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VIEWPOINT

Gut feelings about the endocannabinoid system

V. DI MARZO & F. PISCITELLI

Endocannabinoid Research Group, Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Pozzuoli, Italy

Abstract

Stemming from the centuries-old and well known effects of Cannabis on intestinal motility and secretion, research on the role of the endocannabinoid system in gut function and dysfunction has received ever increasing attention since the discovery of the cannabinoid receptors and their endogenous ligands, the endocannabinoids. In this article, some of the most recent developments in this field are discussed, with particular emphasis on new data, most of which are published in Neurogastroenterology & Motility, on the potential tonic endocannabinoid control of intestinal motility, the function of cannabinoid type-1 (CB1) receptors in gastric function, visceral pain, inflammation and sepsis, the emerging role of cannabinoid type-2 (CB2) receptors in the gut, and the pharmacology of endocannabinoid-related molecules and plant cannabinoids not necessarily acting via cannabinoid CB1 and CB2 receptors. These novel data highlight the multi-faceted aspects of endocannabinoid function in the GI tract, support the feasibility of the future therapeutic exploitation of this signaling system for the treatment of GI disorders, and leave space for some intriguing new hypotheses on the role of endocannabinoids in the gut.

Keywords *cannabinoid, cannabinoid type-1, cannabinoid type-2, endocannabinoid, inflammation, motility, transient receptor potential vanilloid type-1, vanilloid.*

Address for Correspondence
Vincenzo Di Marzo, Endocannabinoid Research Group, Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, Comprensorio Olivetti, 80078 Pozzuoli, Italy.
Tel.: +39 081 867 5093; fax: +39 081 804 1770;
e-mail: vdimarzo@icmib.na.cnr.it
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INTRODUCTION

Apart from being the most widely used recreational drug in the Western world since the 1960s, *Cannabis* has been very popular in Chinese and ayurvedic traditional medicines for centuries.[1] *Cannabis* preparations were applied as palliatives to treat a wide array of health problems, including gastrointestinal (GI) disorders, and extracts from this plant were still indicated for diarrhea a century ago, whereas anecdotal reports exist for their use during dysentery and cholera.[2,3] Although the medicinal as well as psychoactive properties of *Cannabis* were both ascribed, until a few years ago, to the same major component of this plant, i.e. (−)-Δ^9-tetrahydrocannabinol (THC), we now know that several other cannabinoids with fewer psychotropic actions, such as, for example, cannabidiol, may contribute to its pharmacology (reviewed in[4]) (Fig. 1). Nevertheless, studies on the molecular mechanism of action of THC were instrumental in identifying in vertebrates an endogenous signaling system, known as the endocannabinoid system (ECS). This system is active in several tissues, including the GI tract, and comprises at least two G-protein-coupled receptors, the cannabinoid CB1 and CB2 receptors, their endogenous ligands, the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) (Fig. 1), and proteins for the metabolic regulation of endocannabinoid levels (reviewed in[5]). It has also become increasingly clear that endocannabinoids, and anandamide in particular, can activate non-CB1, non-CB2 receptors, the most studied of which is the Transient Receptor Potential Vanilloid type-1 (TRPV1) channel, and that several other endocannabinoid-like molecules, often exhibiting low affinity for CB1 and CB2 receptors, also occur in mammals (reviewed in[6]). Furthermore, we now know that cannabinoids can interact with proteins of the ECS and other targets, in particular TRPV1 and other TRP channels, to the point that many researchers

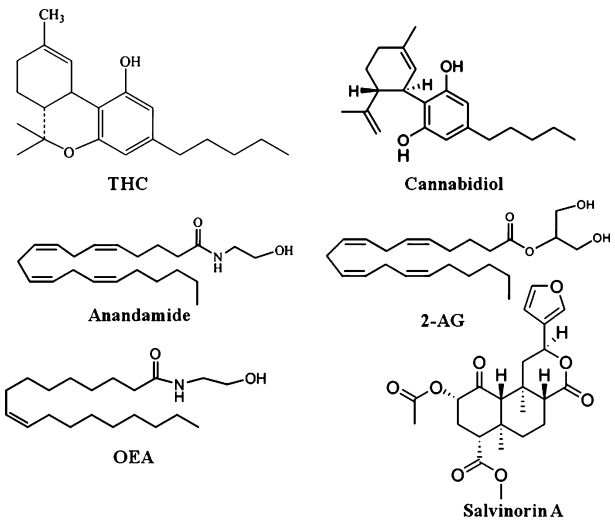

Figure 1 Chemical structures of (−)-Δ^9-tetrahydrocannabinol (THC), (−)-cannabidiol, anandamide, 2-arachidonoyl-glycerol (2-AG), oleoylethanolamide (OEA) and salvinorin A.

now consider these channels as part of the ECS.[6] However, the physiological and pathological significance of these latter discoveries, particularly in the gut, has not yet been investigated. In the present article, we review some of the most recent developments in the research of the function of the ECS in the GI tract, with particular emphasis on data published in the *Journal*.

