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# The gastrointestinal pharmacology of cannabinoids: an update

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Recent work in the field of gastrointestinal pharmacology of cannabinoids has focused on enteric endocannabinoid and endovanilloid systems and their modulation in pathophysiological conditions. CB<sub>1</sub> receptor immunoreactivity was detected on enteric cholinergic neurones and vasoactive intestinal peptide-containing submucosal ganglion cells, on discrete nuclei of the dorsovagal complex (involved in emesis) and on central and peripheral vagal terminals, thus controlling gastroesophageal reflux and gastrointestinal motility. CB<sub>1</sub> receptor activation by endocannabinoids inhibited induced fluid secretion and inflammation in animal models and reduced proliferation of cultured colorectal cancer cells. Endocannabinoids also activate cannabinoid CB<sub>2</sub> and vanilloid VR1 receptors in certain inflammatory states. Thus endocannabinoid metabolism could provide a useful therapeutic target for many gastrointestinal disorders.

## Addresses

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## Abbreviations

<b>2-AG</b>	2-arachidonoyl glycerol
<b>CB<sub>1</sub>IR</b>	CB <sub>1</sub> receptor immunoreactivity
<b>DMNX</b>	dorsal motor nucleus of the vagus
<b>EFS</b>	electrical field stimulation
<b>FAAH</b>	fatty acid amide hydrolase
<b>NANC</b>	non-adrenergic non-cholinergic
<b>VR1</b>	vanilloid receptor
<b>Δ<sup>9</sup>-THC</b>	Δ <sup>9</sup> -tetrahydrocannabinol

## Introduction

For centuries, various preparations derived from the Indian hemp plant (*Cannabis sativa*) have been used medicinally to treat a wide range of disorders, including some of the gastrointestinal tract. The pharmacology of the active components of cannabis (marijuana) and synthetic cannabinoids indicates that these compounds act via at least two types of cannabinoid receptors, both coupled to G proteins. CB<sub>1</sub> receptors are located primarily

on central and peripheral neurons where they modulate neurotransmitter release, whereas CB<sub>2</sub> receptors are associated with immune function [1–4]. The discovery of endogenous ligands (anandamide and 2-arachidonoyl glycerol [2-AG]) for these receptors indicates the presence of a functional endocannabinoid system.

In addition to anandamide and 2-AG, more endocannabinoids (noladin ether, virodhamine, N-arachidonoyl dopamine) have been isolated, although apart from noladin ether, which reduces defaecation rate in mice [3], their roles in the gastrointestinal tract have not been investigated. Radioligand binding studies were used to determine the relative affinities of cannabinoids for CB<sub>1</sub> and CB<sub>2</sub> receptor binding sites [2]. The resulting K<sub>i</sub> values for the most commonly used cannabinoids and endocannabinoids are shown in Table 1. Similarly, cannabinoid antagonists show selectivity for CB<sub>1</sub> or CB<sub>2</sub> receptor binding sites (Table 1) and have been widely used both to identify cannabinoid-receptor-mediated functional responses to exogenous agonists and, when used alone, to indicate the possible existence of ongoing endocannabinoid tone. The actions of anandamide and 2-AG are terminated through hydrolysis by fatty acid amide hydrolase (FAAH) in microsomes following a carrier-mediated uptake process. Therefore, endocannabinoid activity can be augmented by uptake inhibitors or FAAH inhibitors [3].

The aim of this article is to provide a summary of recent findings in the field to update an earlier review published in this journal [3].

