HEP (2005) 168:573–598
© Springer-Verlag 2005

# Cannabinoids and the Digestive Tract

A.A. Izzo[1] · A.A. Coutts[2] (✉)

[1]Department of Experimental Pharmacology, University of Naples Federico II, via D Montesano 49, 80131 Naples, Italy

[2]School of Medical Sciences, College of Life Sciences and Medicine, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, UK
*a.a.coutts@abdn.ac.uk*



**Abstract** In the digestive tract there is evidence for the presence of high levels of endocannabinoids (anandamide and 2-arachidonoylglycerol) and enzymes involved in the synthesis and metabolism of endocannabinoids. Immunohistochemical studies have shown the presence of $CB_1$ receptors on myenteric and submucosal nerve plexuses along the alimentary tract. Pharmacological studies have shown that activation of $CB_1$ receptors produces relaxation of the lower oesophageal sphincter, inhibition of gastric motility and acid secretion, as well as intestinal motility and secretion. In general, $CB_1$-induced inhibition of intesti-

nal motility and secretion is due to reduced acetylcholine release from enteric nerves. Conversely, endocannabinoids stimulate intestinal primary sensory neurons via the vanilloid VR1 receptor, resulting in enteritis and enhanced motility. The endogenous cannabinoid system has been found to be involved in the physiological control of colonic motility and in some pathophysiological states, including paralytic ileus, intestinal inflammation and cholera toxin-induced diarrhoea. Cannabinoids also possess antiemetic effects mediated by activation of central and peripheral $CB_1$ receptors. Pharmacological modulation of the endogenous cannabinoid system could provide a new therapeutic target for the treatment of a number of gastrointestinal diseases, including nausea and vomiting, gastric ulcers, secretory diarrhoea, paralytic ileus, inflammatory bowel disease, colon cancer and gastro-oesophageal reflux conditions.

**Keywords** Cannabinoid receptors · Intestinal motility · Intestinal secretion · Emesis · Intestinal inflammation · Feeding

## 1 Introduction

Preparations of *Cannabis sativa* (Indian hemp) have been used medicinally for the treatment of a variety of gastrointestinal disorders, including gastrointestinal pain, flatulence, gastroenteritis, Crohn's disease, diarrhoea and diabetic gastroparesis (Di Carlo and Izzo 2003). The main psychotropic constituent of *Cannabis sativa* is $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), which exerts its biological effects mainly by activating two G protein-coupled cannabinoid receptors (Pertwee and Ross 2002). These are $CB_1$ receptors, present in central and peripheral nerves, including the enteric nervous system, and $CB_2$ receptors, expressed mainly in immune cells. A general feature of $CB_1$ activation is the reduction of the release of a variety of neurotransmitters (e.g. acetylcholine from enteric nerves), whereas there is currently no evidence for a role for $CB_2$ receptors in the gastrointestinal (GI) tract (Di Carlo and Izzo 2003). Endogenous ligands for the cannabinoid receptors have been identified, the best-known being anandamide, 2-arachidonoyl glycerol (2-AG) (non-selective cannabinoid receptor agonists), noladin ether ($CB_1$ receptor agonist) and virodhamine ($CB_1$ receptor antagonist/$CB_2$ receptor agonist) (De Petrocellis et al. 2004). When released, anandamide and 2-AG are removed from extracellular compartments by a carrier-mediated re-uptake process. Once within the cell, endocannabinoids are hydrolysed by the enzyme fatty acid amide hydrolase (FAAH, also named anandamide amidohydrolase) (Sugiura et al. 2002). Also, 2-AG has been shown to be degraded by monoglyceride lipase (monoacyl glycerol lipase). Both FAAH and monoglyceride lipase have been demonstrated in the intestine (Oleinik 1995; Katayama et al. 1997). In addition to the two cannabinoid receptors, anandamide and 2-AG can also activate transient receptor potential vanilloid subtype 1 (VR1, also known as TRPV1) receptors, the molecular target for the pungent plant compound capsaicin (Zygmunt et al. 1999). Cannabinoid receptors, their endogenous ligands (endocannabinoids) and the proteins involved

in endocannabinoid inactivation (cellular reuptake and enzymatic degradation) are collectively referred to as the endogenous cannabinoid system (ECS).

Although cannabinoids have a wide variety of biological actions, this article will summarise the main studies dealing with the role of the ECS in the gut, including the effects of cannabinoids on emesis.

## 2 The Endogenous Cannabinoid System in the Gut

There are several lines of evidence for a functional ECS in the GI tract. The enteric responses to exogenous cannabinoid drugs show all the hallmarks of a receptor-mediated mechanism, namely, high potency, chemical and stereo-selectivity and structure–activity relationships (Coutts et al. 2000; Coutts and Pertwee 1997; Pertwee 2001). This is coupled with the identification of high-affinity specific binding sites that are saturable at low ligand concentrations and whose characteristics resemble those in the brain (Casu et al. 2003; Ross et al. 1998). The presence of $CB_1$ receptors in rat intestine was demonstrated by radioligand autoradiography with [$^3$H]-CP 55,940 (Lynn and Herkenham 1994) and, more recently, in other species by immunohistochemistry with selective antibodies raised against the N- or C-terminus of the receptor (Casu et al. 2003; Coutts et al. 2002; Kulkarni-Narla and Brown 2000; MacNaughton et al. 2003, 2004; Pinto et al. 2002b; Storr et al. 2004). $CB_1$ receptor protein was found to be associated with cholinergic neurons in both the submucous and myenteric plexuses in the pig, guinea-pig, rat and mouse (Casu et al. 2003; Pinto et al. 2002b). Cholinergic neurons are identified by the presence of cholinacetyl transferase (ChAT), the enzyme responsible for the synthesis of acetylcholine (ACh). The GI tract of the pig, an omnivorous animal, shares many similarities with that of humans. In cross-sections of the porcine gut, colocalisation experiments indicated that $CB_1$ receptors were not expressed by nitrergic nor vasoactive intestinal peptide (VIP)-immunoreactive inhibitory neurons (Kulkarni-Narla and Brown 2000). This was also true in guinea-pig tissue, where all $CB_1$ receptor immunoreactivity was associated with excitatory neurons (Coutts et al. 2002; Kulkarni-Narla and Brown 2000). In primary culture, porcine myenteric $CB_1$-positive cells also expressed $\kappa$- or $\partial$-opioid receptor-like immunoreactivity, in line with their functional sensitivity to opioid ligands (Poonyachoti et al. 2002). Unlike those from the guinea-pig, pig myenteric neurons do not appear to express μ-opioid receptors (Brown et al. 1998). Analysis of the $CB_1$ receptor immunoreactivity of myenteric ganglionic neurons in whole mounts of the guinea-pig myenteric plexus-longitudinal muscle preparation (MP-LMP) allowed visualisation of the cellular morphology, unavailable in cross sections. Images showed $CB_1$ receptor expression in the somata of both Dogiel cell types I and II and punctate expression on neurites of sensory neurons, interneurons and motoneurons, as identified by colocalisation with selective neuronal markers, e.g. calbindin, neurofilament proteins and calretinin (Coutts et al. 2002). There was also a close association with the synaptic protein, synapsin 1, although the limited resolution of the confocal microscope proscribed analysis of the synaptic distribution

of these receptors. Similar results were found in guinea-pig colon and rat ileum preparations, though the quantitative distribution of cholinergic subpopulations varied between tissue types (Coutts 2004; Coutts et al. 2002). In mouse intestine, $CB_1$ receptor labelling was found throughout the GI tract but was most intense in the ileum. In the stomach, the receptors occurred in submucosal ganglia adjacent to the gastric epithelium and also between the smooth muscle layers (Casu et al. 2003; Storr et al. 2004).

