

Role of cannabinoids in gastrointestinal mucosal defense and inflammation

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Abstract

Modulating the activity of the endocannabinoid system influences various gastrointestinal physiological and pathophysiological processes, and cannabinoid receptors as well as regulatory enzymes responsible for the synthesis or degradation of endocannabinoids represent potential targets to reduce the development of gastrointestinal mucosal lesions, hemorrhage and inflammation. Direct activation of CB₁ receptors by plant-derived, endogenous or synthetic cannabinoids effectively reduces both gastric acid secretion and gastric motor activity, and decreases the formation of gastric mucosal lesions induced by stress, pylorus ligation, nonsteroidal anti-inflammatory drugs (NSAIDs) or alcohol, partly by peripheral, partly by central mechanisms. Similarly, indirect activation of cannabinoid receptors through elevation of endocannabinoid levels by globally acting or peripherally restricted inhibitors of their metabolizing enzymes (FAAH, MAGL) or by inhibitors of their cellular uptake reduced the gastric mucosal lesions induced by NSAIDs in a CB₁ receptor-dependent fashion. Dual inhibition of FAAH and cyclooxygenase induced protection against both NSAID-induced gastrointestinal damage and intestinal inflammation. Moreover, in intestinal inflammation direct or indirect activation of CB₁ and CB₂ receptors exerts also multiple beneficial effects. Namely, activation of both CB receptors was shown to ameliorate intestinal inflammation in various murine colitis models, to decrease visceral hypersensitivity and abdominal pain, as well as to reduce colitis-associated hypermotility and diarrhea. In addition, CB₁ receptors suppress secretory processes and also modulate intestinal epithelial barrier functions. Thus, experimental data suggest that the endocannabinoid system represents a promising target in the treatment of inflammatory bowel diseases, and this assumption is also confirmed by preliminary clinical studies.

Key words: gastrointestinal injury, colitis, inflammatory bowel disease, cannabinoids, endocannabinoid system, FAAH, MAGL inhibitors

Running title: Cannabinoids in gastrointestinal inflammation and ulcer

1. INTRODUCTION

Direct activation of cannabinoid receptors by plant-derived, endogenous and synthetic cannabinoids

Cannabinoids were primarily discovered in marijuana (cannabis flower) and hashish (compressed cannabis resin) from the plant of *Cannabis sativa* [1]. This plant contains more than 80 phytocannabinoids [2]. The main active constituent of marijuana is the psychoactive Δ^9 -tetrahydrocannabinol (Δ^9 -THC), which acts at cannabinoid 1 (CB₁) and cannabinoid 2 (CB₂) receptors as a partial agonist. Other important natural cannabinoids present in marijuana are the non-psychoactive cannabidiol (CBD), Δ^9 -tetrahydrocannabivarin (Δ^9 -THCV) and cannabichromene (CBC) [1-3]. Among them CBD has attracted the greatest attention thus far. It was shown to antagonize the effects of CB₁/CB₂ receptor agonists, to counteract the psychotropic and other negative effects of Δ^9 -THC and several data suggest that it behaves as an inverse agonist of CB₁ and CB₂ receptors [4-6].

Some of these plant-derived cannabinoids are used in the medical praxis, such as Δ^9 -THC (dronabinol) and its synthetic analogue, nabilone against chemotherapy-induced nausea and emesis, and as appetite stimulants (e.g. in AIDS patients), and CBD combined with Δ^9 -THC (nabiximols) to relief neuropathic pain and spasticity in multiple sclerosis, and as an adjunctive analgesic treatment in advanced cancer pain [7].

Besides phytocannabinoids another group of naturally occurring substances that interact with cannabinoid receptors are the endocannabinoids. These lipid mediators are not stored but synthesized on demand in a site- and time-dependent manner and are rapidly degraded after exerting a transient and localized effect [8]. Interestingly, discovery of cannabinoid receptors preceded the isolation of their endogenous ligands, the endocannabinoids. Namely, while CB₁ receptor was isolated in 1988 and cloned in 1990 [9, 10], the first endocannabinoid, N-arachidonylethanolamine or anandamide (AEA) was isolated from the porcine brain only in 1992, and it showed high binding affinity to the brain CB₁ receptor [11]. CB₂ receptor was cloned in 1993 [12] and the second endocannabinoid, 2-arachidonoylglycerol (2-AG) was identified in 1994 - 1995 [13, 14]. Since then further endogenous cannabinoids have been identified, such as homo- γ -linolenylethanolamine, 7,10,13,16-docosatetraenylethanolamide, 2-arachidonoylglycerol ether (2-AGE, noladin ether), O-arachidonoyl ethanolamine (virodhamine) and N-arachidonoyl dopamine (NADA) (see review [15]).

Synthetic cannabinoid derivatives may differ from the natural ones in several aspects, e.g. in pharmacokinetic properties or in binding affinity to the different cannabinoid receptors. For example methanandamide, an amidase resistant chiral analogue of AEA [16] possesses higher metabolic stability than its parent compound. WIN 55,212-2, an aminoalkylindole derivative is a potent agonist at both CB₁ and CB₂ receptors and one of the most frequently used synthetic cannabinoids. It produces effects similar to those of Δ^9 -THC, although it has an entirely different chemical structure [17, 18]. Differences in binding affinity to different cannabinoid receptors may result in selective agonists at CB₁ or CB₂ receptors. For example, ACEA (arachidonyl-2'-chloroethylamide) is selective for CB₁ receptors [19], while JWH 133 (3-(1',1'-Dimethylbutyl)-1-deoxy-delta⁸-THC) [20] or GP1a (1-(2',4'-dichlorophenyl)-6-methyl-N-piperidin-1-yl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide) [21] for CB₂ receptors. Moreover, differences in distribution may result either in global actions or peripherally restricted effects, such as the peripherally acting CB_{1/2} agonist AZD 1940 and AZD 1704 developed by Astra Zeneca (see review [22]).

Besides cannabinoid CB₁ and CB₂ receptors, endogenous cannabinoids and synthetic derivatives can act

on other receptors as well. For example, AEA, its synthetic analogue methanandamide as well as the AEA uptake inhibitor AM404 (N-arachidonoylaminophenol, see later) activate TRPV1 receptor, and AEA and various synthetic cannabinoids can also act on central putative non-CB₁, non-CB₂, non-TRPV1 receptors, putative non-11, non-12 imidazoline receptors and allosteric sites on muscarinic M1 and M4 receptors and on 5-HT₃ receptors (see review: [23]).

Indirect activation of cannabinoid receptors by inhibition of endocannabinoid metabolism or uptake

Activation of CB₁ and CB₂ receptors can be achieved not only directly by the natural and synthetic cannabinoids, but also indirectly, by elevation of the level of endocannabinoids in the vicinity of cannabinoid receptors, either by blocking their degradation or uptake. AEA and 2-AG levels are regulated *in vivo* by catabolic enzymes, like the intracellular fatty acid amide hydrolase (FAAH), which hydrolyzes AEA into arachidonic acid and ethanolamine [24], and monoacylglycerol lipase (MAGL) [25], which is the main contributor to 2-AG hydrolysis. However, additional enzymes - cyclooxygenases (COX), lipoxygenases and cytochrome P450 enzymes - may also have role in the degradation of endocannabinoids [26]. Moreover, both AEA and 2-AG are removed from the extracellular space by a process of cellular uptake (and metabolism); however the transporter involved in this uptake mechanism has not yet been cloned [27-29].