CB1 RECEPTORS: FROM MOTILITY TO PAIN AND INFLAMMATION

A CB1 'tone' controlling intestinal motility: to be or not to be?

Anatomical and functional evidence suggests the presence of CB1 receptors in neurons of the myenteric plexus in a variety of species, including humans. Activation of prejunctional CB1 receptors reduces excitatory enteric transmission (mainly cholinergic transmission) in different regions of the GI tract, thereby leading to inhibition of motility (reviewed in[7]). There has been a debate as to whether, under physiological conditions, endocannabinoids tonically activate CB1 receptors to control small intestine and colon motility. Initial studies had suggested this possibility based on the observation that: (i) endocannabinoid levels in several districts of the GI tract are sufficient to constitutively activate CB1 receptors; (ii) CB1 antagonists increase motility, which parallels both their stimulation of electrically induced contractions of the guinea pig ileum *in vitro* and the finding of increased motility in CB1 receptor knockout mice; and (iii) blockade of endocannabinoid catabolism with selective inhibitors reduces intestinal and colonic motility.[7] Importantly, as shown in a paper published in the *Journal*, CB1 antagonists as well as 'knockout' of CB1 also modulate other neurophysiological correlates of small intestine propulsion, such as the ascending neuronal contraction following electrical field stimulation of the rat ileum.[8] The use of this set-up allowed the measurement of peristaltic activity and to separate the aboral stimulation site from the oral one, and led the authors to confirm that endocannabinoids and CB1 receptors are physiologically involved in the control of small intestine motility by inhibiting activity at the neuromuscular junction.[8,9] Finally, an elegant *in vitro* study, published again in the *Journal*,[10] showed how, in primary cultures of guinea pig myenteric neurons, CB1 receptor antagonists increase, and agonists decrease, spontaneous network activity as well as the number of: (i) synaptic vesicles being recycled during electrical stimulation; (ii) synaptophysin-immunopositive release sites; and (iii) mitochondria transported towards enteric fiber terminals, which are all specific indicators of prejunctional synaptic activity of myenteric neurons. The effects of the agonists could also be reproduced with two inhibitors of anandamide inactivation, thus again pointing to a constitutive control of myenteric neuron activity by the ECS.[10]

The conclusion from studies using cannabinoid antagonists that endocannabinoids exert tonic modulation of CB1 to inhibit motility was recently questioned on the basis that these compounds are not 'neutral' antagonists, but behave as inverse agonists *in vitro* at concentrations not too far from those corresponding to their K_i's for CB1 receptors. This would suggest that the observed stimulation of motility by these compounds is not the result of their antagonizing the effects of endocannabinoid levels, but instead is due to their stabilization of a receptor conformation that has stronger affinity for the inactive form of the G-protein. It was argued that such possibility could be investigated using a new generation of 'neutral' CB1 antagonists now available,[11,12] as these compounds would produce stimulation of GI transit only in the presence of endocannabinoid levels sufficiently high to activate CB1 receptors. Indeed, Storr *et al.*, reported that one such compound, AM4113, unlike the widely used inverse agonist AM251, was devoid of any stimulatory activity on electrically induced contractions of the mouse ileum *in vitro*, although, somehow paradoxically, it did enhance upper intestinal transit, whereas it produced no stimulation

of whole gut transit.[11] The issue of 'neutral' *vs* 'inverse agonism' is tricky and very difficult to investigate *in vivo*, and a mathematical model has been proposed recently according to which all supposedly 'neutral' antagonists would exert inverse agonism *in vitro* provided that a sufficiently high, but still specific, concentration is used.[13] Nevertheless, a somehow conservative interpretation of these results is that, while there might still be a tonic ECS controlling motility in the upper GI tract, further investigations are needed for the large intestine, although a study showed the depressant effect of an inhibitor of endocannabinoid inactivation on colonic propulsion.[14]

Pancreatitis, irritable bowel syndrome and septic ileus: is CB1 the 'bad guy'?