$CB_1$ receptor mRNA was detected in the GI tract of the rat, mouse and guinea-pig (Izzo et al. 2003; Storr et al. 2002). In whole gut homogenates from the guinea-pig, $CB_1$ receptor and $CB_2$ receptor-like mRNA transcripts were detected, whereas only $CB_1$ receptor mRNA was found in the myenteric plexus (Griffin et al. 1997). $CB_1$ receptor mRNA was also detected in human colon (Shire et al. 1995). Reverse transcription-polymerase chain reaction (RT-PCR) found both $CB_1$ receptor and $CB_2$ receptor mRNA in the rat stomach and mouse small intestine (Izzo et al. 2003; Storr et al. 2002). The expression level of $CB_1$ receptor mRNA in the latter was upregulated after treatment with cholera toxin (Izzo et al. 2003).

Burdyga and colleagues have recently reported that vagal afferent neurons projecting to the rat stomach and duodenum co-express cholecystokinin (CCK)-1 and $CB_1$ receptors and that the expression of $CB_1$ receptors was increased by withdrawal of food and decreased after refeeding (Burdyga et al. 2004). Changes in $CB_1$ expression were blocked by administration of the CCK-1 receptor antagonist lorglumide (i.p.) and mimicked by administration of CCK (a satiety factor). Rat intestinal anandamide levels also increased after food deprivation (with normalisation after refeeding) and peripheral (but not central) administration of the $CB_1$ antagonist SR141716A-suppressed food intake (Gomez et al. 2002). This is consistent with the observation of an anorexic action of SR141716A in obese humans (Heshmati et al. 2001), suggesting a role for peripheral $CB_1$ receptors in the regulation of feeding.

Of the endogenous ligands mentioned in the introduction, to date the effects of anandamide and its analogues, 2-AG, which was first isolated from canine ileum, and noladin ether, have been investigated in the GI tract. Noladin ether (i.p.) significantly reduces the defaecation rate in mice (Hanus et al. 2001). Interestingly, intestinal anandamide levels increase after food deprivation (Gomez et al. 2002) or in some pathophysiological states, including experimental ileus (Mascolo et al. 2002), cholera toxin-induced diarrhoea (Izzo et al. 2003) and cancer (patients with adenomatous polyps and carcinomas) (Ligresti et al. 2003). Unlike most hydrophilic neurotransmitters, lipophilic endocannabinoids are not stored in synaptic vesicles, but appear to be synthesised and released on demand. Both anandamide and 2-AG are metabolised by the microsomal enzyme FAAH (Katayama et al. 1997; Ueda and Yamamoto 2000) following uptake by selective membrane uptake processes (Izzo et al. 2001c). This uptake carrier mechanism can be inhibited by AM404 (Pertwee 2001) or VDM11 (Izzo et al. 2003; Mascolo et al. 2002), thus preventing metabolism and potentiating any agonist effect. Although FAAH can catalyse both the synthase and hydrolase reactions, the synthase/hydrolase ratio (5.0) is particularly high in the rat small intestine compared with other rat tissues (Katayama et al. 1997). In the same study, FAAH mRNA was confirmed by Northern blots. This enzyme is thought to exert tonic control of local anandamide

levels, and its activity can be reduced by exogenous phenylmethylsulphonyl fluoride (PMSF) (Pertwee et al. 1995) and thus can potentiate the weak agonist activity of anandamide observed in vitro. The presence of specific receptors and endogenous ligands together with their synthetic and catabolic enzymes is strong support for a functional endocannabinoid system in the GI tract.

However, more persuasive evidence for ongoing activity in this system can be derived from the responses to selective $CB_1$ receptor antagonists, mainly SR141716A, but also AM281 or AM630, in the absence of any exogenous agonist. The direction of these responses is invariably opposite to that which would be expected of a cannabinoid receptor agonist and a useful summary is provided by Pinto et al. (2002a). In mice and rats, SR141716A increased motility, transit, defaecation, fluid accumulation and peristaltic contractions (Casu et al. 2003; Colombo et al. 1998; Izzo et al. 1999b, 2003, 2000a,b; Mancinelli et al. 2001; Pinto et al. 2002b). In the rat stomach, SR141716A increased the occurrence of transient lower oesophageal sphincter relaxations (Lehmann et al. 2002), and AM630 potentiated nonadrenergic–noncholinergic (NANC)-evoked relaxations of the fundus (Storr et al. 2002). SR141716A was first shown to increase neurotransmission and ACh release in the guinea-pig MP-LMP (Coutts et al. 2000; Coutts and Pertwee 1997; Pertwee et al. 1996). SR141716A increased maximal ejection pressure during the emptying phase of peristalsis in the guinea pig ileum (Izzo et al. 2000a) and both tonic and phasic motor activity in the colonic longitudinal smooth muscle in the isolated colon of mouse subjected to electrically evoked peristalsis (Mancinelli et al. 2001). These data suggest that peristaltic activity may be tonically inhibited by the endocannabinoid system. Interestingly, the facilitation of peristalsis in the guinea-pig was not observed by Heinemann (1999), suggesting a possible variability of endocannabinoid tone. Facilitatory effects of SR141716A have also been found on the cholinergic and NANC-mediated contractions of the circular muscle (Izzo et al. 1998). However, in view of the reported inverse agonist properties of SR141716A, it is not possible to determine, conclusively, whether its GI actions are due to antagonism of endocannabinoids or to the presence of $CB_1$ receptors that are precoupled to their effector mechanisms (inverse agonism). When tested on human innervated longitudinal muscle strips, SR141716A alone appeared to have no discernable effects (Croci et al. 1998; Manara et al. 2002).

## 3 Gastrointestinal Motility

The predominant action of cannabinoid receptor agonists on the GI tract is an inhibitory effect on gastrointestinal motility, reminiscent of the neuromodulatory response to presynaptic μ-opioid receptor or $\alpha_2$-adrenoceptor activation of cholinergic, postganglionic parasympathetic neurons. The mechanisms underlying this effect have been studied chiefly in the GI tract of small rodents, but also in man and the pig. Here we shall review the findings of studies carried out in vitro (Sect. 3.1, below) and in vivo (Sect. 3.2).