## Localisation of cannabinoid receptors in the gut

The presence of cannabinoid receptors in the gastrointestinal tract has been demonstrated by anatomical and functional evidence. In earlier studies, autoradiography showed the presence of CB<sub>1</sub> receptors in the rat, and immunohistochemistry identified CB<sub>1</sub> receptor immunoreactivity (CB<sub>1</sub>IR) in neural plexuses in cross-sections of pig gastrointestinal tract [5]. Recent studies [6–12, 13\*,14,15] have confirmed colocalisation of CB<sub>1</sub>IR with cholinergic neurones in a variety of species (Table 2) and these constitute the majority of neurones in the gut. In the guinea-pig myenteric plexus, sensory, interneuronal and motoneuronal cell bodies and nerve fibres expressed CB<sub>1</sub> receptors [7], whereas CB<sub>1</sub>IR colocalised with vasoactive intestinal peptide (non-cholinergic) and neuropeptide Y (cholinergic) secretomotor neurones in the submucous plexus. This distribution confirmed the inhibitory effects of SR141716A-sensitive CB<sub>1</sub> receptor activation on motility and secretory processes. *In vivo*,

**Table 1**

**Main cannabinoid receptor ligands evaluated in the digestive tract and their  $K_i$  values (nM) for the *in vitro* displacement of [ $^3$ H]CP55 940, [ $^3$ H]WIN55 212 or [ $^3$ H]HU-243 from CB<sub>1</sub>- and CB<sub>2</sub>-specific binding sites.<sup>a</sup>**

Ligand	Chemistry	CB <sub>1</sub> $K_i$ value	CB <sub>2</sub> $K_i$ value
Non-selective cannabinoid receptor agonists			
Anandamide	Eicosanoid derivative, endogenous ligand	543 (61–89) <sup>b</sup>	581–1940 (279–1930) <sup>b</sup>
2-AG	Eicosanoid derivative, endogenous ligand	58–472	145–1400
HU-210	Dibenzopyrane derivative, synthetic	0.06–0.73	0.17–0.22
CP55 940	Analog of $\Delta^9$ -THC lacking a pyran ring, synthetic	0.58–5	0.69–2.55
$\Delta^9$ -THC	Dibenzopyrane derivative, plant-derived	35.3–80.3	3.9–75.3
WIN55 212-2	Aminoalkylindole, synthetic	1.89–123	0.28–16
Selective CB <sub>1</sub> receptor agonists			
ACEA	Eicosanoid, synthetic	1.4 <sup>b</sup>	>2000
Noladin ether	Lipid-ether, endogenous ligand	21.2	>3000
Methanandamide	Eicosanoid, synthetic	1.4 <sup>b</sup>	815
Selective CB <sub>2</sub> receptor agonists			
JWH-015	Aminoalkylindole, synthetic	383	13.8
Selective CB <sub>1</sub> receptor antagonists			
SR141716A	Diarylpyrazole, synthetic	1.8–12.3	702–13200
AM281	Diarylpyrazole, synthetic	12	4200
Selective CB <sub>2</sub> receptor antagonists			
SR144528	Diarylpyrazole, synthetic	437	0.60

<sup>a</sup>Data extracted from Howlett *et al.* 2002 [2]. The potency of some cannabinoid receptor agonists in inhibiting intestinal motility (i.e. anandamide, WIN55 212-2, cannabinoil,  $\Delta^9$ -THC and CP55,94) can be found elsewhere [22]. <sup>b</sup>With phenylmethylsulphonyl fluoride, a FAAH inhibitor.

noxious stimuli [12,13<sup>\*</sup>,16,17,18<sup>\*\*</sup>,19<sup>\*</sup>], food deprivation [20] or clinically diagnosed colorectal cancer [21<sup>\*\*</sup>] produced measurable increases in the expression of CB<sub>1</sub> receptors (or mRNA), FAAH expression/activity or endocannabinoid levels (Table 3).

### Gastric secretion

Cannabinoids possess a CB<sub>1</sub>-mediated antiulcer activity that might be related to their antisecretory effect [3,22].