Seminal studies carried out in the mid-2000s (reviewed in[15]) showed for the first time that the ECS, both in terms of endocannabinoid levels and CB1 receptor expression, is up-regulated with pro-homeostatic and protective function during several different types of experimental small intestine and colon inflammation, and that such up-regulation occurs also in human inflammatory bowel diseases (IBD). Recent reports (see below) have highlighted the role that CB2 receptors may also play in the taming of colonic inflammation and its consequences on motility, a possibility that, given the potential central side effects of CB1 receptor agonists, opens the way to the possible use of non-psychotropic CB2 agonists for the treatment of IBD, along with compounds that inhibit endocannabinoid inactivation. The anti-inflammatory effects of CB1/CB2 agonists have been recently studied also in the pancreas, as described in an article published in the *Journal*, through experiments carried out both *in vitro*, in isolated pancreatic acini, and *in vivo*, in experimental pancreatitis in rats.[16] The authors showed that the cannabinoid receptor agonist, WIN55,212-2, inhibits the release of interleukin-6 (IL-6) and monocyte chemotactic protein-1 (MCP-1) from acinar cells obtained from untreated rats, and reduced serum amylase, pancreatic edema and IL-6 and MCP-1 acinar content in rats with caerulein-induced pancreatitis, whilst also improving pancreatic damage in these animals. Interestingly, however, these protective effects were observed *in vivo* only when the CB1/CB2 agonist was given before the inflammatory stimulus, whereas when WIN55,212-2 was administered afterwards, the pancreatitis was worsened. While the protective effect observed with pretreatment was antagonized by a selective CB2 receptor blocker, the worsening effect was instead antagonized by a CB1-selective blocker.[16] The authors suggested that, in the context of pancreatitis *in vivo*, CB1 activation might concur to oxidative stress or exert chemoattractant activity on macrophages, thus contributing to inflammation. An alternative explanation, however, might lie in the previous observation that, in the same experimental model of pancreatitis, TRPV1 channels participate in inflammation via a sensory mechanism leading to the production of pro-inflammatory peptides,[17] and the same has also been reported for TRP channels of ankirin-1 type (TRPA1) in mice.[18] The possibility exists that WIN55,212-2 might worsen pancreatitis through the sensitization of TRPV1 either via a direct interaction with this channel in a complex with TRPA1,[19] or, indirectly, by activating CB1 receptors.[20,21]

In view of the very efficacious effects of endocannabinoid-based drugs in animal models of visceral pain, the role of the ECS in the control of irritable bowel syndrome (IBS) has also been proposed (reviewed in[22]). This hypothesis is supported by the recent finding of an association between a polymorphism in the *Cnr1* gene encoding for CB1 receptors and the occurrence of IBS in the Korean population.[23] Two studies published in the *Journal* have now addressed this possibility, using completely different approaches. Yüce *et al.*, investigated the effect of CB1 agonists and antagonists/inverse agonists on afferent nerve discharges from rat myenteric neurons stimulated with either serotonin or bradykinin, two mediators known to activate sensory GI afferents and participate in visceral sensitivity.[24] The results were intriguing and perhaps surprising in as much as the authors reported different effects of the agonist WIN55,212-2 (the activity of which on peristaltic activity was shown by the same group to be mostly mediated by CB1 receptors[8]) depending on the type of the stimulus, and possibly in a direction opposite to what expected. While WIN55,212-2 *enhanced* the effect of serotonin and did not influence that of bradykinin, the CB1 inverse agonist SR141716A (rimonabant) *reduced* the effect of bradykinin without affecting that of serotonin. Although counterintuitive, the findings with rimonabant might help to explain some anti-inflammatory effects observed *in vivo* with this compound in mice treated with lipopolysaccharide (LPS).[25] The lack of effect of WIN55,212-2 on bradykinin, and its stimulation of the serotonin effect, instead, might argue against the possible therapeutic use of CB1 agonists in IBS, although of course studies in more specific animal models of this disorder should be carried out before reaching this conclusion. Interestingly, however, in the other study on this issue published very recently in the *Journal*, THC failed to produce any relief of visceral sensitivity after rectal

distension in both healthy volunteers and IBS patients.[26]

