a global epidemiology study, which used the stringent Rome IV criteria, the prevalence of IBS in adults was on average 4.1%[2,3], but higher rates of 10.1% and up to 17.6% prevalence are obtained when using the Rome III or simple self-report definitions, respectively[2,180]. Owing to its high prevalence, the lack of diagnostic biomarkers and the incomplete efficacy of available treatments, IBS leads to increased health, economic and societal burden[180,181].

Pathophysiology of IBS. The pathophysiology of IBS is complex and entails multiple factors along the gut–brain–microbiota axis. According to Rome IV criteria, patients with IBS have altered stool frequency or form associated with abdominal pain[1]. Based on the prevalent bowel habits, patients can be subcategorized into constipation-predominant IBS (IBS-C), diarrhoea-predominant IBS (IBS-D) or IBS with a mixture of stool patterns (IBS-M)[1]. Conventionally, gastrointestinal dysmotility, psychological factors and visceral hypersensitivity have been evoked as major mechanisms involved in generating symptoms[1,5,182,183]. However, low-grade inflammatory changes of the intestinal mucosa, increased mucosal permeability, bile acid malabsorption, alteration in gut microbiota composition and sensitivity to certain food components have also been proposed[5,89,129]. It is still unknown which of these factors can elicit symptoms or intensify IBS, mainly because the symptoms show great interindividual and intraindividual variability and likely reflect the interactions among these different mechanisms[184].

Visceral hypersensitivity, resulting in an enhanced perception of luminal stimuli, is considered the cornerstone of the pathophysiology of several DGBIs, including IBS[185] (BOX 2). Indeed, increased sensitivity to rectal distension might reach a prevalence as high as 94% in patients with IBS in barostat studies, leading some authors to postulate an altered rectal perception as a 'biomarker' of the disorder[186]. Although subsequent studies scaled back on the prevalence of hypersensitivity in IBS, this is still recognized as one of the main pathogenetic factors that trigger abdominal pain and discomfort[187]. Visceral hypersensitivity is a multifactorial process itself and can occur at any level along the gut–brain–microbiota axis[188,189]. It might arise from microbiota alterations, subtle mucosal inflammation, sensitization of peripheral nerve endings, or altered spinal and/or central processing of visceral stimuli[122,123,187,190]. Interestingly, compared with 22 healthy controls, a 3.5-fold increase in TRPV1-immunoreactive nerve fibre density was described in colonic biopsy samples from 23 patients with IBS and was directly linked to the severity of abdominal pain[191]. Low-grade mucosal inflammation can also have a relevant role in sensitizing primary afferent neurons. Barbara et al. found an increased density of mast cells close to sensory neurons, suggesting that mediators released from resident immune cells could participate in determining visceral hyperalgesia[97]. Furthermore, bile acid malabsorption, leading to accelerated colonic transit and hypersensitivity, was also observed in a subset of patients with IBS-D (38%, $n = 53$ of 139) despite its lack of correlation with abdominal pain[192].

Altered gut microbiota has also been proposed as one of the possible causes of IBS, especially in patients with post-infectious IBS[129,190,193]. The change of intestinal microbiota due to acute gastroenteritis, such as a 12-fold increase in members of the Bacteroidetes phylum, or a

course of antibiotic therapy, was associated with an increased risk of IBS, with dysbiosis being acknowledged by the Rome Foundation Working Team as a plausible contributing factor to the disorder[130,194,195]. Although specific bacteria have been observed in patients with IBS, the data are still conflicting, and it remains to be determined whether these microbes (for example, *Enterobacteriaceae*, *Lactobacillaceae* and *Bacteroides*, which are increased in patients with IBS) are a product or cause of IBS[129]. However, studies evaluating the contribution of most proposed pathophysiological factors are inconsistent and the aetiology is often unrelated to specific gut symptoms. For example, some IBS studies demonstrated gut microinflammation, whereas others could not confirm this despite similar gastrointestinal symptoms. These discrepancies, amongst others, strongly suggest the existence of IBS subpopulations, which, despite being similar in gut symptoms, can be defined and distinguished by their pathophysiology and in-depth characterization[129,190].

