
Many beneficial effects of herbal and synthetic cannabinoids on gut motility and inflammation have been demonstrated, suggesting a vast potential for these compounds in the treatment of gastrointestinal disorders. These effects are based on the so-called ‘endocannabinoid system’ (ECS), a cooperating network of molecules that regulate the metabolism of the body’s own and of exogenously administered cannabinoids. The ECS in the gastrointestinal tract quickly responds to homeostatic disturbances by de novo synthesis of its components to maintain homeostasis, thereby offering many potential targets for pharmacological intervention. Of major therapeutic interest are nonpsychoactive cannabinoids or compounds that do not directly target cannabinoid receptors but still possess cannabinoid-like properties. Drugs that inhibit endocannabinoid degradation and raise the level of endocannabinoids are becoming increasingly promising alternative therapeutic tools to manipulate the ECS.

Key Words: Cannabinoid receptors; Colon cancer; Emesis; Inflammatory bowel disease; Irritable bowel syndrome; Rimonabant; THC

THE ENDOCANNABINOID SYSTEM IN THE GASTROINTESTINAL TRACT

Interest in the therapeutic use of the hemp plant Cannabis sativa underwent a renaissance in the early 1960s with the discovery of delta-9-tetrahydrocannabinol (THC) as the major bioactive constituent of cannabis, and in the 1990s with the description of the first cannabinoid (CB) receptor – now known as CB1. Another boost to CB research was the introduction of the concept of the endocannabinoid system (ECS), which consists of the endogenously produced CBs (ie, endoCBs), their receptors (CB receptors), the enzymatic machinery for the production and degradation of endoCBs, and the proteins that regulate uptake and transport of endoCBs (1) (Figure 1). In the past few years, it has become evident that the ECS plays an important role in the pathophysiology of gastrointestinal (GI) diseases and in the protection against GI inflammation (2).

CB receptors

In the GI tract, CB1 expression is present at prejunctional sites of cholinergic, but not nitricergic, neurons of the enteric nervous system (ENS) (3-6), in the mucosa of the stomach and the colon (4,7,8), and in extrinsic fibers that originate from nodose and dorsal root ganglia (9-11). In the ENS, activated CB1 receptors inhibit the release of contractile transmitters and the downstream signalling of P2X purinoreceptors in cholinergic neurons, leading to relaxation of smooth muscle (12,13). Therefore, CB1 appears to exert tonic control over ENS circuits, and operates as a ‘brake’ for neural over-reactivity (14). Manipulation of CB receptors profoundly affects GI motility (15). In the human gut, agonists of CB1 were shown to inhibit muscle contractions in the ileum and the colon (16,17). CB1 agonists also increase food intake and inhibit vomiting, while antagonists of CB1 inhibit food intake and induce vomiting (18-21). In contrast to agonists, antagonists of CB1 increase GI motility in rodents – interestingly, diarrhea was one of the major side effects reported in clinical trials using the CB1 antagonists rimonabant and tavanabant (22-24). The second CB receptor (CB2) has a different distribution in the GI tract and is mainly present in immunocytes (4,25), but has also been observed in colonic epithelium and ENS neurons (26,27).

Novel CB receptors

Many pharmacological effects of CBs cannot be explained solely by the activity of classical CBs (ie, CB1 and CB2), but rather through actions of unknown, novel CB receptors (28). Several G-protein coupled receptors have been suggested to function as non-CB1/CB2 targets (eg, GPR119 and GPR55), which are activated by the endogenous lipids oleoylethanolamide and palmitoylethanolamide, respectively (29). Another potentially novel CB receptor, GPR18, is activated by N-arachidonoylglycerine and abanom cannabidiol (30).