Pharmacological blockade of the degradation of endocannabinoids is an attractive strategy for enhancing endocannabinoid signaling. It is supposed that increasing endocannabinoid tissue levels would induce less psychoactive effects (such as catalepsy, hypothermia, or hyperphagia) than the direct stimulants of CB₁ receptors [30], while the beneficial effects due to activation of CB₁ and/or CB₂ receptors would be retained [31]. However, it also has to be considered that inhibitors of the degradation or uptake are not entirely selective for endocannabinoids, e.g. FAAH hydrolyzes not only AEA, but also other bioactive lipids, such as N-palmitoyl-ethanolamine and oleamide [32, 33], and the AEA uptake inhibitor AM404 also activates TRPV1 receptor and inhibits COX-1 and COX-2 enzymes [34, 35].

Several FAAH inhibitors have been developed [36], among which the most widely used is URB 597, an irreversible inhibitor of FAAH both in the CNS and in the periphery [37, 38]. URB 937, an *O*-aryl carbamate derivative, is also a potent FAAH inhibitor, but it is extruded from the CNS by membrane transporter ATP-binding cassette, thus it inhibits the inactivation of AEA only in peripheral tissues [39]. Recently, the first class of systemically active multitarget FAAH/COX inhibitors has been developed. The class prototype ARN2508 is a potent inhibitor of FAAH, as well as of COX-1, and COX-2 enzymes [40].

The development of FAAH inhibitors was later followed by the introduction of selective MAGL inhibitors. In 2009 Long *et al.* published [41] that JZL 184 (4-nitrophenyl 4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate) irreversibly, selectively inhibits MAGL, and elevates the brain level of 2-AG by 8-fold without affecting the level of AEA.

However, when analyzing the biological actions of the degradation inhibitors of endocannabinoids it should be considered that elevation of the tissue levels of endocannabinoids may increase the formation of cyclooxygenase-, lipoxygenase- and cytochrome P450-derived metabolites, which are bioactive and may have pro-inflammatory properties as well, such as prostamide F_{2α} [26, 42, 43].

Besides inhibition of degradation, another way to increase the level of endocannabinoids is to interfere

with their cellular uptake mechanism. AM404 is an AEA analogue and the active metabolite of paracetamol [44], which is the best characterized AEA uptake inhibitor *in vivo*. It inhibits the carrier-mediated transport of AEA into presynaptic neurons and other related compounds back from the synaptic cleft without affecting AEA hydrolysis [45]. As mentioned above, it is also an inhibitor of COX-1 and COX-2 enzymes and an agonist on TRPV1 receptors [34, 35]. VDM11, an AEA derivate, is as potent membrane transporter inhibitor as AM404, but it has no agonistic activity at TRPV1 receptors and is a weaker CB₁ receptor agonist than AM404 [34].

Originally definitive differences in distribution of CB₁ and CB₂ receptors have been suggested. While CB₁ receptors have been shown to be widely distributed throughout the central, peripheral and enteric nervous system [1], CB₂ receptors were thought to be located peripherally mainly in the immune tissues (spleen and macrophages) [46]. However, recent findings suggest functionally relevant expression of CB₂ receptors in specific regions of the brain, such as in primed microglia [47] and in neurons in the brainstem [48]. Moreover, this subtype was also shown in several peripheral non-immune tissues, e.g. in myocardium, gut, endothelial, vascular smooth muscle, pancreas, bone, reproductive organs/cells, and in different tumors [49]. Furthermore, inflammation or tissue injury results in increase of local endocannabinoid levels and changes in CB₂ receptor expressions. Such alteration was observed not only experimentally but also in several human diseases, for example in cardiovascular, gastrointestinal, kidney, neurodegenerative, psychiatric, bone, skin, autoimmune and pulmonary disorders (see review [49]).

2. GASTROINTESTINAL ACTIONS OF CANNABINOIDS

Modulating the activity of the endocannabinoid system (ECS), which comprises CB₁ and CB₂ receptors, the endocannabinoids and their synthetic and metabolizing enzymes, may have therapeutic potential in numerous diseases including obesity/metabolic syndrome, diabetes, neurodegenerative, inflammatory, cardiovascular and psychiatric disorders, liver and skin diseases, pain, cachexia, cancer, as well as chemotherapy-induced nausea/vomiting (see review of [22]).

The role of ECS in the physiology and pathophysiology of gastrointestinal (GI) tract has also been extensively studied [50-54]. In the digestive tract high levels of the endocannabinoids, and of the enzymes responsible for their synthesis and metabolism can be detected. The presence of CB₁ receptors on myenteric and submucosal nerve plexuses along the alimentary tract has been shown by immunohistochemical studies (see review of [55]). Co-localization of CB₁ receptor with the cholinergic marker choline acetyltransferase in neural elements innervating smooth muscle, mucosa and submucosal blood vessels of rat stomach fundus, corpus and antrum was shown [56].

There is a number of evidence that activation or inhibition of peripheral (e.g. via enteric neurons) and/or central (vagal, brainstem and spinal nerves) cannabinoid receptors may substantially influence the physiological and pathophysiological processes of the GI tract.

The aims of this review are 1) to summarize the effects of cannabinoids on gastric functions (i.e. on gastric acid secretion, gastric motor activity and emptying, as well as on gastric mucosal integrity), and 2) to provide an overview of current knowledge on the cannabinoid receptor-mediated beneficial effects in inflammatory bowel diseases (IBDs).

2.1. EFFECTS OF CANNABINOIDS ON GASTRIC FUNCTIONS

Cannabinoids and gastric acid secretion

Data of the literature suggest that cannabinoids inhibit gastric acid secretion. The antisecretory effect of cannabinoids (non selective CB-receptor agonist WIN 55,212-2 and the selective CB₁-receptor agonist HU-210 given intravenously /i.v./) in the rat was shown to be related to suppression of the vagal drive to the stomach. This effect was mediated by CB₁ receptors located on pre- and postganglionic cholinergic neurons [56]. While bilateral cervical vagotomy significantly reduced, but not abolished the antisecretory action of synthetic cannabinoids, atropine failed to modify it. These findings suggest that the release of non-cholinergic excitatory neurotransmitters may be regulated by CB₁ receptors [56]. Moreover, since the CB-receptor agonist given intracerebroventricularly (i.c.v.) failed to affect the acid output, it may be concluded that inhibition of gastric acid secretion in the rat is mainly peripherally located [57]. Interestingly, in contrast with the *in vivo* data, in isolated gastric fundus synthetic cannabinoids (WIN 55,212-2 and HU-210) did not change the basal or stimulated acid output to histamine, pentagastrin or electrical field stimulation [58].

Cannabinoids and gastric motor activity and emptying

The psychoactive major constituents of marijuana and the synthetic cannabinoid nabilone were demonstrated to slow the rate of gastric emptying in mice and rats, however, the non-psychoactive CBD given intravenously (i.v.) failed to affect it [59]. In contrast, both psychoactive and non-psychoactive cannabinoid agonists were found to delay gastric emptying through activation of cannabinoid CB₁ receptors. Since neither CB₁ nor CB₂ receptor antagonists affected gastric emptying alone, endogenous cannabinoid system does not seem to modulate gastric motor activity tonically [60].