## 3.1 In Vitro Studies

### 3.1.1 Effects on Excitatory Neuronal Pathways

The depressant effects of cannabinoid receptor activation on gastrointestinal motility, as observed in vitro are, principally, the inhibition of evoked cholinergic and NANC contractile responses. Studies have focussed on the inhibition of the peristaltic reflex in segments of whole intestine, on the inhibition of evoked contractions of longitudinal or circular smooth muscle preparations or on the reduction of excitatory neurotransmitter release. Early experiments with $\Delta^9$-THC and some of the more non-polar organic fractions of tincture of *Cannabis* (British Pharmaceutical Codex) indicated the ability of putative cannabinoid receptor agonists to inhibit the contractile responses of the guinea-pig ileum without affecting responses to exogenous ACh (see review by Pertwee 2001). The peristaltic reflex can be reproduced in intestinal segments maintained in vitro. The synthetic cannabinoid receptor agonists WIN 55,212-2 (0.3–300 nM) significantly decreased longitudinal muscle reflex contraction, compliance and maximal ejection pressure, while increasing the threshold pressure and volume required to elicit peristalsis in guinea-pigs (Izzo et al. 2000a). At maximal agonist concentrations, peristalsis was completely prevented. These effects were insensitive to the opioid antagonist naloxone, the $\alpha_2$-adrenoceptor antagonist, phentolamine or the $CB_2$ receptor selective antagonist SR144528 (0.1 μM). However, blockade was achieved with the $CB_1$ receptor-selective antagonist SR141716A (0.1 μM), thus indicating selective activation of cannabinoid $CB_1$ receptors. Methanandamide, a more stable analogue of anandamide, similarly increased the peristaltic pressure threshold and inhibited the ascending circular muscle contraction (Heinemann et al. 1999). The methanandamide response was antagonised by SR141716A and also by apamin and reduced by the NO synthase inhibitor, *N*-nitro-L-arginine methyl ester (L-NAME) implying a possible involvement of apamin-sensitive $Ca^{2+}$-activated $K^+$ channels and nitric oxide (Heinemann et al. 1999). Thus, inhibition by cannabinoids may affect excitatory or inhibitory components of the reflex. These data are consistent with the ability of apamin to reduce cannabinoid $CB_1$-mediated inhibition of cholinergic transmission in the guinea-pig ileum (Izzo et al. 1998).

Paton and Zar (1968) described the dissection of the MP-LMP of the guinea-pig small intestine. This preparation has been invaluable in the study of neurotransmission from the myenteric plexus to the longitudinal smooth muscle, particularly by opioids and cannabinoids, without the confounding effects of the peristaltic reflex. A similar preparation has been used to study neuromuscular transmission to the circular smooth muscle (Izzo et al. 1998). Contractions of MP-LMP induced by electrical field stimulation (EFS) were potently inhibited in a concentration-dependent fashion by the cannabinoid receptor agonists CP 55,940, CP 50,556, WIN 55,212-2, nabilone, CP 56,667, $\Delta^9$-THC and cannabinol (Coutts and Pertwee 1997; Pertwee 2001). This inhibition was competitively and reversibly antagonised by SR141716A, without any effect on the inhibitory responses to normorphine

(μ-opioid receptor agonist) or clonidine ($\alpha_2$-adrenoceptor agonist) and indicated an involvement of $CB_1$ receptors. Therefore, electrically stimulated isolated preparations from the guinea-pig ileum have been used to demonstrate the high potency and stereoselectivity of $CB_1$ receptor agonists (Nye et al. 1985; Pertwee 2001; Pertwee et al. 1992, 1995, 1996). The rank order of potency of agonists correlates well with their affinities for $CB_1$ receptor binding sites in brain tissue and their known psychotropic effects (Pertwee 1997; Pertwee et al. 1992, 1996). The findings that the cannabinoid-induced inhibition of the guinea-pig MP-LMP was augmented by lowering the extracellular calcium concentration or attenuated by incubating the tissue with forskolin, 8-bromo-cyclic adenosine monophosphate (8-bromo-cAMP) or with the phosphodiesterase inhibitor 3-isobutyl-1-methyl xanthine supports the known signal transduction mechanisms for $CB_1$ receptors (Coutts and Pertwee 1998). Similar cannabinoid inhibitory effects on evoked responses have been reported for longitudinal strips of human tissue (Croci et al. 1998).

In a single electrophysiological analysis of intracellular recordings from myenteric neurons of the guinea-pig MP-LMP, WIN 55,212-2 or CP 55,940 were found to inhibit fast and slow excitatory synaptic transmission. In a subset of the neurons tested, this effect was reversed by SR141716A (López-Redondo et al. 1997). Both cholinergic and NANC responses of circular smooth muscle due to EFS were presynaptically inhibited by cannabinoids by a mechanism that was sensitive to SR141716A but not L-NAME or naloxone (Izzo et al. 1998). Only the cholinergic component of this response was sensitive to attenuation by apamin, suggesting the involvement of $Ca^{2+}$-activated $K^+$ channels. The contractile responses to $\gamma$-aminobutyric acid or 5-hydroxytryptamine, agents that release ACh in the intestine, have been shown to be reduced by $\Delta^9$-THC or its analogues (Rosell and Agurell 1975; Rosell et al. 1976). There is some evidence that the release of adenosine, which also inhibits cholinergic neuromuscular transmission in this preparation, is susceptible to modulation via $CB_1$ receptor activation (Begg et al. 2002a).

### 3.1.2 Effects on Inhibitory Neurotransmission

There is evidence that cannabinoids affect enteric inhibitory transmission in rodents. Storr and colleagues used standard intracellular recording techniques to study the effect of cannabinoid drugs on enteric transmission (Storr et al. 2004). Focal electrical stimulation of intrinsic neurons of isolated strips of the mouse proximal colon induced a transient excitatory junction potential (EJP, abolished by atropine) followed by a fast (transient) inhibitory junction potential (fIJP, which represents the apamin-sensitive component of inhibitory transmission) and a slow (sustained) inhibitory junction potential (sIJP, which represents the nitric oxide-dependent component of inhibitory transmission). WIN 55,212-2 significantly reduced EJP and the fIJP (an effect sensitive to the $CB_1$ receptor antagonist SR141716A), but not sIJP; given alone, SR141716A significantly increased EJP, while fIJP and sIJP remained unchanged (Storr et al. 2004). These data suggest that

cannabinoids, via $CB_1$ receptor activation, might reduce the apamin component (which is mediated by ATP or related purines) of the inhibitory transmission in the mouse colon. Other indirect evidence was provided by Heinemann and colleagues, which showed that methanandamide depressed intestinal peristalsis with a mechanism involving, at least in part, facilitation of inhibitory pathways operating via apamin-sensitive $K^+$ channels and nitric oxide (Heinemann et al. 1999) as mentioned above (Sect. 3.1.1). The effects of cannabinoids on the smooth muscle relaxation of the isolated gastric fundus in response to EFS of NANC innervation are not clear. In rat preparations (Storr et al. 2002), both excitatory cholinergic and NANC transmission were reduced by WIN 55,212-2 and anandamide. Only the anandamide responses were antagonised by the cannabinoid receptor antagonist AM630. By itself, AM630 had no effect on the contractile responses but facilitated the relaxation. This latter effect implied the presence of an ongoing endocannabinoid tone that reduced the NANC neurotransmission. In contrast, Todorov et al. (2003) found no response to anandamide (0.1–10 μM) in the isolated gastric fundus of the guinea-pig. Whether this is due to a species difference or whether the anandamide was metabolised before it could produce a measurable response is unclear. No other, more potent cannabinoid receptor agonist was tested in this study, in which evidence suggested that the NANC response was mediated by nitric oxide and cyclic guanosine monophosphate (cGMP).