Sex differences in chronic visceral pain. Most patients with chronic visceral pain and with IBS are women. Evidence suggests that women with IBS tend to have lower pain thresholds and less tolerance to nociceptive stimuli, whereas no sex differences in visceral sensitivity are observed in healthy controls[196]. Furthermore, brain imaging studies indicate sex-related differences in regional brain responses to provocative stimuli in patients with IBS[197] and functional MRI studies in rats showed that noxious colonic distension enhanced the activation of the insula, anterior cingulate, amygdala, parabrachial nuclei and cerebellum in a sex-dependent manner[198]. Although growing evidence has shown a female predominance in the prevalence of chronic visceral pain, the molecular mechanisms underlying pain vulnerability in women are poorly understood. A most widely accepted explanation for sex differences in visceral pain sensitivity is the cyclical changes in ovarian hormones reviewed by Jiang et al.[199]. In many pain disorders, including IBS, women reported symptom exacerbation and increased rectal sensitivity when oestrogen and progesterone levels were high[199,200]. Moreover, female patients with IBS frequently reported a history of early-life adversity, including general trauma, physical punishments, and emotional and sexual abuse[201]. The activational effect of oestradiol is a key modulator of visceral sensitivity in adulthood following exposure to unpredictable early-life adversity[202]. The role of androgens, such as testosterone, in IBS is less well understood; however, a mouse study showed that reduced androgen levels were linked to the diagnosis and severity of IBS through gonadal androgen signalling to the ENS[203]. The importance of the ECS in sex-related differences in visceral pain reporting in IBS has received very little attention; future research is required to determine whether modifications in the ECS between men and women could explain the female predominance of IBS or serve in the identification of potential biomarkers that could be useful in the diagnosis and therapeutic response to new drug approaches directed at the ECS. However, interactions between the ECS and sex hormones as well as sex differences in cannabinoid metabolism and receptor expression in the central pain matrix likely underly sex differences in cannabinoid antinociception, at least in somatic pain reporting. The reader is referred to an excellent review on the topic by Blanton et al.[204].

Current management of visceral pain in IBS

Within the symptomatic treatment of IBS, drugs specifically targeting visceral pain or pain processing pathways in the gut–brain axis and central regions involved in pain memory are lacking and remain a major unmet need. Supplementary Box 3 provides an overview of IBS drugs that target motility and their effect on IBS-related pain. Opioids appear less suitable owing to poor controllability, the possibility of central adverse effects and, for eluxadoline, serious adverse effects like spasms of the sphincter of Oddi and the development of pancreatitis resulting in contraindications that need to be noted[205]. Neuromodulators targeting visceral pain are recommended though some of the evidence is poor as recommendations are frequently based on extrapolations from other indications with similarities in pain pathophysiology like fibromyalgia or back pain[6]. Neuromodulators are presently underused owing to patient unwillingness, the lack of confidence by the prescriber or fear of side effects in a disease frequently considered not severe.

Over the past two decades, several classes of pharmacological agents targeting pain in IBS, aimed at improving visceral hypersensitivity, have been developed and studied. These studies comprise mechanistic and clinical investigations involving targets such as κ-opioid receptors[206–210], tachykinin receptors[211–219], α2 and β3 adrenoceptors[220], and 5-HT_{1A} receptors[221]; the studies and their outcomes are summarized in TABLE 1. The fact that none of these agents has been successfully further developed for IBS therapy illustrates the challenges related to targeting pain and visceral hypersensitivity in IBS (Supplementary Box 1), which includes the substantial placebo effect encountered in IBS[121].

In the first decade of this century, basic research provided a broad scientific basis for targeting the ECS in IBS (summarized in REF.[14]) and clinical endocannabinoid deficiency was proposed as a pathophysiological mechanism underlying many of the IBS manifestations (discussed later), partly based on the anecdotal use of phytocannabinoids for IBS symptom relief[222]. However, further development of these pathways required non-psychoactive drugs to more selectively target the ECS in the gastrointestinal tract. Developing peripherally restricted molecules enabled research to enter the clinical trial phase with an agent targeting the gastrointestinal ECS[223].

Therapeutic use of cannabinoids. Cannabinoid agonists are used on-label or off-label as anti-emetics or appetite stimulants or in treating neuropathic pain and spasticity in multiple sclerosis, chronic non-cancer pain, within palliative cancer care or for intractable childhood epilepsy (BOX 3).

Various putative health effects have been attributed to the recreational and therapeutic use of cannabis and related substances[224]. Over the past years, medical

Table 1 | **Overview of drugs developed to target pain and visceral hypersensitivity in IBS and the outcomes of clinical studies**