EndoCBs in the GI tract: Synthesis, degradation and reuptake

EndoCBs (as opposed to herbal and synthetic CBs) are produced ‘on demand’ from fatty acid precursors and are released into the extracellular space to bind to their respective receptors (Figure 1). The best-studied endoCBs of the bowel wall are N-arachidonoyl ethanolamine (anandamide [AEA]) and 2-arachidonoylglycerol (2-AG), from which 2-AG is expressed at a higher level (16,31). Both AEA and 2-AG are known to bind to CB receptors; however, AEA also binds to the...
and 2-AG bind to cannabinoid receptors 1 and 2 (CB1, CB2), the transient glycerol (2-AG). After diffusion into the extracellular space, anandamide lyse the final reaction in the production of anandamide. Diacylglycerol phospholipase D (NAPE-PLD) and lysophospholipase D (lyso-PLD) cata-

membrane phospholipids as substrates. N-acyl phosphatidylethanolamine inhibitors. The synthesis of endocannabinoids has several steps involving figure 1)

tetrahydrocannabinol; URB597 Fatty acid amide hydrolase inhibitor; Oleoylethanolamide; PEA Palmitoylethanolamide; THC Delta-9-

cannabinoid receptor GPR55 or GRP119. Actions of the endocannabinoids receptor potential receptor vanilloid 1 receptor (TRPV1) and to the novel cannabinoid receptor GPR55 or GRP119. Actions of the endocannabinoids are terminated by cellular reuptake via an extraneuronal monoamine membrane transporter (EMT) or by passive diffusion. Anandamide is subse-

toratory bowel disease (IBD) (4,8). CB receptors display a certain degree of expression is increased in colonic epithelium of patients with inflamma-

dation. According to experimental models of intestinal inflammation, this increase seems to occur predominantly in myenteric plexus neurons of the ENS and in colonic epithelium (22,25,36,37). However, levels of CB1 remained unaltered in patients with diverticular disease (16) and colorectal carcinoma (38), but were elevated in mucosal biopsies of duodenum from patients with active celiac disease (31). The involve-

capsaicin receptor transient potential vanilloid receptor 1 (TRPV1 formerly known as VR1), which is an important player in gut inflam-

mation (32). The key enzyme for the production of AEA is N-acyl phosphatidylethanolamine phospholipase D, while diacylglycerol lipase is responsible for the synthesis of 2-AG (Figure 1). The fatty acid amide hydrolase (FAAH) is the main enzyme for the degradation of AEA (33), but is also able to break down 2-AG (34). The principal enzyme responsible for the degradation of 2-AG is monoacylglycerol lipase (MAGL) and FAAH, respectively. OEA Oleoylethanolamide; PEA Palmitoylethanolamide; THC Delta-9-

tetrahydrocannabinol; URB597 Fatty acid amide hydrolase inhibitor; VDM11 Reuptake inhibitor

canabis in gi disease

Similar to CBs, endoCB levels increase during inflammation of the GI tract, and it is believed that this increase contributes to GI protection (42). Thus, AEA, but not 2-AG, was found to be elevated in ulcerative colitis patients and rodents with trinitrobenzenesulfonic acid-induced colitis (42). Levels of 2-AG increased in a mouse model of acetylsalicylic-acid-induced colon cancer (43), which is consistent with previous findings in biopsies from patients with adenomatous polyposis (38). Patients with celiac disease reportedly had elevated mucosal levels of AEA and palmitoylethanolamide, which returned to normal when a gluten-free diet was initiated, suggesting a link between the pathophysiology of celiac disease and the ECS (31). Similar to other ECS components, expression and activity of FAAH and MAGL are subject to alterations during inflammation. The number of FAAH-positive cells in the colonic lamina propria was found to be higher in patients with ulcerative colitis than in healthy persons (8). The obser-

vation that blockade of FAAH with the compound URB597 attenuated experim ental colitis in mice also fits well with the hypothesis that high levels of endoCBs, caused by blockade of their degrading enzyme, are protective against inflammation (44). While the presence of an EMT is still not clarified, some reports have demonstrated that the assumed EMT inhibitor VDM11 improved the severity of trinitro-

benzenesulfonic acid-induced colitis (42,44). Nevertheless, little is known about the role of MAGL in the GI tract, except for a study showing high expression of MAGL in immune cells and colon epithelium during human ulcerative colitis (8).