## 3.2 In Vivo Studies

### 3.2.1 Lower Oesophageal Sphincter

Lower oesophageal sphincter (LOS) relaxation is the chief mechanism for gastro-oesophageal reflux, and thus represents a potential target in the treatment of gastro-oesophageal reflux disease. The principal anatomical components of LOS relaxation are afferent gastric pathways, brainstem integrative centre, and efferent inhibitory pathways to the lower oesophageal sphincter. Functional studies have shown that i.v. administration of the cannabinoid receptor agonists WIN 55,212-2 and $\Delta^9$-THC inhibited (via $CB_1$ receptor activation) LOS relaxation in dogs (Lehmann et al. 2002) and ferrets (Partosoedarso et al. 2003), the effect being associated, at least in the dog, with the inhibition of gastro-oesophageal reflux (Lehman et al. 2002). The $CB_1$ receptor antagonist SR141716A, administered alone, stimulated the LOS relaxation incidence and increased the number of reflux episodes and swallowing rate, suggesting an involvement of endocannabinoids in ongoing suppression of LOS relaxation. The most likely site of action is via the $CB_1$ receptor within the central pattern generator thought to control LOS relaxation. Indeed (1) direct application of $\Delta^9$-THC to the dorsal hindbrain surface attenuated LOS relaxation in ferrets (Partosoedarso et al. 2003) and (2) WIN 55,212-2 reduced the rate of LOS relaxation without altering other characteristics of simultaneous oesophageal contraction in dogs (Lehmann et al. 2002). This is in agreement with the observation that $CB_1$ receptor staining is present in cell bodies within the area

postrema, nucleus tractus solitarius and nodose ganglion (Partosoedarso et al. 2003).

### 3.2.2 Gastric Motility

Experimental studies performed in the rat have shown that $CB_1$ receptors modulate gastric motility. A number of cannabinoid receptor agonists, including $\Delta^9$-THC, WIN 55,212-2, CP 55,940 and cannabinol reduced gastric motility, and this effect was antagonised by the $CB_1$ receptor antagonist SR141716A, but not by the $CB_2$ receptor antagonist SR144528 (Izzo et al. 1999a; Krowicki et al. 1999; Landi et al. 2002). However, in contrast to the small intestine and the colon, SR141716A, administered alone to the stomach, does not produce any inverse cannabimimetic effects. Most notably, intravenous $\Delta^9$-THC inhibited gastric motility and decreased intragastric pressure in anaesthetised rats. Also, the application of $\Delta^9$-THC directly to the dorsal surface of the medulla evoked very slight changes in gastric motor activity. Both ganglionic blockade and vagotomy, but not spinal cord transection, abolished the gastric motor effects of peripherally administered $\Delta^9$-THC (Krowichi et al. 1999). Taken together, these data indicated that the gastric effects of systemically administered $\Delta^9$-THC depend on intact vagal circuitry.

In agreement with animal data, a double-blind randomised placebo-controlled study performed on 13 healthy volunteers showed that oral $\Delta^9$-THC, at a dose used for preventing chemotherapy-induced nausea and vomiting (10 mg/m$^2$), significantly delays gastric emptying of solid food in all subjects (McCallum et al. 1999). In contrast, Bateman (1983) found that, in humans, gastric emptying (monitored by a real real-time ultrasound technique) of liquids was unaffected by $\Delta^9$-THC (0.5 and 1 mg/kg i.v., a dose that produced cannabis-like psychomotor and psychological effects). Apart from the different doses and techniques used to measure motility in the two studies, it should be noted that gastric emptying of liquids is mediated by a different mechanism from emptying of solids.

### 3.2.3 Upper Intestinal Motility

The effect of cannabinoid drugs on upper intestinal motility has been generally studied by evaluating the distance travelled by a non-absorbable marker (e.g. charcoal) from the pylorus to the caecum. Since the marker was given intragastrically, this method does not distinguish between an effect on stomach emptying and transit through the small intestine. Exceptions are the studies by Shook and Burks (1989) and Landi and colleagues (2002) in which the marker was given intraduodenally and motility measured along the small intestine only.

Dewey et al. (1972) first reported that $\Delta^9$-THC delayed gastrointestinal transit in mice. These results were confirmed by Chesher and colleagues (1973) who also showed that $\Delta^8$-THC and three different *Cannabis* extracts dose-dependently

reduced the passage of a charcoal meal in mice. $\Delta^8$-THC and $\Delta^9$-THC were shown to be equipotent, while cannabidiol was inactive (Chesher et al. 1973). In a more complete study, Shook and Burks (1989) showed that $\Delta^9$-THC and cannabinol slowed small intestinal transit when injected intravenously in mice and rats, with $\Delta^9$-THC being equipotent to morphine.

More recently, the ability of cannabinoids to reduce intestinal motility has been related to their ability to activate cannabinoid $CB_1$ receptors. Studies have shown that the endogenous ligand anandamide, the natural agonist cannabinol and the synthetic agonists WIN 55,212-2 and CP 55,940 inhibited gastrointestinal transit motility in mice (Calignano et al. 1997; Colombo et al. 1998b; Izzo et al. 1999b, 2000b, 2001b) an effect counteracted by SR141716A, but not by SR144528. Notably, the inhibitory effect of anandamide was not reduced by the VR1 receptor antagonist capsazepine or by a chronic treatment with capsaicin (a treatment which ablates capsaicin-sensitive afferent neurons) (Izzo et al. 2001a), thus implying that the effect of anandamide on intestinal transit is independent of VR1 receptor activation. SR141716A, but not SR144528, administered alone, increased upper gastrointestinal transit, implying the existence of ongoing background activity of $CB_1$ receptors due to either tonic release of endocannabinoids or precoupled $CB_1$ receptors.