Another GI disorder that might be ameliorated by antagonizing, rather than enhancing, the activity of CB1 is ileus, a pathological state consisting of decreased intestinal motility following peritonitis, surgery, or other noxious situations. Mascolo *et al.*, showed that, in acetic acid-induced ileus in mice, reduced intestinal motility was accompanied by increased levels of anandamide compared with control mice, and by over-expression of CB1 receptors in myenteric nerves.[27] Importantly, reduced transit was alleviated by rimonabant, but not by a CB2-selective antagonist, and was worsened by VDM11, a selective inhibitor of anandamide cellular uptake.[27] In an article published in the *Journal*, Li *et al.*, show that not only CB1, but also CB2 receptors might participate in LPS-induced ileus in rats, a model of septic ileus.[28] In this case, the authors monitored not only upper intestinal motility but also spontaneous jejunal myoelectrical activity and IL-6 and tumor necrosis factor (TNF)-α release, and found that antagonism not only of CB1, but also of CB2 receptors, prevented LPS-induced reduction of myoelectrical activity and of upper GI transit. CB1 and CB2 antagonists also tended to reduce the elevation of IL-6 induced by a low dose of LPS.[28] These data indicate that, contrary to ileus induced by a chemical irritant, also CB2 receptors participate in the etiopathology of septic ileus, possibly because of their role in inflammation. Furthermore, they also confirm the role of CB2 receptors in regulating intestinal motility under inflammatory conditions (or perhaps not just [see below]?).

Gastric motility

Although initially neglected, the study of the role of the ECS in the control of gastric motility has been recently investigated in several studies, two of which published in the *Journal*. The existence of a CB1 tone controlling gastric emptying was first suggested by data indicating that: (i) anandamide inhibits this function in a way counteracted by the CB1 receptor antagonist rimonabant, but not by the CB2 receptor antagonist SR144528 or by TRPV1 antagonist 5′-iodoresiniferatoxin; (ii) inhibition of anandamide degradation by fatty acid amide hydrolase (FAAH) also reduces gastric emptying in a way partly reduced by rimonabant; and (iii) rimonabant *per se* increases gastric motility.[29] Interestingly, the inhibitory effect on gastric transit by CB1 activation, as recently investigated by the use of WIN55,212-2 and the CB1 antagonst AM251, does not undergo tolerance following chronic stimulation, unlike the inhibition of upper intestinal or colorectal transit, or the psychotropic effects of chronic CB1 agonism.[30] This finding should open the way to future mechanistic studies investigating the molecular bases of this lack of tolerance, which might be due, for example, to impaired CB1 receptor internalization following repeated stimulation in the stomach. Furthermore, since delayed gastric transit may contribute to satiety and emesis, the authors suggested that the lack of tolerance to inhibition of gastric motility following chronic administration with CB1 agonists might reduce the efficacy of these compounds as anti-anorexiant and anti-emetic therapies.[31] Nevertheless, WIN55,212-2 was recently shown to inhibit gastric myoelectric function, in terms of reduction of the frequency of antral pacemaker activity, both in vehicle- and apomorphine-treated ferrets.[31] Although no CB1 antagonist was used in this study to ascertain the involvement of CB1 receptors in the effects of the compound, these data provided further substantiation to the well-known anti-emetic actions of CB1 receptor activation (reviewed in[32]), and in fact WIN55,212-2 was found by the authors to inhibit also the apomorphine-induced emetic response.[31] On the other hand, contrary to previous findings obtained in the ferret using a different pro-emetic stimulus,[33] the authors found that the FAAH inhibitor URB597 did not reduce retches and vomits induced by the non-selective dopamine receptor agonist.[31]

CB2 RECEPTORS: INFLAMMATION AND BEYOND

The role of the CB2 receptor in the GI tract has been investigated more recently than that of its cognate cannabinoid receptor (reviewed in[34]). It is now clear that CB2 receptors can become activated by elevated endocannabinoid levels in several types of experimental colitis, and that mutated mice lacking this receptor are more sensitive to the inflammatory effects of trinitrobenzene sulfonic acid.[35,36] More recent data, published in the *Journal*, suggest a wider role of this receptor than just the control of gut inflammation. Hillsley *et al.*, reported that the CB2-selective agonist, AM1241, is capable of blocking bradykinin-induced elevation of mesenteric afferent nerve activity, a neurophysiological correlate of small intestine sensitivity, monitored *in vivo* in the mouse jejunum.[37] This inhibitory effect was fully antagonized by a CB2 antagonist and was absent in $CB2^{-/-}$ mice. Given the role of bradykinin in pain and inflammation, this finding was interpreted by the authors as further confirmation of the analgesic and anti-inflammatory effects of CB2 agonists during IBD. However, whilst the observed effect was clearly of peripheral nature, no experiment was performed in

order to assess whether AM1241 was acting at the level of sensory neurons or immune cells.[37] The former possibility should not be excluded since there is evidence, albeit still controversial, that some sensory fibers involved in pain perception do express CB2 receptors.[38]