Adami *et al.* [8] showed that CB<sub>1</sub> activation by the cannabinoid agonists WIN55 212-2 and HU-210 decreased the acid secretion induced by cholinergically mediated secretagogues, such as 2-deoxy-D-glucose and pentagastrin, but not that induced by histamine, which activates H<sub>2</sub> receptors on parietal cells. Bilateral cervical vagotomy and ganglionic blockade, but not atropine treatment, significantly reduced (but did not abolish) the inhibitory effect of HU-210. These data suggest a predominant location for

**Table 2**

**Localisation of cannabinoid CB<sub>1</sub> receptors in the gastrointestinal tract.**

Animal species/region of the gut	Technique	Findings	Reference
Pig; all regions	IHC	CB <sub>1</sub> on cholinergic cells; some colocalised with substance P, but not with nitrenergic or VIP-positive neurons	[5]
Guinea-pig submucosa	IHC	Colocalises with VIP or NPY secretomotor neurones and with VR1 on paravascular fibres	[6]
Guinea-pig and rat MPLMP	IHC	CB <sub>1</sub> on cholinergic Dogiel types I and II neurones and fibres	[7]
Rat stomach	IHC	CB <sub>1</sub> on cholinergic cells innervating muscle and mucosa	[8]
Rat stomach/ duodenum	IHC	CB <sub>1</sub> on vagal afferents to both tissues; colocalised with cholecystokinin	[9]
Rat nodose ganglion	IHC/ RT-PCR	CB <sub>1</sub> expression on ganglion cells increased with fasting	[9]
Rat stomach	RT-PCR	CB <sub>1</sub> and CB <sub>2</sub> mRNA present	[10]
Mouse; all regions	IHC, RT-PCR	Highest expression in neurones of stomach and colon	[11]
Mouse small intestine $\pm$ acetic acid	IHC	CB <sub>1</sub> on cholinergic myenteric neurones and myenteric and submucosal fibres; some colocalisation with substance P (myenteric)	[12]
Mouse small intestine $\pm$ cholera toxin	IHC, RT-PCR	CB <sub>1</sub> on cholinergic myenteric and submucosal neurones	[13 <sup>*</sup> ]
Mouse colon MPLMP	IHC	CB <sub>1</sub> on cholinergic myenteric and submucosal neurones; no colocalisation on nitrenergic neurones	[14]
Mouse colon	IHC	CB <sub>1</sub> on myenteric cholinergic but not nitrenergic neurones	[15]

IHC, immunohistochemistry; MPLMP, myenteric plexus longitudinal muscle preparation; NPY, neuropeptide Y; RT-PCR, reverse transcription polymerase chain reaction for cannabinoid mRNA; VIP, vasoactive intestinal peptide.

**Table 3****Intestinal endocannabinoids levels, receptor expression and FAAH activity/expression in experimental studies or clinical conditions.**

Experimental/clinical condition	Animal species/region of the gut	Endocannabinoid levels	Cannabinoid expression; FAAH expression/activity	Reference
Croton oil-induced intestinal inflammation	Mouse, small intestine	No changes in endocannabinoid levels; decreased level of PEA	Increased CB <sub>1</sub> expression; increased FAAH activity	[16,17]
Cholera toxin-induced diarrhoea	Mouse, small intestine	Increased levels of anandamide (but not 2-AG)	Increased CB <sub>1</sub> mRNA expression; no changes in FAAH activity	[13*]
Colorectal cancer/ adenomatous polyps	Humans, colon	Increased levels of anandamide and 2-AG	No changes in CB <sub>1</sub> , CB <sub>2</sub> and FAAH expression	[21**]
Acetic acid-induced ileus	Mouse, small intestine	Increased levels of anandamide (but not 2-AG)	Increased CB <sub>1</sub> expression; no changes in FAAH activity	[12]
Toxin A-induced inflammation	Rat, ileum	Increased levels of anandamide and 2-AG	Not measured	[19*]
Colitis induced by DNB or by dextrane sulphate sodium	Mouse, colon	Not measured	Increased number of CB <sub>1</sub> -expressing cells	[18**]
Food deprivation	Rat, small intestine	Increased anandamide levels	Not measured	[20]

DNB, dinitrobenzene; PEA, palmitoylethanolamide.