Agent	Pharmacological action	Study	Outcome
Fedotozine	κ-Opioid receptor agonist	Dapoigny et al. 1995 (REF.[206])	In a 6-week, placebo-controlled, phase III study (evaluating 3.5, 15 and 30 mg three times per day) in 238 patients with IBS, fedotozine dose-dependently improved daily pain and bloating scores
		Delvaux et al. 1999 (REF.[207])	Fedotozine 100 mg acutely administered IV decreased sensitivity to isobaric colonic distention in a controlled crossover trial in 14 patients with IBS
Asimadoline	κ-Opioid receptor agonist	Mangel et al. 2008 (REF.[208])	In a 12-week, placebo-controlled, phase II study (evaluating 0.15, 0.5 and 1 mg two times per day) in 596 patients with IBS, the primary end point of adequate relief was not met; in an IBS-D subgroup with at least moderate pain, improvements in pain and overall symptoms were observed
		Szarka et al. 2007 (REF.[209])	In a 4-week, placebo-controlled study of 100 women with IBS and on-demand asimadoline up to 1 mg four times per day taken upon pain, the primary end point of control of pain in the first 2 h was not met; post-hoc analysis suggested potential efficacy on pain in the IBS-mixed subgroup
		Delvaux et al. 2004 (REF.[210])	Asimadoline 0.5 mg acutely orally administered decreased pain intensity area under the curve during isobaric colonic distention in a controlled crossover trial in 20 patients with IBS
Solabegron	β3 Receptor agonist	Kelleher et al. 2008 (REF.[220])	In a 6-week, placebo-controlled, crossover study evaluating solabegron 100 mg two times per day in 99 patients with IBS, the evaluation of the first, in parallel, study phase showed a tendency for solabegron to provide more adequate relief; benefit in adequate relief and in pain control was shown in women with IBS-mixed
Talnetant	Neurokinin 3 receptor antagonist	Dukes et al. 2008 (REF.[211])	Two dose-finding, phase II, placebo-controlled studies (5–100 mg four times per day and 100–400 mg two times per day, respectively) in a total of 1,350 patients with IBS failed to demonstrate benefit in IBS in terms of adequate relief of pain or overall symptoms
		Houghton et al. 2007 (REF.[212])	Talnetant 25 or 100 mg or placebo, administered for 2 weeks to 102 healthy individuals, did not alter rectal compliance or sensitivity ratings during isobaric balloon distention
Nepadutant	Neurokinin 2 receptor antagonist	Delvaux 2002 (REF.[213])	Increase of rectal compliance after administration of glycerol but no effect on rectal sensory thresholds in healthy controls
		Lecci 2008 (REF.[214])	Prevention of reduction in bowel movements during the first day of observation in a clinical trial unit in healthy controls
Ibodutant	Neurokinin 2 receptor antagonist	Clinicaltrials.gov NCT02107196 & NCT02120027	A 12-week, placebo-controlled, phase III study of ibodutant 10 mg four times per day in 535 women with IBS-D failed to meet the primary end points of abdominal pain and stool consistency response; the second study with 558 participants was prematurely interrupted because of this negative result
		Tack et al. 2017 (REF.[215])	In a phase II study in IBS-D, ibodutant 10 mg (a selective NK2 receptor antagonist) was superior to placebo in women but not in men with IBS-D
Ezlopitant	Neurokinin 1 receptor antagonist	Lee et al. 2000 (REF.[216])	Ezlopitant decreased rectosigmoid sensitivity in 14 patients with IBS
AV608	Neurokinin 1 receptor antagonist	Tillisch et al. 2012 (REF.[217])	AV608 during 3 weeks in a placebo-controlled, crossover study reduced anxiety, negative affect, and pain ratings and brain activity during both noxious and innocuous rectal distension in 11 patients with IBS
Aprepitant	Neurokinin 1 receptor antagonist	Akyuz et al. 2007 (REF.[218])	Acutely administered aprepitant 80 or 125 mg did not alter pressure thresholds to rectal balloon distention but reduced rectal compliance and volume thresholds compared with placebo in 16 healthy participants
DNK-333	Neurokinin 1 and neurokinin 2 receptor antagonist	Zakko et al. 2011 (REF.[219])	Two placebo-controlled, clinical trials evaluating 25 and 100 mg two times per day for 2 or 4 weeks in 315 women with IBS-D failed to show adequate relief over placebo; combining data from both trials showed an efficacy signal in providing adequate relief for the 25 mg dose
Robalzotan	5-HT_{1A} receptor antagonist	Drossman et al. 2008 (REF.[221])	In a 12-week, placebo-controlled, phase II study (evaluating 5 and 20 mg two times per day) in 402 patients with IBS, the primary end point of adequate relief was not met; the active groups had a higher rate of central nervous system-related adverse events
AGN203818	α2 Receptor agonist	Clinicaltrials.gov NCT00441766	Two dose-finding, phase II, placebo-controlled study phases of 4 or 12 weeks duration (3–60 mg four times per day and 60–160 mg two times per day, respectively) in a total of 533 patients with IBS failed to demonstrate benefit in IBS in terms of pain scores and global impression of change

IBS, irritable bowel syndrome; IBS-D, diarrhoea-predominant irritable bowel syndrome; IV, intravenous.

cannabis has been made available to patients by physician prescription in several countries and many states in the USA[225]. There are multiple medical conditions for which therapeutic effects have been claimed with medical cannabis, including chronic pain and IBS[224,226]. In a 2017 review by the National Academies of Sciences, it was concluded that there is insufficient evidence to support or refute the conclusion that cannabis might be an effective treatment for the symptoms of IBS[224]. Based on observations in hospitalized patients, it was suggested that IBS is associated with increased cannabinoid use in the general population, possibly with