Although it efficaciously counteracts alterations of intestinal motility during inflammatory conditions,[39] activation of CB2 is known not to affect this function in healthy animals. This is true also for gastric emptying, although a recent study, published in the *Journal*, seems to cast some doubts over this last concept. Indeed, whilst most reports investigating the selective CB2 inverse agonist, SR144528, in the context of gastric emptying found no effect of this compound *per se*, and no antagonism of the inhibitory effects of CB1/CB2 agonists, Abalo *et al.*[40] showed that this compound, at a rather selective dose (1 mg kg^{-1}, i.p.), significantly potentiates the inhibitory effect of the CB1/CB2 agonist, WIN55,212-2, while exerting a little, and not-statistically significant, inhibitory effect *per se*. SR144528 enhancement of WIN55,212-2-induced inhibition of gastric emptying was so strong to result also in delayed emptying of the small intestine, cecum and colon, and it is certainly surprising that such a phenomenon had never been reported before. However, the authors reported that another CB2 inverse agonist, AM630, was not endowed with the same property, thus leaving open the possibility that SR144528 acts via a non-CB2-mediated mechanism.[40] Alternatively, it is possible that the use by Abalo and colleagues of radiographic methods to study GI transit, and of longer observation periods, unmasked a previously undetected and intriguing tonic stimulatory function of CB2 receptors on gastric motility, which could be exploited for the development of new satiety- and weight loss-inducing drugs from CB2 antagonists. Indeed, $CB2^{-/-}$ mice are resistant to weight gain following a high fat diet,[41] which in mice also leads to higher levels of the endocannabinoid 2-AG and lower levels of CB1 receptor expression in the stomach.[29]

ENDOCANNABINOID-RELATED MOLECULES AND PHYTOCANNABINOIDS: NEW MECHANISMS AWAITING TO BE DISCOVERED

The identification of anandamide opened the way to the finding of several anandamide-like molecules that are metabolically related to this endocannabinoid but act mostly via non-CB1 and non-CB2-mediated mechanisms.[6] One of the most studied of these compounds is oleoylethanolamide (OEA) (Fig. 1), an anorexigen mediator acting mostly at peroxisome proliferator-activated receptor-α (PPAR-α) nuclear receptors and, to some extent, TRPV1 channels (reviewed in[42]). Oleoylethanolamide was originally reported to inhibit small intestine motility[43] in a manner insensitive to a TRPV1 antagonist and only partly attenuated by a CB1 antagonist. This effect was shared with other fatty acid amides with little affinity for cannabinoid, PPAR-α and TRPV1 antagonists, and suggested to be mediated in part by inhibition of FAAH through substrate competition, thus potentially leading to elevated levels of endocannabinoids in the small intestine.[43] A study recently appeared in the *Journal*, using different types of mutated mice in which CB1, CB2, or PPAR-α receptors are absent, showed the lack of involvement of these proteins in the effect of OEA, despite the finding of PPAR-α immunoreactivity in the myenteric plexus of the stomach, duodenum, jejunum, ileum and distal colon of the mouse.[44] Moreover, a glucagon-like peptide-1 receptor antagonist did not reverse the inhibitory effect of OEA, which, however, was statistically significant in this study only at i.p. doses fourfold higher than those used in the previous study.[43,44] Interestingly, OEA also inhibits gastric transit, again in a manner not antagonized by cannabinoid, PPAR-α or TRPV1 receptor antagonists, and since the levels of this compound are increased in the stomach of mice subjected to a chronic high fat diet, this effect was suggested to be responsible for the decreased gastric transit observed in these mice, and perhaps to contribute also to a part of the satiety-inducing effects of OEA.[45]