CB<sub>1</sub> receptors on vagal efferent pathways to the gastric mucosa.

### Intestinal secretion

Recently, endogenous anandamide was found to inhibit, via CB<sub>1</sub> activation, secretion in mice treated with cholera toxin [13\*]. Oral cholera toxin increased fluid accumulation in the mouse small intestine, was associated with increased levels of anandamide, and increased cannabinoid CB<sub>1</sub> mRNA expression. The link between overstimulation of endocannabinoid signalling and an antisecretory role was strengthened by the following pharmacological experiments: the cannabinoid antagonist SR141716A further increased fluid accumulation; the anandamide reuptake inhibitor VDM11 reduced fluid accumulation; and the cannabinoid agonist CP55 940 or the selective CB<sub>1</sub> agonist ACEA inhibited secretion in a CB<sub>1</sub> antagonist-sensitive manner.

Studies monitoring electrolyte movement in muscle-stripped sheets of tissues mounted in Ussing chambers revealed the involvement of CB<sub>1</sub> receptors located on submucosal neurones and extrinsic primary afferents in the submucosa in regulating secretory processes [6]. Indeed, the cannabinoid receptor agonist WIN55 212-2 reduced both electrical field stimulation (EFS) secretion, mediated mainly by acetylcholine release from submucosal secretomotor neurones, and capsaicin-induced secretion, caused by evoked neurotransmitter release from extrinsic primary afferents in the guinea-pig ileum, without affecting the response to forskolin or carbachol, which act directly on the epithelium to elicit secretion [6]. Moreover, in extrinsically denervated tissues, the inhibitory effect of WIN55 212-2 on the response to EFS was lost, suggesting that extrinsic nerves are responsible for the CB<sub>1</sub> receptor sensitivity to EFS.

### Lower oesophageal sphincter

In the lower oesophageal sphincter, CB<sub>1</sub> receptor activation might be beneficial in gastro-oesophageal reflux disease [22]. Functional studies have shown that intravenous administration of the cannabinoid agonists WIN55 212-2 and  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) inhibited (via CB<sub>1</sub> activation) lower oesophageal sphincter relaxation in dogs [23] and ferrets [24], the effect being associated, at least in the dog, with inhibition of gastro-oesophageal reflux [23]. Cannabinoid agonists act via modulation of vagal activity at peripheral and central levels. This is confirmed by the observation that CB<sub>1</sub> receptor staining is present in cell bodies within the dorsal vagal complex (i.e. the area postrema, nucleus of the solitary tract and nodose ganglion) [24].

### Gastrointestinal motility

Cannabinoid agonists act on prejunctional CB<sub>1</sub> receptors to reduce smooth muscle contractility and peristalsis in different regions of the gastrointestinal tract [3], including the human ileum and colon [25\*,26]. Mechanisms by which CB<sub>1</sub> activation reduces contractility include reduction of acetylcholine release from enteric nerves, although other mechanisms, such as inhibition of non-adrenergic non-cholinergic (NANC) excitatory transmission, modulation of adenosine release and activation of apamin-sensitive K<sup>+</sup> channels, have been proposed [3,27,28]. A recent report suggested that CB<sub>1</sub> activation might reduce the apamin component (mediated by ATP or related purines) of the NANC inhibitory transmission [15]. Indeed, WIN55 212-2 significantly reduced the transient atropine-sensitive excitatory junction potential and the fast (apamin-sensitive) inhibitory junction potential, but not the slow (nitric oxide-dependent) inhibitory junction potential, in the mouse colon. The effect of WIN55 212-2 was counteracted by the CB<sub>1</sub> antagonist SR141716A,

which by itself increased the excitatory junction potential, but not the fast or slow inhibitory junction potential [15].