**TARGETS OF THE ECS IN SPECIFIC DISEASES**

**Nausea and vomiting**

Cannabis has been known for years to reduce emesis in patients undergoing cancer chemotherapy. This was recently evaluated in a randomized study (45) in which whole-plant cannabis led to clear improvement in chemotherapy-induced nausea and vomiting when applied in conjunction with known antiemetics. The receptors responsible for the emesis-reducing effects are CB1, TRPV1 and, most likely, CB2 – all of which are located in the brainstem (21,46). Physiologically, AEA controls emesis by maintaining an endogenous tone through stimulation of CB2 (21). This suggests that high levels of AEA could be therapeutically useful to control emesis during pathophysiological states. Increased levels of AEA, for instance, can be generated by EMT blockers or by URB597, which inhibits activity of FAAH, thereby raising AEA levels (47). Because serotonin recepto r antagonists or dexamethasone do not provide effective control of chemotherapy-induced emesis in patients with cancer, alternative therapies, such as CBs, are required. The CB compound nabilone (Cesamet [MEDA Pharmaceuticals, USA]) has already been approved for the treatment of chemotherapy-induced nausea. According to a meta-analysis (48), nabilone and the synthetic THC product dron-

abiol (Marinol [Unimed pharmaceuticals Inc, USA]) were shown to be vastly superior to current antiemetic drugs such as neuroleptics. Nevertheless, the use of CBs in emesis remains unsatisfactory due to severe central side effects, such as anxiety, depression and hallucina-

ations, that occur during treatment. Another caveat in the long-term administration of CBs may be the incidence of paroxysmal vomiting, which has been noted in patients with a history of chronic cannabis use (49).
Gastroesophageal reflux disease and gastric ulcer

Transient lower esophageal sphincter relaxation is caused by a derangement of the sphincter mechanism and underlies the symptoms of gastroesophageal reflux disease in a significant proportion of patients. THC inhibited the increase in meal-induced transient lower esophageal sphincter relaxation and reduced lower esophageal sphincter pressure in humans (50), confirming the results of CB1 agonist studies in animals (51).

IBS

Almost 20% of adults in western countries suffer from IBS, which is a functional GI disorder characterized by abdominal discomfort, bloating, altered bowel habits and the absence of organic abnormalities (52). Its etiology is complex, because it incorporates biological and psychosocial factors. There is principle agreement that alterations in gut motility, disturbances in gut-brain signalling, visceral hypersensitivity and bacterial overgrowth contribute to its pathophysiology (53). A recent study in the Korean population (40) suggested that a polymorphism of the CNR1 gene (which encodes the CB1 receptor) could also play a role in the development of IBS. In addition, an FAAH gene polymorphism (C385A) has been associated with accelerated colonic transit in patients with diarrhea-predominant IBS (D-IBS) and mixed-form IBS (54).

Meal motility and progression of visceral pain within the central nervous system have been extensively investigated in IBS (55). Thus, the occurrence of disturbed phasic contractions of the colon after a meal is typical of IBS patients, ie, colonic transit time is accelerated in D-IBS whereas transit is slower in constipation-predominant IBS (C-IBS) (56,57). Based on the fact that CBs are major relaxant drugs of GI motility and, that peristalsis is under tonic control of CB1 (58), CB1 agonists could treat D-IBS diarrhea while CB2 antagonists could be valuable for the treatment of constipation in C-IBS and of dyspepsia, which frequently overlaps with IBS (59). Despite the absence of organic abnormalities, certain aspects of inflammation are involved in IBS. Elevated levels of the proinflammatory cytokines interleukin (IL)-6 and IL-8 have been detected in the plasma of IBS patients, while those with comorbidities had elevated levels of tumour necrosis factor-alpha and IL-1-beta in addition to IL-6 and IL-8 (60). Furthermore, an epidemiological study (61) revealed a large overlap of patients with microscopic colitis and IBS symptoms. In view of these observations, it would be worthwhile to target CBs during IBS treatment.

It is assumed that exaggerated visceral sensation generated either by central and/or peripheral mechanisms underlie the symptoms of abdominal pain in IBS (62). CBs have been demonstrated to provide analgesia, possibly indicating involvement of the ECS in visceral sensation (63). A very recent study (64), however, indicated that visceral sensitivity to rectal distension was unaffected by THC administration to IBS patients and healthy individuals, arguing against the possibility of using CB agonists to treat visceral hypersensitivity.