Box 3 | **FDA and/or EMA-approved cannabinoid drugs and their indications**[a]

Drug	Indication
Cannabidiol (USA: Epidiolex, Jazz Pharmaceuticals; EU: Epidyolex, GW Pharma)	CBD does not act largely as a CB_1 and CB_2 receptor agonist but at additional receptors, including serotonin 1A ($5\text{-}HT_{1A}$) receptors and opioid receptors, and several G protein-coupled receptors can additionally be activated[266,267]. The differentiated pharmacology still needs to be fully characterized. Being a negative allosteric modulator at CB_1 receptors, CBD inhibits endocannabinoid signalling[268]. CBD received approval for medical use in 2017 (EMA) and 2018 (FDA). Indications approved are the combined use with clobazam in treating childhood seizures in Lennox–Gastaut syndrome or Dravet syndrome in patients 2 years and older. The plant-derived CBD solution is given orally twice daily, ranging from 5–20 mg per kg daily.
Dronabinol (USA: Marinol (capsule), Alkem Labs, Syndros (solution), Benuvia Therapeutics)	(-)-Trans-Δ^9-tetrahydrocannabinol is extracted from plants and can be considered a pure THC product. Dronabinol acts as a partial agonist at CB_1 and with lower affinity as a full agonist at CB_2 (REF.[15]). Dronabinol was approved for medical use in 2016 (FDA) for the treatment of anorexia associated with weight loss in patients with AIDS, and nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional anti-emetic treatments. In Europe, an additional approved indication is the treatment of central and peripheral neuropathic pain. Oral capsules or an oral solution is dosed once or twice per day at doses ranging from 2.5 to 20 mg per day.
Nabilone (Cesamet, Bausch Health; Canemes)	Nabilone ((±)-trans-3-(l,l-dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-l-hydroxy-6-6-dimethyl-9H-dibenzo[b,d] pyran-9-one) is a synthetic cannabinoid with structural similarities to THC. Nabilone can be regarded as a THC analogue, a pure THC product with partial agonist activity at CB_1 and lower affinity as a full agonist at CB_2 (REF.[15]). From a clinical standpoint, nabilone is an anti-emetic used in capsule form as a second-line agent for treating nausea and vomiting associated with chemotherapy in patients with cancer. Capsulated nabilone is taken in doses ranging from 0.5 to 8 mg per day[269]. An application for approval for the treatment of amyotrophic lateral sclerosis to EMA in 2011 was not approved.
Nabiximols (EMA: Sativex, GW Pharma)	Nabiximols is an extract from leaves and flowers of the hemp plant *Cannabis sativa* with standardized, approximately equal levels of THC and CBD. Nabiximols is an oromucosal spray approved by EMA in 2011 as a second-line agent to improve symptoms in patients with moderate to severe spasticity due to multiple sclerosis. In the USA, nabiximols is an investigational drug[270].
Rimonabant (CB_1 antagonist; USA: Zimulti; EU: Acomplia; Sanofi-Aventis)	Rimonabant is a CB_1 antagonist, approved in 2006 (FDA, EMA) for the treatment of obesity in accompaniment to diet therapy and exercise. An additional application for smoking cessation was not approved. In 2007, rimonabant did not receive approval in patients with obesity and associated risk factors. Owing to serious psychiatric adverse effects like anxiety, panic attacks, depression, insomnia and suicidality, rimonabant was withdrawn from the North American market in 2007 and, in 2008, Sanofi-Aventis announced the closure of all clinical trials with rimonabant, and the European approval was suspended. Rimonabant was taken in tablets of 20 mg, 1 per day, with breakfast. Given the multiple central sites of CB_1 expression and the adverse effect profile of rimonabant, no further CB_1 antagonist for human use is presently being developed[271].
Investigational and off-label uses[b]	
CBDV	CBDV is a non-psychoactive cannabinoid found in *C. sativa* and is a homologue of CBD. CBDV in a phase II trial investigating ad-on treatment effects of CDBV on focal seizures was generally well tolerated but not effective in reducing seizure frequency[272].
Pure THC formulation (Namisol)	Namisol is a pure THC formulation with >98% THC content. THC acts as a partial agonist at CB_1 and CB_2. Namisol is delivered in an oral tablet and is under investigation in dementia[273].
Olorinab	Olorinab is a highly selective, peripherally restricted, full agonist at CB_2. In preclinical investigations, olorinab proved to be a visceral analgesic. In patients with quiescent Crohn's disease, relief of abdominal pain was reported[274].
Palmitoylethanolamide	Palmitoylethanolamide is an endogenous cannabinoid receptor agonist acting as a PPARα ligand. In preclinical trials, functions related to chronic pain and inflammation were established. In patients with IBS and 12 healthy controls, palmitoylethanolamide effectively reduced the severity of abdominal pain and discomfort[77]. A 12-week, phase II trial in patients with Tourette syndrome found a THC combination with palmitoylethanolamide effective in improving the Yale Global Tic Severity Scale total tic score[275].
Cannabinoid delivery	Current research also includes attempts to increase the bioavailability of cannabinoids to allow peripheral restriction, to use other chemical compounds contained in *C. sativa*, or to develop methods of topical application like the development of emulsions, hydrogels and other delivery systems like bioactive encapsulates or transdermal application[276–278].