Consistent with these *in vitro* results, several cannabinoid agonists including anandamide, cannabitol, WIN55 212-2, CP55 940 and ACEA (CB<sub>1</sub>-selective), but not JWH-133 (CB<sub>2</sub>-selective), inhibited gastric and intestinal motility in rats and mice; this effect was inhibited by the CB<sub>1</sub> antagonist SR141716A, which by itself increased motility (see also Update), but not by the CB<sub>2</sub> antagonist SR144528 [3,22]. By blocking autonomic ganglia and by giving cannabinoids intracerebroventricularly, it has been shown that at least part of the inhibitory effect of cannabinoid agonists involves enteric CB<sub>1</sub> receptors [3,29].

In mice, immunohistochemical and pharmacological evidence supports a role for endocannabinoids and myenteric CB<sub>1</sub> receptors in regulating colonic motility *in vivo* [14]. The cannabinoid agonists cannabitol, anandamide, WIN 55 212-2 and ACEA decreased motility in an SR141716A-sensitive manner. The hypothesis that local endocannabinoid tone controls propulsion was strengthened by the following findings: unusually high amounts of endocannabinoids were present in the mouse colon; a stimulatory action on colonic propulsion occurred after selective blockade of CB<sub>1</sub> receptors with SR141716A; and an inhibitory effect on colonic propulsion occurred after inhibition of endocannabinoid re-uptake with VDM11.

Finally, palmitoylethanolamide, a fatty acid co-released with anandamide from nerves, reduces gastrointestinal transit in mice through a mechanism independent of CB<sub>1</sub> or CB<sub>2</sub> receptor activation, both in physiological states and in the experimental model of inflammation induced by croton oil [17].

### Motility in pathophysiological states

Depending upon the experimental model, both CB<sub>1</sub> and CB<sub>2</sub> receptors can limit the increase in intestinal motility induced by an inflammatory stimulus. Whereas previous studies showed the importance of overexpressed CB<sub>1</sub>, but not CB<sub>2</sub>, receptors in reducing the increased transit associated with oral croton oil [16], a recent report demonstrated that CB<sub>1</sub>-mediated reduction of gastrointestinal transit was absent in rats treated with an endotoxic inflammatory agent, and was replaced by CB<sub>2</sub>-mediated inhibition of stimulated transit [30••]. Indeed, the CB<sub>2</sub> agonist JWH-133 (but not the CB<sub>1</sub> agonist ACEA) reduced the increase in intestinal transit induced by lipopolysaccharide; this effect was counteracted by the selective CB<sub>2</sub> receptor antagonist AM-630. Indomethacin abolished the inhibitory effect of JWH-133, whereas neither the platelet-activating factor receptor antagonist PCA 4248 nor the inducible nitric oxide synthase inhibitor SATU had any effect. These results indicate that

the CB<sub>2</sub> agonist acted via cyclooxygenase metabolites, independently of inducible nitric oxide synthase and platelet-activating factor.

Mascolo *et al.* [12] provided evidence for the involvement of the enteric endocannabinoid system in the induction of experimental paralytic ileus by peritoneal irritation. Reduced gastrointestinal motility associated with intraperitoneal acetic acid in mice was restored by the CB<sub>1</sub> receptor antagonist SR141716A, whereas it was exaggerated by the cellular re-uptake inhibitor VDM11. Experimental paralytic ileus was characterised by increased intestinal levels of anandamide (but not 2-AG) and an increase in the number and density of CB<sub>1</sub> receptors on cholinergic and substance P-containing neurones. Because CB<sub>1</sub> receptor activation reduced excitatory transmission [3], it was hypothesized that, following peritonitis-induced ileus, overactivity of CB<sub>1</sub> receptors on the enteric cholinergic/substance P neurones reduced the release of both neurotransmitters, with subsequent delayed motility.