TRPV1, which is activated by the endocannabinoids, has been implicated in visceral hypersensitivity to colon distension in rodents (65). This finding is of importance for the human gut, which shows an increased density of TRPV1-immunoreactive fibres in patients with IBS (66). Pharmacological manipulation of TRPV1 may be superior to that of CB1, partly because of the aforementioned reason but also because of the observation that rats treated with the CB2 antagonist rimonabant develop hypogalgesia during repeated colorectal distension—a phenomenon that has not been observed with TRPV1 antagonists (67). In this context, the use of FAAH blockers that raise AEA levels—but also act as TRPV1 antagonists—could be an interesting option for the treatment of visceral hypersensitivity.

IBD

IBD is a chronic and relapsing inflammation of the GI tract, and comprises two clinical forms: Crohn's disease and ulcerative colitis. The etiology of IBD remains unresolved, although it is commonly agreed that it is a multifactorial disease caused by an uncontrolled immune response to microbial antigens (68). There are anecdotal reports (69) that people with IBD experience relief when smoking marijuana, suggesting a protective role for CB receptors and the ECS in intestinal inflammation. An anti-inflammatory role for CB receptors has been demonstrated in several studies of chemically induced colitis in mice (25,36,70) – the ECS may, therefore, play a protective role in IBD (ie, that upregulation of ECS components could positively influence disease progression) (42). Evaluation of colonic biopsies from patients with ulcerative colitis revealed high levels of AEA (42), a large influx of CB1-positive cells (4,8) and an increased expression of MAGL and diacylglycerol lipase in the colonic epithelium (8). The exact mechanisms by which CB receptors and other ECS components confer protection during colitis has not been determined. Because CB2 receptor activity promotes the reconstitution of injured colonic epithelium (4), it is possible that CBs accelerate wound closure during colitis, although an inhibitory effect on the release of proinflammatory cytokines is also conceivable (71).

Genetic susceptibility is a major etiological factor in the development of IBD. Accordingly, polymorphisms in the FAAH and CNR1 genes have been investigated in human IBD. A FAAH gene polymorphism has been detected in patients with D-IBS (54), but not in Crohn's disease (44). A CNR1 gene polymorphism (CNR1 p.Thr453Thr) may be associated with genetic susceptibility in ulcerative colitis and phenotypic changes in Crohn's disease (41). Collectively, results from animal and human studies strongly suggest that pharmacological manipulation of the ECS could be of great therapeutic value in patients with IBD.

Colon cancer

CBs have shifted into the field of cancer research mainly because of their ability to reduce proliferation and induce apoptosis of tumour cells, suggesting a protective role for the ECS in colon cancer patients (38). In colorectal tumour cells, apoptotic and antiproliferative effects are mediated by CB2; however, CB1 receptors may also be involved depending on the cell line tested (38,72). AEA has been shown to inhibit migration of colon carcinoma cells via CB2, and to cause nonapoptotic and non-necrotic cell death in apoptosis-resistant colon cancer cells that depend on the presence of cyclooxygenase-2 (73). Because colorectal tumour cells express high levels of cyclooxygenase-2 (which is known to promote tumourigenesis), AEA could be a valuable therapeutic tool against colon tumours resistant to apoptosis (73). In HT-29 and DLD-1 cells, CB1 and CB2 activation caused apoptosis via TNF-alpha-sensitive ceramide synthesis (74). Similar to GI inflammation, changes in the levels of ECS components also occur during the development of colon cancer: levels of AEA and 2-AG were increased in colorectal adenomas and adenocarcinomas compared with normal mucosa (31). The CB1 receptor may provide the strongest link to colon cancer because CB1, but not CB2, was shown to be downregulated in colon carcinoma (75), indicating that absence of the CB1 receptor promotes tumourigenesis. It should be mentioned that a different study (38) failed to observe a downregulation of CB1 in colonic biopsies of patients with colorectal cancer. Other ECS components, such as FAAH, may also play a role in tumourigenesis, probably via the increase of endocannabinoids. For example, in a mouse model of azoxymethane-induced carcinogenesis (43), the FAAH inhibitor N-arachidonoylsperotonin reduced aberrant crypt foci (early neoplastic lesions) in the colon and increased 2-AG levels. In the APC gene knock-out model (75), mice with an additional deletion in the CNR1 gene or that were subjected to pharmacological blockade of the CB1 receptor, demonstrated a higher colorectal tumour burden than their littermates. The authors also demonstrated an increase in DNA methylation of the CNR1 promoter, indicating that epigenetic silencing of the CNR1 gene may have been responsible for the enhanced growth of tumours. In contrast to these findings, the CB1 antagonist rimonabant inhibited the growth of cancer cells and the development of precancerous lesions in mice (76).