Δ^9 -THC exerted inhibitory effect on gastric motility and emptying and this effect was abolished by bilateral cervical vagotomy, suggesting the involvement of a central component (the dorsal vagal complex) in the observed effect. It was supposed that cannabinoids modulate the vagal (parasympathetic) outflow to gastric smooth muscle [61]. Furthermore, i.c.v. administration of WIN 55,212-2 inhibited the gastric emptying in rats [62]. However, i.c.v. injection of the CB₁ receptor antagonist SR141716A failed to affect the inhibitory action of peripherally injected WIN 55,212-2, which argues against the role of central cannabinoid receptors and suggests that peripheral CB₁ receptors are primarily responsible for the inhibition of gastric emptying [62].

Several studies aimed to clarify whether tolerance develops to the inhibitory effect of GI motor activity of cannabinoids. Based on *in vivo* and *in vitro* studies in mice and guinea pigs it was concluded that cannabinoid pretreatment induces tolerance to the inhibitory actions of cannabinoid receptor agonists on GI motility (see review of [63]). However, Abalo *et al.* (2011) found that after chronic intermittent administration of WIN 55,212-2 to the rat its inhibitory effect on gastric emptying was intensified, indicating that hypersensitization may develop to some of the effects of cannabinoids, particularly to the delayed gastric emptying [64].

Cannabinoids and gastric mucosal integrity

CB₁ and CB₂ receptors and enzymes involved in regulation of synthesis and degradation of endocannabinoids are all potential targets, which can be modulated to protect the gastric mucosa against erosions,

mucosal lesions and inflammation (Table 1).

Activation of cannabinoid receptors by exogenous or endogenous ligands has been shown to decrease the formation of different types of experimental gastric ulcers. For example Δ^9 -THC reduced mucosal damage induced by pylorus ligation [65]. It also attenuated diclofenac-induced gastric mucosal lesions given either orally or intraperitoneally (i.p.) in a CB₁ receptor-dependent fashion, and it proved to be more potent in exerting gastroprotective effect than producing classical cannibimimetic effects, such as locomotor immobility, antinociception, hypothermia and catalepsy [66, 67]. Gastric lesions induced by water immersion and restraint stress were reduced by AEA as well as by WIN 55,212-2 (both given i.p.), and their gastroprotective action was mediated also by CB₁ receptors [68, 69]. The protective effect of AEA was associated with an increase in gastric mucosal blood flow and mucosal DNA synthesis and with reduced level of pro-inflammatory interleukin-1 β (IL-1 β) [69]. Involvement of CB₁ receptors in gastroprotection was further supported by the results with the selective cannabinoid CB₁ receptor agonist ACEA, which effectively reduced the aspirin-induced gastric mucosal lesions (given i.p.) [70].

As discussed above, cannabinoids inhibit also gastric acid secretion via CB₁ receptors. Acid secretion is involved in the pathomechanism of gastric damage induced by pylorus ligation, cold restraint test as well as in NSAID-induced mucosal damage. According to the original concept, cytoprotective (gastroprotective) effect was unrelated to inhibition of acid secretion, and acid-independent ulcer models induced by absolute ethanol, 0.6 N hydrochloric acid, 0.2 N sodium hydroxide and 25% sodium chloride were used to demonstrate the cytoprotective effect of prostaglandins [71]. Cannabinoids, such as AEA, methanandamide and WIN 55,212-2, have been shown to reduce the absolute ethanol-induced gastric lesions after both peripheral (i.v.) and central (i.c.v.) administration in the rat by a CB₁ receptor-mediated mechanism [72]. Moreover, the role of endogenous opioids (endomorphin 2) was suggested by the findings that the gastroprotective effect of AEA was antagonized by naloxone and by pretreatment with endomorphin 2 antiserum [72]. Involvement of a central component in the gastroprotective action of cannabinoids was confirmed by the findings that the protective effect of methanandamide injected i.v. was reversed by i.c.v. injected SR141716A, a CB₁ receptor antagonist [72]. 2-AG prevented also ethanol-induced lesions, when given i.c.v. in the rat. Moreover, 2-AG was shown to be involved in the centrally initiated gastroprotective effect of angiotensin II, which was reversed by the CB₁ receptor antagonist/inverse agonist AM 251, as well as by tetrahydrolipstatin, an inhibitor of diacylglycerol lipase (DAGL), the principle enzyme responsible for the synthesis of 2-AG [73].

As described above, the cannabinoid system can be activated not only by direct stimulation of cannabinoid receptors, but also via the inhibition of enzymes that inactivate the endocannabinoids. Inhibition of FAAH by URB 597 resulted in inhibition of diclofenac-induced gastric mucosal lesions. Similarly, reduction of the mucosal lesions was observed in transgenic, FAAH (-/-) mice. URB 597 retained its efficacy in CB₂ (-/-) mice, but was ineffective in CB₁ (-/-) mice, indicating that the gastroprotective effect was mediated entirely by CB₁ receptors [74].

In contrast to URB 597, which is a global FAAH inhibitor, URB 937 inhibits FAAH only in peripheral tissues. It reduced the gastric lesions induced by indomethacin. It also exerted antinociceptive and anti-inflammatory activity and its combination with indomethacin synergistically attenuated pain-related behaviors [75].

Recently, compounds that block both FAAH and COX enzymes have been developed. The hypothesis

was that multitarget FAAH/COX blockade may result in substantial anti-inflammatory efficacy and decrease of gastrointestinal toxicity [40]. Namely, it is well-documented that COX-2 has pathogenetic role in inflammatory processes. On the other hand, increased FAAH-mediated degradation of AEA was observed in some inflammatory conditions [76]. Since several data suggest the anti-inflammatory and mucosal protective effect of AEA, reduction of its level by increased FAAH activity may weaken its ability to attenuate the inflammation and gastrointestinal injury. Data of the literature suggest that both COX-2 [77] and FAAH are expressed at abnormally high levels [78, 79] in IBD and increased COX-2 activity in gastrointestinal mucosa may mediate not only protective actions but also formation of bioactive metabolites and inflammatory mediators generated by COX-2-dependent oxygenation of AEA, such as prostamide F2 α [26]. The prototype of ligands that target FAAH, COX-1, and COX-2 is ARN2508, which besides exerting profound inhibition of dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis (see also below), did not cause gastric mucosal injury and protected the stomach from the damaging effect of flurbiprofen through a mechanism that requires FAAH inhibition and elevation of AEA level [40]. These results suggest that FAAH/COX blockade may provide a new therapeutic strategy for the treatment of inflammatory diseases in which both enzymes are overactive.

Elevating the level of the other principle endocannabinoid, 2-AG by inhibiting MAGL with JZL 184 also induced significant protection and reduced the diclofenac-induced gastric hemorrhages injected i.p. to the mouse. JZL184 increased significantly the gastric levels of 2-AG, but did not influence that of AEA, free arachidonic acid, PGE₂, or PGD₂ suggesting that prostaglandins are not involved in the protective action of MAGL inhibitors against NSAID-induced gastric ulcers. It was found that the protective effect may be related to the reduction of diclofenac-induced elevation of proinflammatory cytokines, such as IL-1 β , IL-6 or tumor necrosis factor- α (TNF- α). Pharmacological or genetic blockade of CB₁, but not CB₂ receptors inhibited the protective effect of JZL 184, which again indicates the predominant role of CB₁ receptors in the regulation of gastric mucosal integrity [67].