### Emesis

Cannabinoids (nabilone,  $\Delta^9$ -THC and levonantradol) are effective antiemetics in humans [31]. CB<sub>1</sub> receptors, as well as FAAH, have been found in areas of the brain involved in emesis, including the dorsal vagal complex and the dorsal motor nucleus of the vagus (DMNX) [32]. CB<sub>1</sub> activation prevented cisplatin- and 5-hydroxytryptophan-induced emesis in the least shrew; opioid- or cisplatin-induced emesis in ferrets; and lithium-induced conditioned rejection reactions (which may reflect a sensation of nausea) in rats (Table 4) [25•,26,32–41]. The CB<sub>1</sub> antagonist SR141716A caused nausea or emesis, or potentiated emetic stimuli, when given alone, suggesting a possible involvement of endocannabinoids. However, the potent ability of the endocannabinoid 2-AG (but not anandamide) to induce emesis in shrews is inconsistent with the putative antiemetic action of the endogenous cannabinoid system (Table 4) [36].

The site of action of cannabinoid agonists has been investigated in ferrets by comparing the effect of  $\Delta^9$ -THC applied locally to the surface of the brain stem with emesis induced by intragastric hypertonic saline and, more importantly, by measuring Fos expression induced by cisplatin in the DMNX and the medial subnucleus of the nucleus of the solitary tract [25•]. Anti-emetic effects of cannabinoids are mediated by CB<sub>1</sub> receptors on pathways related to vagal gastric function either centrally, in the area postrema and dorsal vagal complex, or at the peripheral endings of abdominal vagal efferents. Because chemosensors of the area postrema are located outside the blood–brain barrier, cannabinoids that do not cross this barrier might have antiemetic actions devoid of psychotropic side effects.

Table 4

Antinausea and antiemetic effects of systemically administered cannabinoid receptor agonists.<sup>a</sup>

Animal species	Emetic stimulus	Cannabinoid agonist studied	Comment	Reference
Ferret	Morphine-6- glucuronide	$\Delta^9$ -THC, methanandamide, WIN55 212-2	CB <sub>1</sub> receptors and FAAH were localized in the dorsal vagal complex, consisting of the area postrema, nucleus of the solitary tract and the DMNX in the brainstem. The CB <sub>1</sub> antagonist AM521, given alone, potentiated vomiting induced by morphine-6-glucuronide	[32]
	Cisplatin hypertonic saline	$\Delta^9$ -THC <sup>b</sup>	Fos expression induced by cisplatin in the DMNX and the medial subnucleus of the nucleus of the solitary tract was reduced by $\Delta^9$ -THC rostral to obex ED <sub>50</sub> was 0.05 mg/kg for retches and 0.03 mg/kg for vomits	[25*]
	Morphine	WIN55,212-2		[26]
Least shrew	Cisplatin	CP55 940	The antiemetic effect of CP55 940 (unlike $\Delta^9$ -THC or WIN55 212-2) occurs at motor-suppressant doses	[33]
	Cisplatin	WIN55 212-2	WIN55 212-2 reduced frequency of vomiting at lower doses relative to its sedative actions	[34]
	SR141716A	CP55 940, $\Delta^9$ -THC, WIN55 212-2	The ability of the CB <sub>1</sub> receptor antagonist SR141716A to induce vomiting suggests an important role for endogenous cannabinoids in emetic circuits	[35]
	2-AG	CP55 940, $\Delta^9$ -THC, WIN55 212-2	The effect of 2-AG was blocked by the CB <sub>1</sub> receptor antagonist SR141716A and indomethacin; it has been hypothesized that the emetic response to exogenous 2-AG may reduce an antiemetic tone by displacing an endogenous CB <sub>1</sub> receptor agonist with greater efficacy in brain areas involved emesis. The emetic effect of 2-AG occurs at lower doses relative to its locomotor suppressant action.	[36]
	5-HT, 2-methylserotonin, serotonin	$\Delta^9$ -THC	$\Delta^9$ -THC prevents serotonergically mediated vomiting via mechanisms that probably involve central and peripheral mechanisms	[37]
Musk shrew	Lithium-induced anticipatory nausea and vomiting	$\Delta^9$ -THC	$\Delta^9$ -THC suppresses anticipatory nausea at a dose that did not suppress general activity	[38]
	Cisplatin	$\Delta^9$ -THC	A combined pre-treatment of doses of $\Delta^9$ -THC and the 5-HT <sub>3</sub> antagonist ondansetron that were ineffective alone completely suppressed vomiting and retching. The non-psychotropic marijuana compound cannabidiol suppressed vomiting at low doses (5 mg/kg) and potentiated it at higher doses (40 mg/kg)	[39]
Rat	Lithium-induced conditioned rejection reactions	$\Delta^9$ -THC, HU-210	SR141716A potentiated rejection reaction, suggesting a role of endogenous cannabinoids in modulation of nausea	[40]
	Lithium-induced-conditioned gaping	$\Delta^9$ -THC, HU-210	The CB <sub>1</sub> receptor antagonist SR141716A potentiated lithium-induced conditioned gaping. The non-psychotropic marijuana compound cannabidiol reduced conditioned gaping	[41]