WIN 55,212-2 and cannabinol were significantly more effective when administered intracerebroventricularly (i.c.v.) than when administered intraperitoneally (Izzo et al. 2000b), suggesting a central site of action. However, central $CB_1$ receptors probably contribute little to the effect of peripherally administered cannabinoids, as the effect of i.p.-injected cannabinoid receptor agonists was not modified by the ganglion blocker hexamethonium (Izzo et al. 2000b). The primary role of peripheral $CB_1$ receptors was emphasised by the observation that i.c.v.-administered SR141716A did not significantly reduce the effect of i.p. WIN 55,212-2 (Landi et al. 2002).

Palmitoylethanolamide (PEA) is an endogenous fatty acid ethanolamide that shares some pharmacological actions with $\Delta^9$-THC and with the endocannabinoids anandamide and 2-AG (Lambert et al. 2002). However PEA does not bind to $CB_1$ and $CB_2$ receptors. Capasso and colleagues (2002) reported that i.p.-injected PEA inhibited upper gastrointestinal transit, both in control and in intestine-inflamed mice, and this effect was not antagonised by the cannabinoid receptor antagonists SR141716A or SR144528; moreover, the PEA effect was unaffected by the NO synthase inhibitor L-NAME, the $\alpha_2$-adrenoceptor antagonist yohimbine, the opioid receptor antagonist naloxone or the nicotinic receptor antagonist hexamethonium.

### 3.2.4 Motility in the Colon

Pinto and colleagues provided immunohistochemical and pharmacological evidence supporting a role for the endocannabinoids and myenteric $CB_1$ receptors in regulating colonic motility in vivo in mice (Pinto et al. 2002b). Motility was assessed by measuring the time required for expulsion of a glass bead inserted

2 cm into the distal colon. It was found that the non-selective cannabinoid receptor agonists cannabinol, anandamide and WIN 55,212-2, as well as the selective $CB_1$ receptor agonist arachidonyl-2-chloroethylamide (ACEA) decreased motility in an SR141716A-sensitive manner. The hypothesis for a local endocannabinoid tone controlling propulsion was strengthened by the following findings: (1) unusually high amounts of endocannabinoids were present in the mouse colon; (2) a stimulatory action on colonic propulsion occurred after selective blockade of the $CB_1$ receptor with SR141716A; and (3) an inhibitory effect on colonic propulsion occurred after inhibition of endocannabinoid re-uptake with VDM11. Consistent with these in vivo results, $CB_1$ receptors mediate the antiperistaltic effects of WIN 55,212-2 in the mouse isolated colon (Mancinelli et al. 2001).

## 4 Intestinal Secretion

Taking short circuit current (Isc) as an indicator of net electrogenic ion transport in Ussing chambers, it was shown that the cannabinoid receptor agonist WIN 55,212-2 reduced (via $CB_1$ receptor activation) the secretory response to EFS (which is mediated mainly by acetylcholine release from submucosal secretomotor neurons) and capsaicin (which evokes neurotransmitter release such as acetylcholine by activating extrinsic primary afferents) in the rat (Tyler et al. 2000) and guinea-pig ileum (MacNaughton et al. 2004). The inhibitory effect of WIN 55,212-2 was on the enteric nerves, and not on the epithelial cells, since the Isc response to forskolin and carbachol, which act directly on the epithelium to elicit secretion, were unaffected by WIN 55,212-2 pretreatment. Moreover, in extrinsically denervated segments of guinea-pig ileum, the inhibitory effect of WIN 55,212-2 on the response to EFS was completely lost, suggesting a predominant role for capsaicin-sensitive extrinsic primary afferent nerves that innervate submucosal secretomotor neurons (MacNaughton et al. 2004). In agreement, immunohistochemical studies have shown that $CB_1$ receptors are present on submucosal neurons and extrinsic primary afferent nerves in the submucosa of the small intestine (MacNaughton et al. 2004).

## 5 Gastrointestinal Signs of Tolerance and Dependence

Chronic treatment with cannabinoids can induce a state of tolerance to their inhibitory effects in the gastrointestinal tract. Studies of this phenomenon have been performed predominantly with pieces of tissue excised from chronically treated animals (ex vivo) or on isolated tissues pretreated in vitro with a cannabinoid receptor agonist. These investigations were comprehensively reviewed by Pertwee (2001) and will be summarised here.

In mice, the inhibition of transit by daily oral $\Delta^9$-THC was reduced for up to 19 days post-treatment (Anderson et al. 1975). Similarly, $\Delta^9$-THC (s.c.) for

3 days reduced the sensitivity of mouse MP-LMP to CP 55,940 compared with vehicle-pretreated littermates, when tested 24–28 h after the final injection (Pertwee et al. 1998). In addition, tolerance to $\Delta^9$-THC and CP 55,940 could be demonstrated in the MP-LMP of guinea-pigs receiving $\Delta^9$-THC (10 mg.kg$^{-1}$) i.p. daily for 2 days. In tolerant animals, a reduction was observed in the maxima of agonist log concentration–response curves. This was thought to indicate a down-regulation of receptor expression and/or coupling efficiency (Pertwee et al. 1998).

A form of tolerance was induced in guinea-pig ileal segments in vitro by incubation with WIN 55,212-2 (50 nM) for 5 h. At the end of incubation, the size of electrically evoked contractions was not significantly different from untreated preparations (Basilico et al. 1999). MP-LMP from human ileum or distal jejunum, pretreated for 48 h with (+)- or (–)-WIN 55,212 (10 μM), or vehicle alone at 18°C were tested for their sensitivity to subsequent doses of the active isomer, (+)-WIN 55,212 or to SR141716A (Guanini et al. 2000). Those preparations pretreated with (+)-WIN 55,212 but not (–) WIN 55,212 were insensitive to the inhibitory effects of (+)-WIN 55,212 on the evoked contractions. In addition, SR141716A (1 μM) significantly enhanced the contractile responses in (+)-WIN 55,212-pretreated preparations but not those treated with the (–) isomer or the vehicle, dimethylsulfoxide (DMSO). Earlier reports had shown SR141716A not to have inverse agonist effects on human fresh innervated preparations (Croci et al. 1998). This in vitro inverse response to SR141716A supports the "withdrawal" diarrhoea observed on treatment of $\Delta^9$-THC-tolerant dogs with SR141716A. Work in non-GI tissues suggests that selective kinases may be involved in the development of cannabinoid tolerance (Lee et al. 2003).