CB_1, cannabinoid receptor 1; CBDV, cannabidivarin; CBD, cannabidiol; IBS, irritable bowel syndrome; PPARα, peroxisome proliferator-activated receptor-α; THC, Δ^9-tetrahydrocannabinol. [a]In December 2020, the UN Commission on Narcotic Drugs removed cannabis from Schedule IV of the Single Convention on Narcotic Drugs, recognizing its medicinal value[268]. Over the past decade, five cannabinoid drugs, including receptor agonists and receptor antagonists, have been approved for medical use in various indications by the FDA and EMA. Approval was granted, although the strength of evidence for the various indications has to be considered overall weak or moderate at best. [b]Available cannabinoids are used off-label for the treatment of anxiety and depression mitigation, appetite stimulation, sleep disorders, and various forms of pain, for example, associated with degenerative or neurological disorders and inflammatory bowel disease. Quantitative data on off-label use are not available.

therapeutic intentions[225,227]. However, although a review published in 2020 stated that the lack of controlled trials with cannabinoid agents in IBS precludes making any conclusions on their efficacy in IBS[228], a study published in 2022 on rates of readmission found that cannabis use was associated with reduced 30-day hospital readmission rates for all causes whereas no statistically significant differences were observed in 30-day readmission rates for IBS-specific causes[229]. A randomized, double-blinded, placebo-controlled crossover trial with cannabidiol-containing chewing gum failed to show a beneficial effect on pain in IBS[179].

Given its role in controlling gastrointestinal motility, there is evidence that the ECS is differentially involved in the IBS subtypes. In 40 patients with IBS-D and IBS-M, dronabinol, a CB_1 and CB_2 agonist, increased colonic compliance and decreased colonic motility without affecting sensation[49]. By contrast, the CB_2 agonist olorinab showed the highest potential for benefit in 136 patients with IBS-C[223]. Interestingly, the patterns of IBS in patients might correlate with serum levels of acylethanolamides. Compared with healthy controls, 7 patients with IBS-D showed lower levels of OEA and PEA, likely reflecting genetic alterations in the *CNR1* and *FAAH* genes modulating endocannabinoid metabolism[230]. Conversely, 7 patients with IBS-C had higher levels of OEA and reduced levels of *FAAH* mRNA in intestinal tissues[230]. Though not confirmed in clinical trials, these findings support the involvement of fatty acid amides in altered motility and nociception symptoms and their pathophysiological involvement in patients with IBS. Sex is another likely important determinant of altered ECS as discussed earlier. Most of the available treatment trials with endocannabinoid ligands in IBS have not targeted subgroups based on sex, stool subtype or genotypes.