Table 1. Gastric mucosal protective effect of direct and indirect stimulants of cannabinoid receptors

Compound	Target within the ECS	Ulcer model	Route of administration, animal	Antagonist or genetic manipulation to clarify the CB receptor type	Receptor type that mediates the effect	Reference
Δ^9 -THC	CB ₁ , CB ₂	pylorus ligation	rat		not detected	Sofia <i>et al.</i> [65]
Δ^9 -THC	CB ₁ , CB ₂	diclofenac	i.p., per os, mouse	CB ₁ , CB ₂ antagonist, deletion of CB ₁ , CB ₂ receptors	CB ₁	Kinsey and Cole [66], Kinsey <i>et al.</i> [67]
WIN 55,212-2	CB ₁ , CB ₂	water immersion restraint stress	i.p., rat	CB ₁ , CB ₂ antagonist	CB ₁	Germano <i>et al.</i> [68]
AEA	CB ₁ , CB ₂	water immersion	i.p., rat	CB ₁ antagonist	CB ₁	Dembinski <i>et al.</i> [69]

		restraint stress				
ACEA	CB ₁	aspirin	i.p., rat		CB ₁	Rutkowska and Fereniec-Goltbiewska [70]
AEA, methanandamide, WIN 55,212-2	CB ₁ , CB ₂	100% ethanol	i.v., i.c.v., rat	CB ₁ antagonist	CB ₁	Shujaa <i>et al.</i> [72]
2-AG	CB ₁ , CB ₂	100% ethanol	i.c.v., rat	CB ₁ antagonist	CB ₁	Gyires <i>et al.</i> [73]
URB 597	FAAH inhibition (both centrally and peripherally)	diclofenac	i.p., mouse	deletion of CB ₁ and CB ₂ receptors	CB ₁	Naidu <i>et al.</i> [74]
URB 937	FAAH inhibition (only peripherally)	indomethacin	p.os, mouse		not analyzed	Sasso <i>et al.</i> [75]
ARN2508	FAAH, COX-1, COX-2 inhibition	flurbiprofen (DSS, TNBS colitis)	p.os, mouse	CB ₁ antagonist	CB ₁	Sasso <i>et al.</i> [40]
JZL 184	MAGL inhibition	diclofenac	p.os, i.p., mouse	deletion of CB ₁ and CB ₂ receptors	CB ₁	Kinsey <i>et al.</i> [67]

Abbreviations: 2-AG: 2-arachidonoylglycerol; AEA: anandamide; COX: cyclooxygenase; ECS: endocannabinoid system; FAAH: fatty acid amide hydrolase; i.c.v.: intracerebroventricularly; i.p.: intraperitoneally; i.v.: intravenously; MAGL: monoacylglycerol lipase; p.os: orally

II.2. CANNABINOIDS IN INFLAMMATORY BOWEL DISEASES

Inflammatory bowel diseases (IBDs) are chronic, relapsing inflammatory conditions of the gastrointestinal (GI) tract. The two major forms are Crohn's disease (CD) and ulcerative colitis (UC), which share similar symptoms, such as diarrhea, abdominal pain and weight loss [80]. The pathogenesis of both forms is complex, involving various predisposing environmental and genetic factors, which together with the altered intestinal flora can induce mucosal disruption and result in penetration of luminal antigens into the gut wall [73, 80]. [80, 81] The activation of immune cells by these antigens and the chronic, uncontrolled inflammation is a key component of the pathogenesis of IBDs, and the patients are mainly treated today with anti-inflammatory and immunosuppressive agents, such as 5-aminosalicylic acid derivatives, corticosteroids, purine antimetabolites,

methotrexate, calcineurin inhibitors or monoclonal antibodies targeting primarily TNF- α [81]. The long-term use of these medications, however, can induce severe adverse reactions, and a large effort is currently put into finding new therapeutic approaches [73][81]. Beside developing new antibodies targeting various anti-inflammatory cytokines and attempting to restore the altered microbiota, one promising approach is the activation of cannabinoid receptors in the gut, which does not only suppresses many of the IBD-related symptoms, such as diarrhea and visceral hypersensitivity, but also inhibits the inflammatory reaction.

The endocannabinoid system in healthy and inflamed gut

All components of the ECS (CB₁ and CB₂ receptors, the endocannabinoids AEA and 2-AG, and proteins responsible for their synthesis and degradation) are widely distributed in the GI tract, and there is plenty of evidence that their expression is substantially altered during inflammation.

Both CB receptors are localized throughout the gut. In non-inflamed tissues CB₁ receptors are mainly localized on excitatory motor neurons, interneurons and intrinsic primary afferent neurons of the enteric nervous system (ENS), although epithelial cells, smooth muscles and immune cells express also this subtype [82-84]. CB₂ receptors, on the other hand, are mainly expressed by subepithelial immune cells (such as macrophages and plasma cells) [82], and also by enteric neurons [85, 86], while they are absent in epithelial cells [82, 85].

As described in the introduction, AEA and 2-AG are synthesized on-demand from membrane lipid precursors [87], and their primary biosynthetic enzymes, N-acylphosphatidylethanolamine selective phospholipase D (NAPE-PLD) and diacylglycerol lipase (DAGL)- α and - β , respectively, have been identified in the small and large intestines of mice and humans [78, 79, 83, 88, 89]. Immunohistochemical studies revealed that both synthetic enzymes are localized in the epithelium, in lamina propria plasma cells, in both layers of muscularis externa and in nerve fibers of the myenteric plexus [78, 83], suggesting an active endocannabinoid synthesis in the healthy gut. Accordingly, 2-AG and (in considerably lower amount) AEA have been demonstrated in the intestines of different species [79, 90-92].

The endocannabinoids are subjected to various degradation pathways [87], and the major enzymes responsible for their hydrolysis, FAAH (AEA) and MAGL (2-AG) show similarly wide GI distribution [83, 90, 93, 94].

The concept that the ECS is altered in IBD is supported by several animal and human studies, and Tables 2 and 3 provide a brief overview of the observed changes. At the first glance these results are rather contradictory, since both elevated, depressed and unchanged expressions of the various components have been described, but a closer look reveals some important similarities.

In the majority of studies, the expression of CB₁ receptor was elevated in the inflamed gut, and this was evident in both epithelial cells, lamina propria mononuclear cells and myenteric neurons [79, 95, 96]. Although in the case of CB₂ receptor the findings are more erratic, an increased epithelial expression has been consistently observed [82, 83, 96]. These findings suggest that disruption of the epithelial barrier and the concomitant inflammatory reaction upregulate the expression of both CB receptors, which may explain the enhanced GI effects of CB receptor ligands in inflammation (see below).

Among the endocannabinoids the intestinal level of AEA during inflammation almost always differed significantly from the level measured in healthy tissues, however, both elevation [92, 93] and reduction [40, 79,

89] have been reported. These diverging results can be partly explained by different experimental conditions. For example, the measured levels of AEA and its synthetic and degrading enzymes seem to vary at different time points during the course of the disease. Storr *et al.* [97] observed significantly reduced FAAH mRNA in the early phase of various colitis models (TNBS, oxazolone, DSS), but this reduction disappeared at later time points, or even changed to an elevation. This finding suggests that the level of AEA increases in the initial phase of colitis, which may have important protective effect, but with the progression of the disease this endogenous protective mechanism deteriorates. Beside this time-dependence AEA levels also differ in various segments of the gut, as well as in different regions of the gut wall. For instance, Izzo *et al.* [89] reported a significant decrease of AEA level in the jejunum of croton oil-treated mice, but not in the duodenum or ileum, and in the study of D'Argenio *et al.* [92] TNBS-treatment induced significant rise of AEA in the submucosa, but not in the mucosa of rat colon. In short, these studies clearly demonstrate that intestinal inflammation alters the tissue level of AEA, but further studies are needed to get a clear picture of its role in the pathomechanism.