Opioids and cannabinoids are among the most widely consumed drugs of abuse in humans; therefore, cross-tolerance or interactivity have been investigated with the two drugs in the GI tract. Basilico et al. (1999) found dextral shifts in the log concentration-response curves for the inhibition of electrically evoked contractions for both (+)-WIN 55,212 and morphine in guinea-pig MP-LMP's that had been preincubated for 5 h with either drug. However, in ex vivo preparations from $\Delta^9$-THC-tolerant guinea-pigs (Pertwee et al. 1998), tolerance was not found to the inhibitory responses to normorphine or clonidine (presynaptic $\alpha_2$-adrenoceptor agonist). Early in vivo studies showed that increases in GI activity (diarrhoea and increased defaecation) and other abstinence signs precipitated by naloxone in morphine-dependent rats could be reduced in a dose-related fashion by $\Delta^9$-THC but not cannabidiol (Hine et al. 1975). Such observations led to hopes for potential treatment of opiate addicts with cannabinoids. An interesting phenomenon observed in the absence of electrical stimulation of morphine-tolerant guinea-pig MP-LMP in vitro is a fast withdrawal contracture in response to naloxone; this is not mimicked by exposure of cannabinoid-tolerant tissues to SR141716A (personal communication). However, the in vitro naloxone "withdrawal" contraction can be significantly reduced by (–)- but not (+)-$\Delta^9$-THC (95 nM) by a presynaptic mechanism (Frederickson et al. 1976). This cross tolerance was confirmed by Morrone et al. (1993) with cannabis extract (equivalent to 5.2 μM $\Delta^9$-THC) in segments of guinea-pig ileum and rabbit jejunum that had been exposed for 5 min to either morphine or the $\kappa$-opioid receptor agonist, U-50,488H. The induction of opioid

and cannabinoid tolerance by incubation of guinea-pig MP-LMP for 5 h with morphine could be prevented by the addition of (+)-WIN 55,212 (50 nM), as shown by loss of the naloxone-precipitated withdrawal response, which is evident as a slow, sustained contraction. The mechanism responsible for this contraction is thought to be a cannabinoid-sensitive release of endogenous ACh, 5-hydroxytryptamine and/or substance P from myenteric neurons into the neuromuscular space (Basilico et al. 1999; Frederickson et al. 1976).

In the CNS, recent work suggests that the endocannabinoid system is involved in the development of opioid tolerance. In morphine-tolerant rats, autoradiographic binding showed a slight but significant reduction in cannabinoid receptor level in the cerebellum and hippocampus, whereas in the limbic area there was a strong decrease (40%) in receptor/G protein coupling (CP 55,940-stimulated [$^{35}$S]GTP$\gamma$S binding). Chronic morphine exposure produced a strong reduction in 2-AG content without changes in anandamide levels in several brain regions (i.e. striatum, cortex, hippocampus, limbic area and hypothalamus) (Vigano et al. 2003).

## 6
## Cannabinoids in Pathological States

### 6.1
### Emesis

Although the antiemetic potential has been recognised for decades, and cannabinoids such as the natural $\Delta^9$-THC or the synthetic cannabinoid nabilone are effectively used in humans (Tramer et al. 2001), the molecular mechanism by which cannabinoids prevent vomiting was only recently ascertained. Immunohistochemistry identified $CB_1$ receptors and FAAH in areas involved in emesis, including the dorsal vagal complex (DVC) (area postrema and nucleus of the solitary tract, NTS) and the dorsal motor nucleus of the vagus (DMN) (Van Sickle et al. 2001). Functional studies aimed at investigating the role of the endogenous cannabinoid system in nausea and emesis have been performed in both vomiting (i.e. least shrews, ferrets) and non-vomiting (i.e. rats) species. Emesis has been induced mainly by cisplatin or opioids in vomiting species, while conditioned rejection reactions, which may reflect a sensation of nausea, have been elicited in rats mostly by lithium chloride.

A number of cannabinoid receptor agonists (given i.p.), including CP 55,940, $\Delta^9$-THC, WIN 55,212-2 and (–)-11-OH-$\Delta$8-THC dimethylheptyl (HU-210) prevented cisplatin-induced emesis in the least shrew (Darmani 2001a,b; Darmani et al. 2003b), opioid-induced emesis in ferrets (Simoneau et al. 2001; Van Sickle et al. 2001) or lithium-induced conditioned rejection reactions in rats (Parker and Mechoulam 2003; Parker et al. 2003). These effects were mediated by $CB_1$ receptors, since they were counteracted by selective receptor antagonists such as SR141716A or AM251. Furthermore, the order of potency for reducing both the frequency of emesis and the percentage of shrews vomiting was CP 55,940>WIN 55,212-2>$\Delta^9$-THC, which is consistent with an action on the $CB_1$ receptor (Darmani 2001a,b).

However, in the least shrew, unlike $\Delta^9$-THC and WIN 55,212-2, the antiemetic activity of CP 55,940 occurs at motor-suppressant doses (Darmani et al. 2003b).

The site of action of cannabinoid receptor agonists has been investigated in ferrets by comparing the effect of $\Delta^9$-THC applied locally to the surface of the brain stem against the emesis induced by intragastric hypertonic saline and, most importantly, by measuring Fos expression induced by cisplatin (Van Sickle et al. 2003). It was found that the anti-emetic effects of cannabinoids are mediated by $CB_1$ receptors on pathways related to vagal gastric function either centrally, in the area postrema and DVC, or at the peripheral endings of abdominal vagal efferents. Specifically, $CB_1$ receptors may be involved at three sites: (1) $CB_1$ receptors on the terminals of primary afferent fibres from the stomach and duodenum could reduce the input indicating intestinal distress and reduce the resulting episodes of emesis, (2) $CB_1$ receptors on the terminals of interneurons within the NTS could reduce the input to the DMN and therefore reduce emesis, and (3) $CB_1$ receptors on the terminals of NTS projection neurons could modulate input from the area postrema or directly reduce excitatory transmission to the DMN. Since the chemosensors of the area postrema are located outside the blood–brain barrier, cannabinoids which do not cross this barrier may have antiemetic actions devoid of psychotropic side-effects.

Experimental evidence suggests that an ECS may be present in the brain stem centres that modulate emesis. Indeed, $CB_1$ receptor antagonists caused emesis when given alone to the least shrews (Darmani 2001a) and also potentiated the emetic response to opioids in the ferret (Van Sickle et al. 2001) as well as lithium-induced nausea in a rat model of nausea (Parker et al. 2003). In the least shrews, the emetic effect of SR141716A was associated with increased forebrain levels of 5-hydroxytryptamine and dopamine (Darmani et al. 2003a). Inconsistent with the putative antiemetic action of the endogenous cannabinoid system is the potent ability of the endocannabinoid 2-AG (but not anandamide) to induce emesis in shrews. This effect is blocked by a non-emetic dose of SR141716A, by the cannabinoid receptor agonist CP 55,940, WIN 55,212-2 or $\Delta^9$-THC and by the cyclo-oxygenase inhibitor indomethacin (Darmani 2002). It has been hypothesised that exogenous 2-AG may elicit its emetic response by acting in brain areas involved in emesis to reduce anti-emetic tone through the displacement from $CB_1$ receptors of an endogenous CB1 receptor agonist with greater efficacy.

Finally, it should be noted that cannabidiol, a natural cannabinoid that does not activate cannabinoid receptors, suppresses lithium-induced conditioned rejection reactions in a rat model of nausea (Parker et al. 2002) and also potentiates the antiemetic effect of ondansetron and $\Delta^9$-THC in the musk shrew (Kwiatkowska et al. 2004).