Cannabinoids in visceral pain. Given the several lines of research linking the ECS to IBS pathophysiology, the concept of 'clinical endocannabinoid deficiency' has emerged[226]. This theory suggests that, under certain conditions, either congenital or acquired, the ECS tone becomes deficient, resulting in several functional disorders featured by pain and hyperalgesia (migraine, fibromyalgia and IBS). Since its first formulation in 2001, this still unverified hypothesis postulates a decreased ECS tone based on the anecdotal evidence that treatment with exogenous cannabinoids frequently provides symptomatic relief[226]. Nevertheless, very little clinical trial data support this notion to date. A randomized controlled trial in 40 healthy participants evaluated the effect of acute exposure of 7.5 mg of dronabinol or placebo on colonic motility and found a significant ($P = 0.045$) increase in colonic compliance, indicating that THC enables colonic relaxation and reduces postprandial colonic contractility[231]. Interestingly, although dronabinol did not affect thresholds for pain sensation, the authors found an increase in sensory rating for pain during random distensions at all tested pressures and hypothesized a role of central sensitization following acute exposure[231]. A further small-sized, placebo-controlled, randomized controlled trial ($n = 10$ patients with IBS and $n = 12$ healthy controls) explored the effects of different doses of dronabinol (5 mg and 10 mg) on colonic sensitivity as assessed by barostat and found that it failed to affect rectal sensitivity to distension, with all participants reporting central adverse events at the highest tested doses of THC[232]. Another trial investigated the effects of a single dose of placebo or 2.5 or 5.0 mg of dronabinol in 75 patients with IBS ($n = 27$, placebo; $n = 24$ with each dose of dronabinol) and examined the effect of IBS subtype and specific genetic variants in *FAAH*, *MGLL* and *CNR1* on colonic motility and sensitivity[49]. It was shown that dronabinol increased colonic compliance ($P = 0.058$) and decreased colonic motility ($P = 0.05$)[49]. These effects, however, were dependent on the IBS subtype, with the greatest effect displayed in IBS-D and patients with IBS with alternating diarrhoea and constipation. As underlined by the authors, an interesting aspect to consider is that genetic variants in *FAAH* and *CNR1* could influence the effects of dronabinol on colonic motility[49]. A survey study involving 721 participants found that regular cannabis users self-medicate themselves for various psychiatric and somatic conditions, including IBS[233]. This number is expected to continuously grow owing to the recent changes in regulation in the USA market. However, a retrospective cross-sectional study computing 31,272 IBS hospitalizations in the USA underlined a 40.7% higher risk for IBS-related hospitalizations in patients with cannabis use disorder[227]. Despite the promising preclinical evidence, the efficacy and safety of cannabinoids and related compounds still need to be assessed in IBS patients. A confounding issue in determining efficacy is the plasticity of the endocannabinoid system, with opposite effects for acute and long-term exposure due to receptor downregulation and desensitization or internalization[234]. In fact, although acute exogenous cannabinoids might offer relief from pain, chronic cannabis exposure could show paradoxical effects that might perpetuate or worsen the underlying pathophysiological mechanisms. An example of this paradoxical effect is cannabinoid hyperemesis syndrome, in which heavy cannabis use results in intractable emesis, dehydration and electrolyte abnormalities, despite the well-recognized anti-emetic and orexigenic properties of cannabis[235]. Although the pathophysiological mechanisms of the disorder are still obscure, the paradoxical effects of chronic cannabis use could shed light on the conflicting results reported in the literature regarding IBS and cannabis use disorder.

There are very few controlled studies with cannabinoids in IBS and other DGBIs. A randomized, double-blind, placebo-controlled crossover trial, published in 2021, with on-demand cannabidiol-containing chewing gum in 32 patients with IBS failed to show a beneficial effect on pain[179]. In a controlled trial in 54 patients with IBS, a dietary supplement containing PEA and polydatin significantly ($P < 0.05$) improved abdominal pain[77].

Olorinab is a peripherally restricted, highly selective CB_2 agonist. In a study in 14 patients with quiescent Crohn's disease, olorinab improved symptoms of abdominal pain[236]. In a randomized, double-blind, placebo-controlled, phase IIb study of olorinab 10,

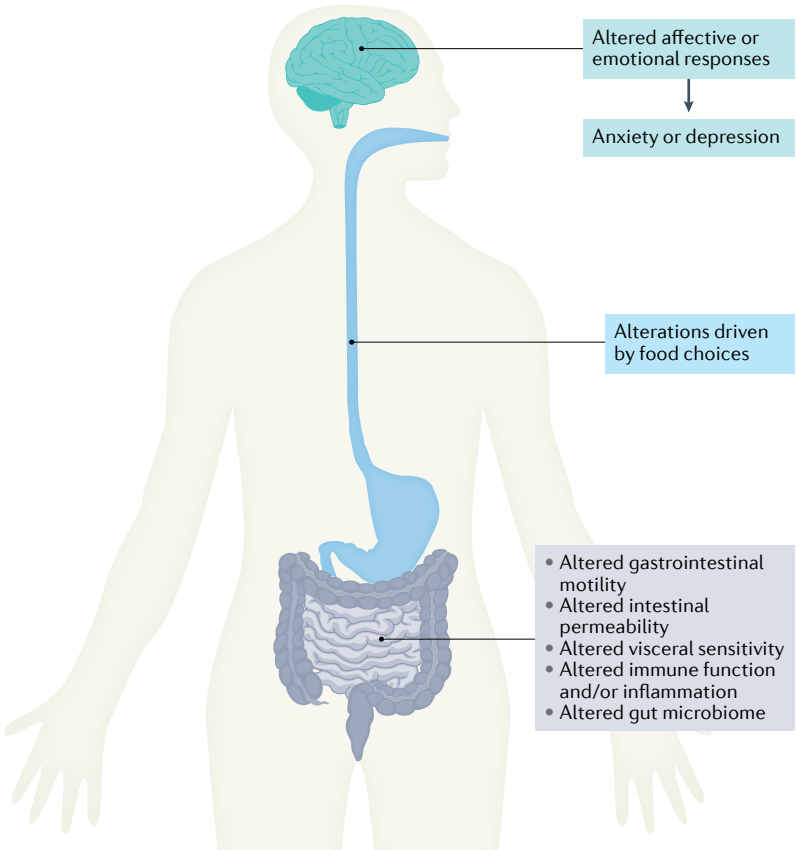

Fig. 4 | **Actions of the endocannabinoid system as they relate to the gastrointestinal tract, motility, gut microbiota, immune function and visceral pain.** The endocannabinoid system acts as a physiological regulator of various processes that affects all of the clinical features of disorders of gut–brain interaction. These processes include critical gut functions within the gut, such as gastrointestinal motility, intestinal permeability, visceral sensitivity, microbial composition and immune responses, as well as extra-intestinal processes such as the hosts' affective or emotional responses, anxiety and depression.