Interestingly, the intestinal level of 2-AG in most studies remained unchanged [79, 91, 92, 98], which implies that this endocannabinoid does not have important role in IBD. An alternative explanation, however, can be that both the synthesis and degradation of 2-AG accelerate in inflammation, which results in a more rapid turnover without altering its tissue level. This assumption is supported by the findings of Marquez *et al.* [83], who measured elevated DAGL- α and MAGL levels in colon mucosal biopsies of patients with UC.

In conclusion, both CB receptors and at least one of the two major endocannabinoids (AEA) show altered intestinal levels in IBD. The upregulation of CB receptors and their activation by AEA and 2-AG promotes epithelial healing and tempers the inflammation, as discussed below. This seems to be a systemic (pan-intestinal) reaction rather than a local response, because alterations were also observed in such parts of the gut, which were not directly exposed to the inflammatory agents [97]. Thus, the ECS may serve as an endogenous gastrointestinal defense system, which shows increased activity in pathological conditions and its impaired function may increase susceptibility to various GI diseases, such as IBD. This is in line with findings that genetic deletion or pharmacological blockade of either CB₁ or CB₂ receptors aggravates the development of murine colitis, while deletion of FAAH confers protection [95, 99, 100].

Unfortunately human studies failed to identify major IBD susceptibility genes related to the ECS so far, since the investigated single nucleotide polymorphisms (SNPs), such as the G1359A SNP (rs1049353) within the CNR1 gene encoding the CB1 receptor [101], the Q63R SNP (rs35761398) in the CNR2 gene [102] or the FAAH C385A variant (rs324420) [97, 100] had only minor influence at best on the pathogenesis of IBD. These gene variants were also not identified as IBD susceptibility loci by recent genome-wide association studies [103].

Table 2. Altered ECS in animal models of IBD

Animal	Model	CB ₁	CB ₂	AEA	2-AG	FAAH	MAGL	Reference
C57BL/6N mouse	DNBS	↑						Massa <i>et al.</i> [95]
C57BL/6 mouse	TNBS	↔	↔		↔	↓ mRNA	↓ mRNA	Alhouayek <i>et al.</i> [98]

						↔ activity	↔ activity	
CD1 mouse	DSS	↑				↑	↑	Matos <i>et al.</i> [104]
C57BL/6N mouse	TNBS DSS		↑					Storr <i>et al.</i> [105]
ICR mouse	croton oil	↑	↓	↓	↔	↓		Izzo <i>et al.</i> [89]
ICR mouse	croton oil	↑		↔	↔	↑		Izzo <i>et al.</i> [91]
CD1 mouse BALB/c mouse	oil of mustard DSS	↑	↑					Kimball <i>et al.</i> [96]
Sprague-Dawley rat	LPS		↔					Duncan <i>et al.</i> [86]
CD1 mouse	TNBS			↓				Sasso <i>et al.</i> [40]
Wistar rat C57BL/6J mouse	TNBS DNBS			↑	↔			D'Argenio <i>et al.</i> [92]
ICR mouse	DNBS			↑	↑	↓		Borrelli <i>et al.</i> [93]
C57BL/6N mouse	TNBS oxazolone DSS					↓ / ↔		Storr <i>et al.</i> [97]
C57BL/6 mouse	TNBS DSS			↑				Alhouayek <i>et al.</i> [33]
C57BL/6 mouse	TNBS				↔			Salaga <i>et al.</i> [106]

Signs and abbreviations: ↑: elevation, ↓: reduction, ↔: no change, 2-AG: 2-arachidonoylglycerol; AEA: anandamide; FAAH: fatty acid amide hydrolase; LPS: lipopolysaccharide; MAGL: monoacylglycerol lipase

Table 3. Altered ECS in IBD patients

IBD type	CB ₁	CB ₂	AEA	2- AG	NAPE- PLD	DAGL- α and - β	FAAH	MAGL	Reference
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CD, UC	↑	↔							Stintzing <i>et al.</i> [107]
UC	↔	↑			↓	↑ (α) ↔ (β)	↔	↑	Marquez <i>et al.</i> [83]
CD, UC	↑	↔	↓	↔	↓		↑		Di Sabatino <i>et al.</i> [79]
UC			↑	↔					D'Argenio <i>et al.</i> [92]
UC					↔ (mRNA) ↓ (protein)		↑ (mRNA) ↔ (protein)		Suarez <i>et al.</i> [78]
CD, UC							↔ (↓) *		Salaga <i>et al.</i> [106]

Signs and abbreviations: ↑: elevation; ↓: reduction; ↔: no change; 2-AG: 2-arachidonoylglycerol; AEA: anandamide; DAGL: diacylglycerol lipase; FAAH: fatty acid amide hydrolase; MAGL: monoacylglycerol lipase; NAPE-PLD: N-acylphosphatidylethanolamine selective phospholipase D

* FAAH mRNA levels showed tendency for reduction, but the difference did not reach statistical significance.

The beneficial effects of cannabinoids in IBD

Cannabis has been used for centuries to alleviate the symptoms of numerous diseases [108] and recent preliminary clinical studies (retrospectal observations, questionnaires and pilot prospective studies) confirmed the anecdotal reports that it may have beneficial effect in IBD as well [109-112]. In these studies the use of cannabis reduced the patients' disease activity index, abdominal pain, diarrhea and improved their quality of life.

In the last decade several experiments have been conducted to analyze the effects of cannabinoids in IBD, mainly by using different colitis models in animals, and the data accumulated thus far indicate that cannabinoids efficiently inhibit the inflammatory reaction, modulate the mucosal barrier functions and also alleviate some IBD-associated symptoms, like diarrhea and visceral pain [82, 95, 96, 113-118].

Inhibition of inflammation

There is a large body of evidence that cannabinoids exert immunomodulatory, mainly immunosuppressive effect. Both synthetic CB receptor agonists and endocannabinoids were shown to impair cellular and humoral immunity by reducing inflammatory cell recruitment, inducing T cell apoptosis and

suppressing the production of numerous pro-inflammatory cytokines and chemokines (e.g. TNF- α , IL-1 β , IL-2, IL-6, IL-17, IFN- γ , CCL2 or CXCL10) (recently reviewed by Turcotte *et al.* [119]). These effects are primarily mediated by CB₂ receptors localized on macrophages and lymphocytes, but some studies underline the importance of CB₁ receptors as well [95, 99, 120]. Furthermore, other, non-cannabinoid receptors, like peroxisome proliferator-activated receptor gamma (PPAR- γ) [121, 122] or adenosine A2A receptor [123] are also involved in the anti-inflammatory action of certain cannabinoids, like WIN 55,212-2 or CBD. Some phytocannabinoids, such as CBD and cannabigerol (CBG), as well as the endocannabinoid AEA also activate TRPV1 receptors [15, 124], but it is still a matter of debate whether this results in pro- or anti-inflammatory action. Namely, it was reported that AEA can induce intestinal inflammation by activating TRPV1 receptors [125] and antagonists of this receptor attenuate the development of DSS colitis [126], but there is also evidence that the presence of these receptors confers protection in dinitrobenzene sulfonic acid (DNBS) colitis [127].