## 6.2 Gastric Ulcer

The gastric antisecretory and antiulcer activity of cannabinoids was first observed in the late 1970s, when it was found that $\Delta^9$-THC reduced gastric juice volume and

ulcer formation after ligation of the pylorus (Shay rat test) (Sofia et al. 1978). More recently, it has been shown that the cannabinoid receptor agonist WIN 55,212-2 reduced, in an SR141716A-sensitive manner, stress-induced gastric ulcers in rats (Germanò et al. 2001). The antiulcerative effect of WIN 55,212-2 may well be related to its antisecretory effect (Adami et al. 2002; Coruzzi et al. 1999). Indeed, the non-selective cannabinoid receptor agonists WIN 55,212-2 and HU-210 decreased (via $CB_1$ activation) the acid secretion induced by indirectly acting secretagogues, such as 2-deoxy-D-glucose (which stimulated acid secretion by increasing the efferent activity of the vagus nerve) and pentagastrin (which acts partly through a cholinergic pathway). These observations were made in anaesthetised rats in which the secretion induced by the activation of parietal cell $H_2$ receptors by histamine was unaffected, which is consistent with the absence of $CB_1$ receptors on parietal cells (Adami et al. 2002). Bilateral cervical vagotomy and ganglionic blockade, but not atropine treatment, significantly reduced, but did not abolish, the inhibitory effect of HU-210. These results indicate that gastric antisecretory effects of cannabinoids are mediated by suppression of vagal drive to the stomach through activation of $CB_1$ receptors, located on pre- and postganglionic cholinergic pathways. In addition, the ineffectiveness of atropine suggests $CB_1$ receptors may regulate the release of non-cholinergic secretory neurotransmitters.

## 6.3 Intestinal Inflammation

Many patients with inflammatory bowel disease anecdotally report that they experience relief by smoking marijuana (Di Carlo and Izzo 2003). Furthermore, some cannabinoid-based preparations are already being evaluated in clinical trials for the treatment of inflammatory bowel disease (Di Carlo and Izzo 2003). Experimental evidence indicates that the ECS, via $CB_1$ activation, mediates protective pathophysiological signals counteracting intestinal inflammatory responses. Enhancement of the cannabinoid signalling, as revealed by the increased expression of enteric $CB_1$ receptors, has been observed following intestinal inflammation induced by a number of irritants, including intra-colonic dinitrobenzene sulfonic acid (DNBS) (Massa et al. 2004), oral croton oil (Izzo et al. 2001b) and intraperitoneal acetic acid (Mascolo et al. 2002). Massa et al. (2004) showed that colitis induced by intra-colonic DNBS was more severe in $CB_1$-deficient mice than in wild-types littermates, while FAAH-deficient mice (which are expected to have higher levels of anandamide) showed significant protection against intestinal inflammation. Consistent with experimental results obtained with genetically modified mice, the cannabinoid receptor agonist HU-210 inhibited, while the $CB_1$ receptor antagonist SR141716A exacerbated, DNBS-induced colonic inflammation (Massa et al. 2004).

The possible involvement of $CB_2$ receptors in inflammatory bowel disease has been hypothesised on the basis of recent in vitro studies; indeed, cannabinoids exert an inhibitory effect on the expression of tumour necrosis factor (TNF)-$\alpha$-induced interleukin-release from a human colonic epithelial cell line HT-29, and this effect was reversed by the $CB_2$ receptor antagonist SR144528 (Ihenetu et al.

2003). Furthermore, Western immunoblotting revealed an immunoreactive protein in this cell line at a region with a size consistent with that of $CB_2$ receptors (Ihenetu et al. 2003). In contrast with a beneficial role of endocannabinoids, Croci and colleagues (2003) reported that the $CB_1$ receptor antagonist SR141716A prevented the intestinal ulcers and the rise in TNF-$\alpha$ and myeloperoxidase activity (a marker of inflammation) induced by indomethacin in rats, while the $CB_2$ receptor antagonist SR144528 reduced the ulcers only (Croci et al. 2003).

Finally, it should be noted that anandamide and 2-AG have been shown to stimulate intestinal primary sensory neurons via the VR1 receptor to release substance P, resulting in ileitis in rats (McVey et al. 2003) and that endocannabinoids may mediate the inflammatory effects of toxin A. Thus, in the intestinal mucosa, endocannabinoids may have both a protective role (via $CB_1$ receptor activation) and produce deleterious effects (via VR1 receptor activation, presumably at higher concentrations).

## 6.4 Paralytic Ileus

Paralytic ileus (i.e. a "non-mechanical" bowel obstruction observed in response to nociception initiated at the abdominal level) is a common complication whose pathogenesis is still under debate. Mascolo and colleagues (2002) provided evidence that alterations in the enteric endocannabinoid system contribute to the onset of experimental paralytic ileus induced by peritoneal irritation. Reduced gastrointestinal motility associated with intraperitoneal acetic acid in mice was restored by the $CB_1$ receptor antagonist SR141716A, while it was worsened by the anandamide cellular re-uptake inhibitor VDM11. Ileus was characterised by increased intestinal levels of anandamide (but not 2-AG) and by an increase in the number and density of $CB_1$ receptors on acetylcholine- and substance P-containing neurons. Because $CB_1$ receptor activation reduced excitatory transmission, it was hypothesised that, following peritonitis-induced ileus, overactivity of $CB_1$ receptors on the enteric cholinergic/substance P neurons lead to a reduced release of both neurotransmitters, with subsequent delayed motility.

## 6.5 Diarrhoea (Cholera Toxin)

Extracts of *Cannabis* were indicated for the treatment of diarrhoea a century ago in the United States, and there are a number of anecdotal accounts of the effective use of *Cannabis* against dysentery and cholera (Di Carlo and Izzo 2003). Cholera toxin (CT) is the most recognisable enterotoxin causing secretory diarrhoea. The profound dehydrating secretory diarrhoea associated with CT may involve several intestinal secretory mechanisms, including activation of enteric neurons and release and/or synthesis of endogenous secretagogues such as 5-hydroxytryptamine, prostaglandins, tachykinins, vasoactive intestinal peptide, and platelet activating

factor (Lundgren 2002). Oral administration of CT to mice increased fluid accumulation in the small intestine, raised anandamide levels and led to overexpression of $CB_1$ receptor mRNA (Izzo et al. 2003). The non-selective cannabinoid receptor agonist CP 55,940 and the $CB_1$ selective agonist, ACEA inhibited CT-induced fluid accumulation, and this effect was counteracted by SR141716A (but not by SR144528 or by the vanilloid receptor antagonist capsazepine). The antisecretory effect of cannabinoids may involve peripheral mechanisms, since CP 55,940 still inhibited CT-induced fluid accumulation after ganglionic blockade. Furthermore SR141716A enhanced, while the inhibitor of anandamide uptake VDM11 prevented, CT-induced fluid accumulation. These results indicate that CT, as well as enhancing intestinal secretion, causes overstimulation of endocannabinoid signalling with an antisecretory role in the small intestine.