<sup>a</sup>Conditioned rejection reactions reflect a sensation of nausea. <sup>b</sup>Applied at the surface of the brain stem when emesis was induced by hypertonic saline. 5-HT, 5-hydroxytryptamine.



## Intestinal inflammation

Enhanced cannabinoid signalling, as revealed by increased expression of enteric CB<sub>1</sub> receptors and/or increased intestinal endocannabinoid levels, has been observed following intestinal inflammation (Table 3). Massa *et al.* [18\*\*] reported that genetic ablation of CB<sub>1</sub> receptors rendered mice more sensitive to colitis induced by intracolonic dinitrobenzene or oral dextrane sulphate, whereas FAAH-deficient mice, which are expected to have higher levels of anandamide [42], showed significant protection against intestinal inflammation. Moreover, the cannabinoid agonist HU-210 inhibited intestinal inflammation, whereas it was exacerbated by the CB<sub>1</sub> receptor antagonist SR141716A. By contrast, Croci *et al.* [43] showed that SR141716A prevented intestinal inflammation induced by indomethacin in rats and mice.

McVey *et al.* [19\*] have shown that anandamide and 2-AG stimulate intestinal primary sensory neurones via the vanilloid receptor (VR1) to release substance P, resulting in ileitis in rats, and that endocannabinoids might mediate the inflammatory effects of toxin A. Thus, endocannabinoids might have both a protective role (via CB<sub>1</sub> activation) and a deleterious one (via VR1 activation, presumably at higher concentrations) in the intestinal mucosa.

Finally, CB<sub>2</sub> receptor activation by cannabinoids exerts an inhibitory effect on tumour necrosis factor- $\alpha$ -induced interleukin-8 release in human colonic epithelial cells, which are recognized to exert a major influence in the maintenance of intestinal immune homeostasis [44]. These studies open the way to investigate the role of CB<sub>2</sub> receptors in gut inflammation *in vivo*.

## Cancer

Cannabinoids exert palliative effects in cancer patients by preventing nausea, vomiting and pain and by stimulating appetite. In addition, these compounds inhibit the growth of tumour cells in culture and animal models [45]. Ligresti *et al.* [21\*\*] showed that the mucosa of colorectal adenomatous polyps and carcinoma contained higher levels of anandamide and 2-AG, with no difference in the expression of CB<sub>1</sub> and CB<sub>2</sub> receptors or FAAH. Moreover, anandamide, 2-AG and HU-210, as well as inhibitors of anandamide inactivation, preferentially inhibited cell proliferation of CaCo2 cells (which express CB<sub>1</sub> receptors) when compared with DLD-1 cells (which express both CB<sub>1</sub> and CB<sub>2</sub> receptors, but with the CB<sub>1</sub> receptor expressed at lower levels than in CaCo-2 cells). Such data suggest that CB<sub>1</sub> receptors are more important than CB<sub>2</sub> receptors in reducing the proliferation of colorectal carcinoma cells. Consistently, in a study performed on SW 480 colon carcinoma cells, Joseph *et al.* [46] reported that CB<sub>1</sub> activation by anandamide inhibited tumour cell migration, which is of paramount importance in metastasis development.