Due to the complex anti-inflammatory action cannabinoids can efficiently inhibit the development of colitis. Table 4 provides a list from different non-selective and selective CB₁ and CB₂ receptor agonists, which proved to be protective in animal models of IBD. In general, these studies demonstrated that cannabinoids given peripherally significantly reduce the animals weight loss and diarrhea, the macroscopic and histological colonic damage, neutrophil migration and MPO activity, as well as the production of various inflammatory cytokines (like TNF- α or IL-1 β). The protective effect was counteracted by pharmacological or genetic blockade of CB₁ [95, 128] or CB₂ receptors [105, 114], confirming the involvement of both receptors in the action.

In most studies cannabinoids were given prophylactically, i.e. starting before or at the time of the colitis induction. Kimball *et al.* [96], however, demonstrated that ACEA was equally effective, while JWH-133 was even more effective, when administered therapeutically, that is 24 h after the colitis induction. Similarly, the covalently acting non-selective CB receptor agonist AM841 significantly attenuated DSS-induced inflammation, when it was given on days 4 – 7 after colitis induction [128]. These results indicate that cannabinoids can not only inhibit the development of colitis, but also able to effectively reduce the already established inflammation, which is in line with the observations in IBD patients.

Table 4. Direct activation of CB₁, CB₂ or both receptors alleviate murine colitis

Animal	Colitis model	Cannabinoid ligand	CB receptor selectivity	Dosage and route of administration	Reference
C57BL/6N mouse	DNBS	HU 210	non-selective	0.05 mg/kg, q.d., s.c.	Massa <i>et al.</i> [95]
CD1 mouse	DSS TNBS	AM 841	non-selective	0.01 – 1 mg/kg, q.d./b.i.d., i.p.	Fichna <i>et al.</i> [128]
AKR mouse	TNBS	AEA	non-selective	5 mg/kg, q.d., i.p.	Engel <i>et al.</i> [129]
Wistar rat	TNBS	THC	non-selective	10 – 20 mg/kg, q.d., i.p.	Jamontt <i>et al.</i> [130]

CD1 mouse	DSS TNBS	WIN 55,212-2	non-selective	2 mg/kg, b.i.d., i.p.	Cluny <i>et al.</i> [131]
C57BL/6J mouse	DSS	WIN 55,212-2	non-selective	5 mg/kg, q.d., i.p.	Li <i>et al.</i> [132]
CD1 mouse BALB/c mouse	oil of mustard DSS	ACEA	CB ₁	2.5 mg/kg, q.d., i.p. 10 mg/kg, b.i.d., i.p.	Kimball <i>et al.</i> [96]
CD1 mouse BALB/c mouse	oil of mustard DSS	JWH-133	CB ₂	2.5 mg/kg, q.d., i.p. 10 – 20 mg/kg, b.i.d., i.p.	Kimball <i>et al.</i> [96]
C57BL/6 mouse	DSS IL-10 (-/-)	JWH-133	CB ₂	10 – 20 mg/kg, q.d., i.p. 2.5 mg/kg, q.a.d., i.p.	Singh <i>et al.</i> [114]
C57BL/6N mouse	TNBS	JWH-133 AM1241	CB ₂	20 mg/kg, q.d./b.i.d., i.p. 10 – 20 mg/kg, b.i.d., i.p.	Storr <i>et al.</i> [105]
C57BL/6 mouse	DSS	Compounds 58 and 64	CB ₂	10 mg/kg, q.d., i.p.	Tourteau <i>et al.</i> [133]

Abbreviations: AEA: anandamide; b.i.d.: *bis in die* (twice daily); i.p.: intraperitoneally; q.a.d.: *quaque altera die* (every other day); q.d.: *quaque die* (once daily); s.c.: subcutaneously

Modulation of intestinal barrier functions

Epithelial damage and breach of the intestinal barrier are important factors in the pathomechanism of IBD, which allow bacterial products and other antigens to cross the epithelium and enter the lamina propria, resulting in inflammation and tissue damage [134-136]. Restoration of the barrier function therefore represents an important approach to treat IBD patients. In this respect, it is of relevance that the endocannabinoids AEA and noladin ether, as well as the CB₁ receptor agonist arachidonylcyclopropylamide (ACPA), but not the CB₂ receptor agonist JWH-133 induced wound-closure in human colonic epithelial cell lines [82], implying that CB₁ receptor activation can improve the impaired mucosal barrier in IBD. This concept is underpinned by studies demonstrating that Δ⁹-THC and CBD prevented EDTA- and cytokine-induced increased paracellular permeability in the Caco-2 cell culture model and increased the expression of the tight-junction protein zonula occludens 1, which effects were sensitive to CB₁-, but not to CB₂-antagonism [118, 137]. On the other hand, the latter studies also showed that apical, but not basolateral application of AEA and 2-AG exerted opposite effect, and increased Caco-2 cell permeability, which was also mediated by CB₁ receptors and was at least partly due to down-regulated expression

of claudin-1, another tight-junction protein [118, 137]. These *in vitro* results suggest that CB₁ receptor ligands can induce opposing effects on inflammation-induced intestinal permeability and may initiate different signaling pathways, leading to changes in different tight junction proteins. The complex (and yet not fully understood) role of cannabinoids on intestinal permeability is also indicated by *in vivo* studies. Zoppi *et al.* [113], for example, provided evidence for the mucosal protective effect of the ECS, because immobilization and acoustic stress induced greater inflammation and colonic barrier dysfunction (characterized by lower IgA secretion, higher paracellular permeability to ⁵¹Cr-EDTA and higher bacterial translocation) in CB₁ (-/-) mice. In contrast, the study of Muccioli *et al.* [138] suggests that CB₁ receptor activation is rather detrimental for the epithelial barrier function, because the CB₁ receptor antagonist SR141716A improved gut barrier functions and reduced gut permeability in obese ob/ob mice, while chronic (4-week) infusion of the CB₁/CB₂ agonist HU-210 increased gut permeability in lean wild-type mice. Thus, the accumulated evidence indicates that CB receptor ligands are able to directly modulate intestinal epithelial permeability by acting mainly on CB₁ receptors, but further studies are warranted to resolve the apparent contradictions reported thus far. It also has to be considered that the anti-inflammatory effect of cannabinoids can indirectly modify their action on intestinal permeability and improve barrier functions [98].

Inhibition of motility and secretion

Beside their potent anti-inflammatory property and modulatory effect on intestinal epithelial permeability, cannabinoids also inhibit gastrointestinal motility and secretion, which both may alleviate diarrhea, a common clinical manifestation of IBD [139, 140].