25 or 50 mg three times per day in 273 patients with IBS, no significant difference in the primary (change in the weekly average abdominal pain scale from baseline to week 12) or secondary (proportion of patients reaching at least a 30% improvement in weekly average abdominal pain scale) end point were obtained[223]. However, a statistically significant ($P=0.014$) improvement in abdominal pain scores was obtained with the 50 mg dose over placebo in the subgroup of patients with greater severity of abdominal pain at baseline (on average >6.5 of 10). Furthermore, response rates to olorinab were better in IBS-C than in IBS-D[223].

Apart from the non-selective and selective CB_1 and CB_2 ligands, several FAAH and MAGL inhibitors are under development for the treatment of chronic painful conditions and various psychiatric or CNS disorders[19]. Especially in DGBIs, where there is indirect evidence of decreased endocannabinoid signalling, inhibitors of the endocannabinoid degrading enzymes might provide an attractive approach with potentially better safety[237]. To date, there are no reports on the use of those inhibitors in IBS or other DGBIs but one small pilot study of four patients evaluating the effect of the MAGL inhibitor ABX-1431 on gastric accommodation in functional dyspepsia showed no significant effects[238].

ECS as a potential therapeutic target. In view of the preclinical research showing involvement of the ECS or beneficial effects of cannabinoids and of in vivo studies focusing on altered gastrointestinal motility, barrier function, low-grade mucosal inflammation, and gut–brain signalling, the ECS is a potential target for the treatment of IBS and other DGBIs[12,36,57–59] (FIG. 4). This suggestion is supported by early observations in humans of cannabinoid effects related to gastrointestinal motility control and gut peptide signalling[58–61,237,239]. The potential approaches to target the ECS in IBS and other DGBIs include using non-selective cannabinoids and selective endocannabinoid receptor ligands and inhibiting the degrading enzymes FAAH and MAGL to raise the levels of endocannabinoids. Owing to the central adverse effects of cannabinoid ligands, peripherally restricted agonists and antagonists seem preferable. Although cannabis can cause cannabinoid hyperemesis syndrome[3,235], cannabinoids have been used for the management of nausea and vomiting, including in patients receiving cancer chemotherapy[240,241]. Taken together, several controlled trials show beneficial effects of cannabinoids for chemotherapy-induced nausea and vomiting but the quality of the trials is often suboptimal[240,242]. Controlled studies are lacking in chronic nausea and vomiting or in gastroparesis, in which nausea is a key symptom and cannabinoid use is high, with patients reporting symptomatic benefit[243,244]. Sleep disturbances are also common in IBS[245] and poor sleep is usually associated with augmented pain sensitivity and central sensitization in patients with IBS[246,247]. The ECS has a role in regulating the sleep–wake cycle[248,249] and cannabis and cannabinoids alter sleep architecture, improving sleep acutely but with variable and often negative effects with chronic use[250,251]. Thus, ECS-mediated mechanisms related to sleep improvement might increase the quality of life in people with IBS, but this remains to be determined until more clinical evidence is accrued.

Genomic and pharmacogenomic considerations. A genome-wide association study reported six genetic susceptibility loci for IBS potentially involved in underlying pathophysiological mechanisms[252]. *NCAM1*, *CADM2* and *PHF2* (also known as *FAM120A*) were associated with mood and anxiety disorders prevalent in patients with IBS and in which ECS mechanisms have a substantial regulatory role[20]. Additionally, there is a strong genome-wide correlation between risk of IBS and risk of anxiety, neuroticism and depression, along with the most severe associated traits with IBS being sleep disorders, tiredness, excessive worrying, trouble relaxing and pain conditions, suggesting central sensitization, strengthens findings from earlier epidemiological research[252]. Furthermore, genes implicated in this study included those regulating neural cell adhesion molecules, which have been identified as important in the actions of the ECS, supporting a potential role of the ECS in IBS at a genomic level[253].