The realization that another cannabinoid constituent of *Cannabis*, namely cannabidiol, possess potential therapeutic properties,[4] suggested the thorough pharmacological exploration of several non-THC cannabinoids also in the GI tract. Cannabidiol has very low affinity for CB1 and CB2 receptors, but was reported to exert either functional enhancement or counteraction of CB1-mediated effects, and to inhibit some of the processes through which endocannabinoids are inactivated, and FAAH in particular.[46] This compound was recently investigated in models of upper intestinal motility disturbances induced by inflammatory stimuli. Thus, cannabidiol reversed croton oil-induced small intestine hypermotility,[47] and worsened LPS-induced hypomotility.[48] While the former effect, based on experiments with CB1 and FAAH inhibitors, was suggested to be due to indirect activation of CB1 receptors subsequent to inhibition of FAAH activity,[47] the effect on LPS-induced hypomotility was accompanied by reduction of FAAH expression in the small intestine (which is up-regulated by LPS), and again antagonized by CB1 receptor blockade.[48] Thus, by

acting as an indirect agonist at CB1 receptors, cannabidiol reproduces some of the effects of selective inhibitors of anandamide hydrolysis or reuptake and ameliorates small intestine motility whilst worsening LPS-induced ileus. Interestingly, cannabidiol also produces anti-inflammatory effects in experimental models of colitis.[49,50] These effects are particularly strong in the mouse and, however, at least in this species, do not seem to involve FAAH inhibition.[49]

Phytocannabinoids have been recently defined as 'any plant-derived natural product capable of either directly interacting with cannabinoid receptors or sharing chemical similarity with cannabinoids, or both'.[51] With this definition in mind, salvinorin A (SA) (Fig. 1), the major active ingredient of *Salvia divinorum* and potent κ-opioid receptor (KOR) agonist, which produces central effects *in vivo* that are partly antagonized by CB1 blockers,[52] but does not bind with appreciable affinity to either CB1 or CB2 receptors,[53] should not be considered a 'phytocannabinoid'. Nevertheless, the GI effects produced by this hallucinogenic compound are antagonized by CB1 inverse agonists. Capasso *et al.*, showed that SA counteracts croton oil-induced hypermotility in a manner attenuated by both KOR and CB1 antagonists,[53] whereas Fichna *et al.* published the results of a thorough investigation of the effects of this compound on GI transit *in vivo* and *in vitro*, and on neurogenic ion transport *in vitro*, in healthy mice.[54] These authors observed that SA inhibits contractions of the mouse stomach, ileum, and colon *in vitro*, and prolongs colonic propulsion and slows upper GI transit *in vivo*, without affecting gastric emptying. It also reduces veratridine-, but not forskolin-, induced epithelial ion transport. The effects of SA on colonic motility *in vitro* were mediated by both KOR and CB1 receptors, as they were inhibited by the antagonists nor-binaltorphimine and AM251, respectively. Perhaps even more intriguing was the finding that AM630, a CB2-selective inverse agonist, also inhibited these effects. However, in the colon *in vivo*, SA actions were almost uniquely mediated by KOR. Finally, the effects of SA on veratridine-mediated epithelial ion transport were inhibited by both nor-binaltorphimine and AM630.[54] These data, bearing in mind the lack of affinity of SA for CB1 and CB2 receptors,[53] point to the existence of a functional cross-talk between KOR and cannabinoid receptors. This possibility is also suggested by the recent finding that CB1 antagonism attenuates the activation of KOR by a selective agonist in a GTPγS binding assay, although SA does not substitute for THC in mice trained to discriminate this compound.[55] It is possible that KOR and CB1 or CB2 receptors form heterodimers with pharmacology different from that of the homodimers, and this could be also a unique way through which CB2 receptors may participate in upper GI motility and epithelial ion transport. Alternatively, KOR and CB1 or CB2 receptors might cross-talk at the level of their signal transduction cascades, as was recently suggested for CB1 and δ-opioid receptors,[56] and previously reported for CB1 and μ-opioid receptors (reviewed in[57]).

CONCLUSIONS

The reports published in *Neurogastroenterology & Motility* and included in this special collection, together with related studies published in other journals over the last 2 years, confirm that the ECS and related emerging signaling systems may play a fundamental role in the control of all aspects of GI physiology and pathology. As with pathological states affecting other vital functions,[5] the available data allow us to predict that strategies that either enhance or curb the activity of the ECS might be both employed for future therapies targeting various GI disorders. Furthermore, the new data discussed in this article allow for speculations on what could be novel physiological and pathological functions in the GI tract of the ECS, particularly at the level of CB2 receptors and TRP channels, and of endocannabinoid-related molecules, while opening the way also to future investigations on non-THC cannabinoids and plant natural products that do not necessarily directly modify the activity of CB1 and CB2 receptors. Future research will tell us if these 'gut feelings' about the ECS will eventually translate into new knowledge of basic and clinical importance.

CONFLICT OF INTERESTS

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