The effect of cannabinoids on gastrointestinal motility is well-documented and has been extensively reviewed [117, 139, 141]. A large amount of data obtained from *in vitro* and *in vivo* studies demonstrate that activation of presynaptic CB₁ receptors by cannabinoids and endocannabinoid degradation inhibitors reduces gastrointestinal smooth muscle contractility, gastric emptying and intestinal peristalsis in both animals and humans. This effect is mainly due to presynaptic inhibition of acetylcholine release from cholinergic nerves, but additional mechanisms, such as inhibition of non-adrenergic-non-cholinergic excitatory and inhibitory transmission have also been described [142-144]. Furthermore, the results of Grider *et al.* [145] suggest that CB₁ receptors inhibit all components of the peristaltic reflex in the rat colon, namely the excitatory cholinergic/tachykininergic motor neurons, the inhibitory VIPergic motor neurons as well as the intrinsic sensory CGRP-containing neurons.

Regarding the inhibitory action of cannabinoids on GI motility, two important differences have been described between normal and inflamed tissues. First, CB₁ receptor-mediated suppression of motility is enhanced during inflammation, which is mirrored by lower ED₅₀ values of cannabinoids in croton oil-treated mice [91, 146]. This phenomenon may result (at least partly) from the overexpression of CB₁ receptors, which was reported by several groups (see above). Second, although under physiological conditions GI motility is predominantly regulated by CB₁ receptors [117], in inflammation CB₂ receptors are also involved in the control of (pathological) motility. The importance of CB₂ receptors in inflammatory state is indicated by studies showing that JWH-133, a selective CB₂ receptor agonist normalized the lipopolysaccharide (LPS)-induced intestinal hypermotility both *in vitro* [86] and *in vivo* [147]. Because CB₂ receptors are upregulated in epithelial cells during inflammation and

also abundantly expressed by immune cells, their activation may effectively reduce the release of various inflammatory mediators from these cells, which would otherwise stimulate intestinal peristalsis.

Another important feature of cannabinoids is that they also decrease intestinal secretions, which may take part in their antidiarrheal action. This effect is predominantly, if not entirely mediated by CB₁ receptors and was observed both *in vivo* [140] and *in vitro* [116, 148, 149]. The latter studies clearly demonstrated that cannabinoids act on the enteric nerves and not on the epithelium, because the cannabinoid agonist WIN 55,212-2 inhibited ileal secretions caused by electrical stimulation and capsaicin, but not by acetylcholine, carbachol or forskolin [116, 149]. Moreover, the colocalization of CB₁ and TRPV1 receptors, and the loss of inhibitory effect in extrinsically denervated ileal segments suggest that CB₁ receptors controlling intestinal secretions are primarily localized on extrinsic primary afferent nerves that innervate submucosal secretomotor neurons [116].

Inhibition of visceral hypersensitivity

Beside inhibiting motility and secretion cannabinoids may possess another important beneficial effect in IBD, namely alleviation of visceral hypersensitivity and abdominal pain. The analgesic effect of cannabinoids is well-described, especially in the case of somatic pains (for reviews see [150-153]), and several lines of evidence suggest that they also potently reduce visceral sensations. Both CB₁ and CB₂ receptor agonists were shown to reduce basal visceral sensitivity, as well as colitis-induced hypersensitivity to colorectal distension in rats and mice [115, 154-156]. In inflammation the antinociceptive effect of CB receptor agonists was enhanced [115], which is in accordance with their increased inhibitory effect on GI motility and may reflect the overexpression of the CB receptors during inflammation. The exact site of this visceral antinociceptive action still remains to be established, but some evidence points to the importance of peripheral CB receptors. For example, the peripherally restricted CB₁/CB₂ receptor ligand SAB-378 inhibited pain-related responses to colorectal distension similarly to WIN 55,212-2 (which readily penetrates the blood-brain barrier) [154], and LPS- and bradykinin-evoked activation of mesenteric afferents was attenuated by CB₁- and CB₂-receptor stimulation, respectively [157, 158]. Increased epithelial expression of CB₂ receptors due to oral administration of the *Lactobacillus acidophilus* NCFM also resulted in reduced visceral perception [159], but it is still not clear, exactly how these epithelial receptors influence the activity of nociceptive pathways. In short, these preclinical data suggest that cannabinoids may serve as useful tools for alleviating visceral hypersensitivity and relieving abdominal pain in IBD, and this assumption is supported by preliminary clinical studies, in which IBD-patients treated with cannabis reported a statistically significant pain reduction [109, 160].

Approaches to avoid central, CB1 receptor-mediated psychotropic effects

On the one hand, cannabinoids effectively reduce intestinal inflammation and several accompanying symptoms, as discussed above. On the other hand, they can induce adverse psychotropic side effects, including anxiety, panic attacks, paranoia and cognitive impairment, which are primarily mediated by central CB₁ receptors and hamper their utilization in the therapy [161].

In order to exploit the beneficial effects of cannabinoids and avoid their unacceptable side effects, four different therapeutic strategies could be pursued.

Selective activation of CB₂ receptors

The first, and probably most evident approach is the selective stimulation of CB₂ receptors, which does not induce psychoactive effects [151], in spite of the presence of functional CB₂ receptors within the central nervous system [48, 162]. As depicted above, CB₂ receptors are abundant on immune cells, their intestinal expression is increased in inflammation and their activation significantly ameliorates the development of colitis in numerous animal models [96, 105, 114, 133]. Moreover, CB₂ receptor agonists also suppress the inflammation-induced hypermotility [86, 147] and visceral hypersensitivity [115, 155-157]. These data, obtained mainly from animal experiments, foreshadow an important role of CB₂ receptor agonists in the therapy of IBD, but future trials are warranted to confirm their clinical efficacy and safety.

Selective activation of peripheral CB receptors

Another obvious approach to avoid central side effects is to use peripherally restricted drugs. Studies using either such compounds (e.g. SAB-378) or centrally acting drugs together with vagotomy or ganglionic blockade demonstrated that selective activation of peripheral CB receptors is sufficient to control both visceral nociception [154], intestinal motility [62, 91, 131] and secretions [140]. An important question is, however, whether the inflammation itself can also be inhibited by selective peripheral CB receptor modulation. The intestinal localization of the ECS and the increased expression of CB receptors and endocannabinoids in inflammation imply that peripheral CB receptors play an important, if not predominant, role in the regulation of inflammatory processes. Interestingly, the results of two recent studies suggest the opposite. Cluny *et al.* [131] reported that intraperitoneal application of SAB-378 did not influence DSS- or TNBS-induced colitis in mice, which was later confirmed by Fichna *et al.* [128]. In contrast, i.c.v. administration of the same compound afforded protection against TNBS [128]. These findings suggest that centrally located CB receptors are crucial for the anti-inflammatory action of cannabinoids, and also question the feasibility of this second strategy in the IBD therapy. On the other hand, it is worthy of note that SAB-378 was used in a relatively low dose range (0.1 – 1 mg/kg), as compared to the anti-inflammatory doses of several other CB receptor ligands (see Table 4), and it can be raised that higher doses could be protective without inducing relevant central effects. The potential inability of peripherally restricted cannabinoids to inhibit intestinal inflammation is therefore yet to be confirmed.

Increasing the level of endocannabinoids

As mentioned before, pharmacological or genetic blockade of either CB₁ [95], or CB₂ [105, 163] or both receptors [99] aggravated the inflammatory reaction in various colitis models, suggesting the existence of an endogenous cannabinoid tone, which confers protection against an inflammatory insult. Thus, boosting the on-demand (intestinal) synthesis of endocannabinoids and/or preventing their degradation theoretically offers the third possibility to harness the CB receptor-mediated beneficial effects and avoid (or minimize) the undesired central psychotropic effects [52].