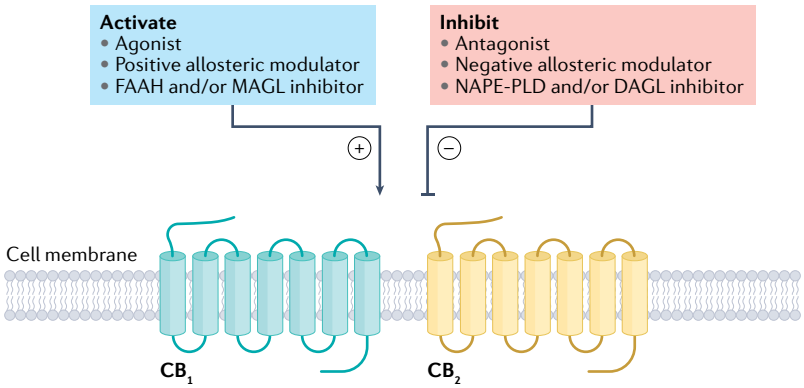

Fig. 5 | **Potential therapeutic intervention strategies to modulate the function of CB_1 and CB_2.** Potential therapeutic approaches to modulate cannabinoid receptor signalling are depicted. Drugs are currently under development that take advantage of these approaches and can offer opportunities to selectively target the endocannabinoid system of the gastrointestinal tract for the treatment of irritable bowel syndrome and other disorders of gut–brain interaction. CB_1, cannabinoid receptor 1.

Genetic variability in the ECS is well documented[122,254]. Polymorphisms in the cannabinoid receptors and metabolic enzymes of the ECS promote susceptibility to psychiatric disorders and other conditions and could, therefore, substantially alter the response of individuals to cannabinoid therapies and/or modulate the physiology of the ECS in patients with IBS and with stress-induced symptomatology that is comorbid with depression and anxiety[20]. Single-nucleotide polymorphisms in *CNR1* (rs806378), *FAAH* (rs324420) and *MAGL* (MGLL rs4881) were investigated[49,59,255]. In general, only modest differences were observed in patients with IBS and with various ECS gene polymorphisms. In a randomized trial of the effects of the agonist dronabinol in 36 patients with IBS and diarrhoea, *CNR1* rs806378 was associated with a modest delay in colonic transit ($P = 0.13$) and, in a separate study, a more pronounced dronabinol-induced reduction in fasting proximal colon motility index in 35 patients with IBS and constipation was observed[59,255]. The *FAAH* polymorphism was associated with all forms of IBS and associated with some motility alterations but not with changes in rectal sensation[59]. When 75 patients with IBS (35 with IBS-C, 35 with IBS-D and 5 with IBS-M) were treated with dronabinol, some substantial changes in colonic motility were observed that varied with the colon region examined, the type of IBS and the genotype of polymorphism[49]. No changes were observed with the *MAGL* polymorphism in patients with IBS[49]. To date, the epigenomics of the ECS have not been studied in patients with IBS, but, given the epigenetic changes in the physiology of the ECS, this is certainly warranted.

Conclusions

Pain is part of the symptom spectrum in many DGBIs and is a major determinant of disease severity and health-care-seeking behaviour[1]. In IBS, the best-studied DGBI, pain is a disease-defining symptom in which its presence is related to visceral hypersensitivity, loss of mucosal integrity and low-grade immune activation[5]. Currently available therapies for IBS, although having demonstrable symptom effects, are less and often suboptimally effective for controlling abdominal pain. A large group of agents aimed at controlling visceral hypersensitivity have been evaluated in clinical trials for the treatment of pain in IBS but none demonstrated sufficient efficacy to allow full clinical development (TABLE 1).

Several animal studies documented the involvement of the ECS in controlling gastrointestinal motility, mucosal barrier and immune function, and gut–brain signalling. Early observations in humans also suggested the involvement of the ECS in DGBI pathogenesis. Animal research demonstrated relevant actions of cannabinoids on epithelial tight junctions, mast cell and macrophage activation in the mucosa, and the interaction between the gut microbiota and the immune system, all relevant activities when aiming at controlling symptom generation in DGBIs. Based on these observations, the ECS, and particularly the cannabinoid receptors of the gastrointestinal tract, are emerging as potentially relevant and attractive targets for the treatment of IBS and other DGBIs. This therapeutic avenue is further supported by anecdotal reports of patients using phytocannabinoid agents for symptom relief, although convincing evidence of efficacy is lacking. Several cannabinoid receptor ligands, some of them peripherally restricted as well as inhibitors of endocannabinoid degradation pathways are being explored in other human disease conditions, including stress-related anxiety disorders[20,256], metabolic disease[257] and neuropathic pain[258]. Many unexplored avenues remain to further examine how modulation of these receptors could alter gut function and visceral sensitivity, building on the homeostatic role of the ECS in the body (FIG. 5). There are also early observations of therapeutic potential in DGBIs of PEA, the endogenous fatty acid, which acts through PPARα and through activation of endocannabinoid receptors by stimulating 2-AG biosynthesis. Emerging evidence supports the putative analgesic properties of a peripherally restricted CB_2 agonist in IBS[223]. Future studies are needed to address in detail the emerging therapeutic benefit of targeting the ECS in IBS and other DGBIs, whereas basic research and animal models continue to provide supporting and clarifying information.

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