## Anandamide as an endovanilloid

There is now strong evidence that anandamide is an agonist at VR1 (also known as the TRP1 receptor). VR1 immunoreactivity was identified in cholinergic enteric neurones from the pig and guinea-pig [47–50]. In the latter, cholinergic VR1-positive fibres in the tertiary plexus co-expressed calretinin, substance P and synapsin 1. These findings support VR1-mediated acetylcholine release from motoneurones of the guinea-pig myenteric plexus [51]. By contrast, in rat preparations expressing CB<sub>1</sub> mRNA, VR1-immunoreactivity was confined to fibres only [49–50], and was increased by inflammation in human colon or in the hypertrophic extrinsic nerve bundles in Hirschsprung's disease [52]. However, Bartho *et al.* [53] could find no evidence for anandamide activation of capsaicin-sensitive receptors in the isolated human sigmoid colon.

Ileitis caused by toxin A depends upon VR1 activation by endocannabinoids [19\*]. Begg *et al.* [54] found that VR1 activation by anandamide predominated at higher concentrations, whereas Mang *et al.* [51] found that pEC<sub>50</sub> values for cannabinoid activation were less than for VR1 activation. There is evidence that VR1 activation by anandamide increases ethylene diamine-induced  $\gamma$ -aminobutyric acid release from guinea-pig myenteric plexus by a capsazepine (VR1 antagonist)-sensitive mechanism [54].

Finally, there is *in vitro* evidence that endocannabinoids can act through non-cannabinoid non-vanilloid mechanisms. Mang *et al.* [51] showed that anandamide inhibited electrically evoked acetylcholine release in the guinea-pig ileum via activation of non-cannabinoid, non vanilloid receptors. Also, 2-AG contracted the longitudinal smooth muscle from the guinea-pig distal colon in a tetrodotoxin-sensitive manner [55]. This response was not mimicked by the CB<sub>1</sub> agonist WIN55 212-2 or the VR1 agonist AM 404, and was not inhibited by antagonists of CB<sub>1</sub> or vanilloid receptors. Because the response to 2-AG was partially reduced by the lipoxygenase inhibitor nordihydroguaiaretic acid, it is possible that leukotrienes contribute to the neurogenic contractile action of 2-AG [55].

## Conclusions

The mechanisms of action of exogenous and endocannabinoids on CB<sub>1</sub> receptors, shown by recent imaging techniques, were associated predominantly with the inhibition of excitatory cholinergic (but possibly also NANC) innervation of smooth muscle and secretomotor cells, thus mediating their relaxant, antisecretory and antiulcerogenic properties. Further, CB<sub>1</sub> receptor expression on peripheral vagal terminals and central areas associated with gastrointestinal motility and emesis correlates with the effects of cannabinoids on these two processes. These effects, together with their analgesic, orexigenic and anti-proliferative actions, raise potential for cancer treatment.

Hence, the modulation of endocannabinoid and endovanilloid activity associated with diseased states through the reduction of endocannabinoid uptake or metabolism could prove preferable to systemic psychotropic cannabinoid drugs in the management of gastrointestinal disturbances refractory to more conventional therapies. Whether such putative treatment could be confined to the peripheral circulation or would exhibit central side effects remains to be discovered.

## Update

Recent work has revealed a rapid tolerance to the gastrointestinal pro-kinetic effect of the CB<sub>1</sub> receptor antagonist SR141716A (rimonabant) in mice *in vivo* [56]. Such information is important because of the proposed clinical introduction of SR141716A to induce weight loss and smoking cessation.

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## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
  - of outstanding interest
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