Indeed, a number of studies have shown that elevating the level of AEA or 2-AG by inhibiting the AEA uptake mechanism, or their major degrading enzymes FAAH and MAGL results in significant protection against colitis in mice (Table 4). Although these studies did not directly address the analysis of potential central side effects, other studies demonstrated that URB 597 [164] or JZL 184 [165] do not produce antinociception, catalepsy or hypothermia in the same dose range.

Beside inhibiting the inflammation, MAGL inhibitors also improved intestinal barrier functions and delayed GI transit, [94, 98], while the AEA uptake inhibitor VDM 11 reduced visceral hypersensitivity by modulating the firing of intestinal afferent nerves [158]. These findings together corroborate the concept that modulation of the ECS might be a promising way to treat IBD.

Finally, it is worthy of note that the FAAH inhibitors URB 597 and AA-5-HT were less effective than the uptake inhibitor VDM 11 to elevate the intestinal level of AEA, to decrease intestinal inflammation [92] and to suppress the activity of intestinal afferents [158], which suggests that the effect of AEA is mainly terminated by uptake rather than FAAH-mediated breakdown in the GI tract.

Table 5. Elevating the level of endocannabinoids alleviates murine colitis

Animal	Colitis model	Target of block	Drug	Dosage and route of administration	Reference
C57BL/6N mouse	DNBS	FAAH	- (genetic deletion)	-	Massa <i>et al.</i> [95]
CD1 mouse	DSS TNBS	FAAH (+ COX-1, COX-2)	ARN2508	1 – 30 mg/kg, q.d., p.os	Sasso <i>et al.</i> [40]
C57BL/6J mouse	DNBS	AEA uptake FAAH	VDM 11 AA-5-HT	5 mg/kg, q.d., s.c. 10 mg/kg, q.d., s.c.	D'Argenio <i>et al.</i> [92]
C57BL/6N mouse	TNBS	AEA uptake FAAH	VDM 11 URB 597	5 mg/kg, q.d./b.i.d., i.p. 5 mg/kg, q.d./b.i.d., i.p.	Storr <i>et al.</i> [97]
C57BL/6 mouse	TNBS	FAAH	compound 39	10 mg/kg, q.d., i.p.	Andrzejak <i>et al.</i> [166]
C57BL/6 mouse	DSS	FAAH	compounds 10 and 11	10 mg/kg, q.d., i.p.	Tourteau <i>et al.</i> [167]
C57BL/6 mouse	TNBS	MAGL	JZL 184	16 mg/kg, b.i.d., i.p.	Alhouayek <i>et al.</i> [98]

C57BL/6 mouse	TNBS	FAAH	PF-3845	10 mg/kg, q.d., i.p.	Alhouayek <i>et al.</i> [33]
C57BL/6 mouse	TNBS	FAAH	PF-3845	10 mg/kg, q.d./b.i.d., i.p., i.c., p.os	Salaga <i>et al.</i> [106]

Abbreviations: 2-AG: 2-arachidonoylglycerol; AEA: anandamide; b.i.d.: *bis in die* (twice daily); COX: cyclooxygenase; ECS: endocannabinoid system; FAAH: fatty acid amide hydrolase; i.c.: intracolonic; i.p.: intraperitoneally; MAGL: monoacylglycerol lipase; p.os: orally; q.d.: *quaque die* (once daily); s.c.: subcutaneously

Using non-psychotropic phytocannabinoids

The fourth approach to minimize cannabimimetic effects is the use of various phytocannabinoids, such as CBD, CBG or CBC. In general, these compounds exert multiple pharmacodynamic actions, which are not or only partially related to modulation of the ECS (see the review of [124]). CBD does not activate CB₁ and CB₂ receptors (or even antagonizes them), but inhibits or activates several enzymes, transporters or receptors, including FAAH (inhibitor), the AEA and adenosine transporters (inhibitor), PPAR- γ receptor (agonist) or TRPV1 (agonist) [124]. CBD possessed potent anti-inflammatory action in DNBS-, TNBS and LPS-induced intestinal inflammation in mice and rats [93, 122, 130, 168], as well as in human colonic cultures derived from UC patients, in which PPAR- γ was identified as a key receptor [122]. In addition, CBD was also shown to inhibit inflammation-induced hypermotility [169] which, together with the anti-inflammatory action, the lack of psychotropic activity and low toxicity [170], makes it a promising candidate for the treatment of IBD.

Another promising phytocannabinoids are CBG and CBC: the first is a partial agonist of CB₁ and CB₂ receptors as well as an AEA reuptake inhibitor [124], which exerted both preventive and curative effects in DNBS-induced colitis, and also attenuated both nitrite production in macrophages and ROS production in intestinal epithelial cells [171], while CBC is a potent TRPA1 agonist and weak anandamide reuptake inhibitor, which also ameliorated DNBS colitis in mice [172] and reduced croton oil-induced increased motility [89].

SUMMARY AND CONCLUSIONS

The cannabinoid receptors, the endocannabinoids AEA and 2-AG, and proteins responsible for their synthesis and degradation are widely distributed in the GI tract and several data suggest that their expressions are substantially altered during inflammatory processes. Consequently, the ECS can be a potential target to reduce the gastrointestinal mucosal lesions, hemorrhage and inflammation. Direct activation of CB₁ receptors by plant-derived, endogenous or synthetic cannabinoids effectively reduces gastric acid secretion, gastric motor activity, formation of gastric mucosal lesions induced by stress, pylorus ligation, NSAIDs or alcohol, as well as inhibits intestinal inflammation in different murine colitis models. While the gastric protective effect is likely to be mediated by CB₁ receptors, involvement of both CB₁ and CB₂ receptors was shown to inhibit intestinal inflammation. Moreover, indirect activation of cannabinoid receptors through elevation of endocannabinoid levels

by inhibition of their metabolizing enzymes (FAAH, MAGL) or cellular uptake reduced the gastric mucosal lesions induced by NSAIDs and intestinal inflammation in colitis models.

However, besides their beneficial effects on gastrointestinal ulcerative and inflammatory conditions, cannabinoids may induce several adverse effects, first of all the central CB₁ receptor-mediated psychotropic effects. Several attempts have been made to develop CB receptor ligands which devoid of central psychotropic effects, such as peripherally acting ligands, CB₂ receptor selective compounds, or inhibitors of degradation or uptake of endocannabinoids. On the other hand, it also should be considered that activation of central CB₁ or CB₂ receptors may also contribute to some beneficial effects of cannabinoids. Or selective CB₂ receptor activation can alleviate intestinal inflammation, but may not influence the development of gastric mucosal damage. In addition, elevation of the tissue levels of endocannabinoids may increase the formation of cyclooxygenase-, lipoxygenase- and cytochrome P450-derived metabolites, which are bioactive and may have pro-inflammatory properties as well. In spite of these concerns, the numerous experimental data and preliminary clinical studies are convincing, and the ECS represents a promising target in the treatment of inflammatory bowel diseases and gastric mucosal lesions, ulceration and inflammation. The intensive research focusing to develop new structures that modulate the ECS without inducing the central undesired side effects, gives the hope that in the near future safe, effective compound(s) could be translated into clinical praxis.

CONFLICT OF INTEREST

No conflict of interest.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Viktória E. Tóth for her valuable help.

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