Collectively, the ECS fundamentally affects colon tumourigenesis. Targeting the CB2 receptor or the FAAH enzyme (to raise endocannabinoids) could be helpful in the protection against colorectal cancer.
TABLE 1
Potential therapeutic effects of drugs targeting the endocannabinoid system in gastrointestinal (GI) diseases

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cannabinoid receptor 1</th>
<th>Cannabinoid receptor 2</th>
<th>Cannabinoid receptor 1</th>
<th>Cannabinoid receptor 2</th>
<th>FAAH blockers</th>
<th>Reuptake inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emesis</td>
<td>Reduction of emesis (18,83)</td>
<td>Inhibition of emesis (46)</td>
<td>Inhibition of toxicity-induced vomiting (82)</td>
<td>Inhibition of apomorphine-induced emesis (84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBS</td>
<td>Reduction of visceral pain (63), secretion (5,85), hypermotility and diarrhea (22,86)</td>
<td>Reduction of visceral hypersensitivity (79), secretion (85) and hypermotility (27)</td>
<td>Reduction of GI inflammation (36,44)</td>
<td>Reduction of GI inflammation (42,44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBD</td>
<td>Reduction of intestinal inflammation, visceral pain, hypermotility and diarrhea (22,36,63)</td>
<td>Reduction of inflammation and visceral hypersensitivity (4,25,70)</td>
<td>Increase in GI transit in paralytic and septic ileus (37,87)</td>
<td>Increase in GI transit in septic ileus (87)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileus</td>
<td>Increase in GI transit (50)</td>
<td>Increase in GI transit (88)</td>
<td>Reduction of intestinal transit and hypermotility (22)</td>
<td>Reduction of intestinal transit (90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GERD</td>
<td>Reduction of TLESR (50)</td>
<td>Increase in GI transit (88)</td>
<td>Reduction of GI inflammation (36,44)</td>
<td>Reduction of GI inflammation (42,44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>Increase in GI transit (88)</td>
<td>Reduction of intestinal transit (90)</td>
<td>Reduction of GI inflammation (36,44)</td>
<td>Reduction of GI inflammation (42,44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Reduction of intestinal transit and hypermotility (22)</td>
<td>Reduction of hypermotility (89)</td>
<td>Reduction of intestinal transit (90)</td>
<td>Reduction of intestinal transit (91)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastroparesis</td>
<td>Increase in gastric emptying (92)</td>
<td>Reduction of GI inflammation (36,44)</td>
<td>Reduction of GI inflammation (42,44)</td>
<td>Reduction of GI inflammation (42,44)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate reference(s). FAAH Fatty acid amide hydrolase; GERD Gastroesophageal reflux disease; IBD Inflammatory bowel disease; IBS Irritable bowel syndrome; TLESR Transient lower esophageal sphincter relaxation.

CURRENTLY AVAILABLE CBs FOR CLINICAL USE
Several CBs have already been marketed and are prescribed in certain countries. A combination of THC and cannabidiol is available in Canada under the brand name Sativex (GW Pharmaceuticals, United Kingdom), which is provided as a sublingual oromucosal spray, and prescribed for pain and spasticity in patients with multiple sclerosis. Dronabinol (Marinol [Abbott Laboratories Inc, Canada]) is a synthetic THC that functions as an agonist for both CB1 and CB2. It has been marketed as an appetite stimulant and antiemetic, and is taken orally as a capsule. Nabilone is a synthetic THC analogue that is marketed in the UK, Canada and other countries. It is used for treating patients with chemotherapy-induced nausea and vomiting, and taken orally as a capsule.

POSSIBLE TARGETS AND EXPERIMENTAL DRUGS
Apart from the classical CB receptors, the ECS may hold additional CB-responsive drug targets for GI disease (eg, novel CB receptors such as GPR55, GPR119 and GPR18). CBs acting via these non-CB1/CB2 sites could become promising therapeutic tools in the future. The phytocannabinoids cannabidiol and delta 9-tetrahydrocannabinol have no or only weak affinity for CB receptors, but may have large potential in the treatment and cure of GI diseases. In rodents, cannabidiol improved experimental colitis (77,78) and reduced hypermotility in ileitis (79), while delta 9-tetrahydrocannabinol reduced food intake and body weight (80). Recently, it was demonstrated that the atypical CB compound O-1602 protected against experimental colitis by a mechanism that involved inhibition of neutrophil recruitment to the site of inflammation (81). The authors showed that the beneficial effect of O-1602 did not involve CB1 or CB2, or the novel CB receptor GPR55, suggesting that an unknown non-CB1/CB2 target could have mediated the protective mechanism. An important approach to the pharmacological exploitation of the ECS was initiated by the blockade of FAAH activity. The desired effect is an increase in endocannabinoid levels that drives CB activity and provides protection against inflammation.

This approach has proven to be very effective in animal models of emesis (82), colitis (36,42,44) and colon cancer (43). The same desired effect has been achieved in a mouse model of experimental colitis by application of the EMT blocker VDM11 (44). Table 1 summarizes the targets and potential beneficial effects of CB drugs in various GI diseases.

SUMMARY AND FUTURE DIRECTIONS
Extracts from Cannabis sativa have been used in traditional medicine to treat inflammation and diarrhea. AEA and 2-AG represent the best-studied endocannabinoids and act via classical CB1 and CB2 receptors, as well as via novel CB receptors such as GPR55, GPR119 or GPR18. Endocannabinoids and CB receptors are integrated in the ECS, which displays a high degree of plasticity in GI diseases with the aim of protecting GI homeostasis. The fact that cells produce their own CBs offers a unique opportunity for future drug targeting – either to manipulate endocannabinoid receptor signaling, or to inhibit the degradation of endocannabinoids to increase their levels in the extracellular space. Available studies suggest that CB1 agonists may be useful in emesis, IBD, colon cancer and functional disorders associated with hypermotility and diarrhea, while CB2 agonists may be future drugs used for the treatment of IBD. Additionally, there are strong arguments supporting the use of CBs for the treatment of IBS.

Pharmacological manipulation of the ECS, however, has its problems and caveats. The CB1 antagonist rimonabant (Acomplia [Sanofi-Aventis, United Kingdom]) had already been introduced as an anti-obesity drug, but was withdrawn from the market due to its serious psychosomatic side effects including sedation, drowsiness, depression and paranoia. This exemplifies the primary obstacle to the pharmacological exploitation of CB1 ligands: unwanted central effects that are only overcome if CB1 ligands are prevented from crossing the blood-brain barrier, or if they are chemically modified such that their psychoactive effects are mitigated. It is not certain whether ligands remain fully effective if their actions are restricted only to peripheral CB receptors. The generation of CB1 ligands that predominantly act at peripheral sites will be an
important step toward the clinical use of these drugs. Because expression of CB2 in the peripheral nervous system is low, targeting of CB2 receptors will be less of a problem. The absence of unwanted psychoactivity suggests that CB2 agonists may be useful drugs for GI inflammation. The discovery of novel CB receptors and proteins that regulate endocannabinoid metabolism will expand the definition of the ECS and will offer new therapeutic targets to reduce the problem of unwanted psychoactive effects associated with CB treatment.

ACKNOWLEDGEMENTS: RS is supported by the Austrian Science Fund (FWF grant P22771-B18) and the F. Lanyar Foundation. Work in the laboratory of MS is supported by the Crohn’s and Colitis Foundation of Canada.

REFERENCES

Cannabinoids in gastrointestinal diseases