### 6.6 Colorectal Cancer

Endocannabinoids are known to inhibit the proliferation of breast cancer cells, prostate cancer cells, and rat thyroid cancer cells (Bifulco and Di Marzo 2002). Ligresti and colleagues (2003) showed that the levels of anandamide and 2-AG were increased relative to controls in adenomatous polyps and carcinomas, but there appeared to be no differences in the expression of $CB_1$ and $CB_2$ receptors or FAAH levels among the tissues. To determine if cannabinoids affect colorectal cancer cell growth, the authors used CaCo-2 (which express $CB_1$ receptor) and DLD-1 cells (which express both $CB_1$ and $CB_2$ receptors, with $CB_1$ receptor less expressed than in CaCo-2 cells). Anandamide, 2-AG and HU-210, as well as an inhibitor of anandamide inactivation, potently inhibited CaCo2 cell proliferation (relative potencies: HU-210>>anandamide$\geq$2-AG), while DLD-1 cells were less responsive to cannabimimetics than CaCo-2 cells (Ligresti et al. 2003). Such data suggest that $CB_1$ receptors are more important than $CB_2$ receptors in reducing the proliferation of colorectal carcinoma cells. Consistent with this, in a study performed on SW 480 colon carcinoma cells, Joseph and colleagues (2004) reported that anandamide (via $CB_1$ activation) inhibited tumour cell migration, which is of paramount importance in metastasis development (Joseph et al. 2004).

## 7 Anandamide as an Endovanilloid

The unexpected revelation that anandamide is also an agonist at VR1 receptors (Zygmunt et al. 1999) has important implications for the physiological roles of endocannabinoid and VR1 receptor systems. Capsaicin has long been known to affect GI motility (Feher and Vajda 1982; Holzer 2001, 2003). VR1 receptor expression has been associated not only with the oesophagus and GI tract and their related ganglia, but also with areas of the CNS concerned with GI activity. In the rat brain, varicose fibres in the commissural, dorsomedial and gelatinosus

subnuclei of the medial solitary tract and lateral area postrema expressed VR1 immunoreactivity that was reduced after vagotomy above the nodose ganglion (Rumessen et al. 2001). A proportion of nodose ganglionic neurons with afferent terminals in the gastric mucosa and vagal afferents from the GI tract overall were found to express VR1 receptors (Rumessen et al. 2001). These fibres were found to traverse both submucous and myenteric plexuses (Akiba et al. 2001) and many individual fibres coexpressed calcitonin gene-related protein (CGRP) (Rumessen et al. 2001). In the pig ileum, some myenteric VR1-positive neurons also expressed $\delta$-opioid and $\kappa$-opioid receptors (Poonyachoti et al. 2002); also, in primary cultures of porcine myenteric ileal neurons, some cholinergic cells with $\delta$-opioid-like immunoreactivity were also immunopositive for $\kappa$-opioid, cannabinoid or vanilloid receptors (Kulkarni-Narla and Brown 2001).

Anavi-Goffer et al. (2002) identified VR1 immunoreactivity in whole mounts of myenteric plexus preparations from the guinea-pig ileum and colon and rat ileum (Anavi-Goffer and Coutts 2003; Anavi-Goffer et al. 2002). They found VR1 immunoreactivity in a subpopulation (47%) of cholinergic myenteric neurons and fibres in the ganglia, the secondary bundles and tertiary plexus. In guinea-pig myenteric ganglia, intrinsic primary afferent neurons (IPAN's) had the chemical signature ChAT/calbindin/$CB_1$ receptor/VR1 receptor. In contrast, in rat and human preparations, VR1-immunoreactivity was confined to fibres only, and was increased by inflammation in human tissue (Anavi-Goffer and Coutts 2003; Yiangou et al. 2001).

In a study of hypo- and aganglionic regions of the large bowel in Hirschsprung's disease, hypertrophic extrinsic nerve bundles showed intense VR1 immunoreactivity compared with normoganglionic regions, which were similar to control large intestine (Facer et al. 2001). Aganglionic tissue was also associated with weak purine P2X(3)-receptor immunoreactivity compared with normal specimens. It has been proposed that ATP can lower the threshold for activation of VR1 receptors (Tominaga et al. 2001). It is possible that the relative down-regulation of purinergic receptors in Hirschsprung's disease may be associated with an increased release of ATP and sensitisation of the sensory nerves. Ileitis due to *Clostridium difficile* toxin A could be mimicked by the intraluminal administration of anandamide and 2-AG in rats (McVey et al. 2003): this effect was reduced by pretreatment with the selective VR1 receptor antagonist capsazepine but not the cannabinoid receptor antagonists SR141716A or SR144528. Indeed, toxin A resulted in increased tissue levels of anandamide and 2-AG in the ileum that were further enhanced when their metabolism was reduced by FAAH inhibitors. Responses to both toxin A and anandamide were associated with capsazepine-sensitive substance P release and activation of specific natural killer (NK)-1 receptors and antagonised by the NK-1 antagonist L-733060 (McVey et al. 2003). These results suggest that enteritis due to toxin A involves the release of endocannabinoids that activate VR1 receptors on enteric primary afferent sensory neurons, resulting in the release of inflammatory mediators such as substance P. Clearly, the relevance of vanilloid receptor activation involvement in this field needs further investigation.

It may be of interest that VR1-immunoreactive cells in the rat dorsal root ganglia coexpress $CB_1$ receptors (Ahluwalia et al. 2000). VR1 mRNA detected by

RT-PCR from rat ileal tissue showed a protein band corresponding to that for VR1 mRNA from rat brain (Anavi-Goffer et al. 2002). Cholinergic VR1 receptor-positive fibres in the tertiary plexus were found to co-express calretinin, substance P and synapsin 1. These findings support results from functional studies indicating that VR1 activation is related to ACh release from motoneurons (Mang et al. 2001). Mang et al. showed that anandamide facilitates spontaneous ACh release from the myenteric plexus by a capsazepine-sensitive mechanism as measured by the release of [$^3$H]-choline. In the same report, Mang et al. demonstrated that SR141716A caused dextral shifts in the log concentration–response curves to CP 55,940 or anandamide for their inhibitory effects on cholinergic transmission. The relative activities of anandamide at $CB_1$ and VR1 receptors in this tissue are concentration dependent (Begg et al. 2002b). Begg's group found that VR1 receptor activation predominated at higher concentrations, whereas Mang et al. found $pEC_{50}$ values for cannabinoid receptor activation to be less than for vanilloid receptor activation. There is also some controversy as to whether anandamide inhibits ACh release via a $CB_1$ or a non-$CB_1$cannabinoid receptor mechanism, since the $K_B$ values differ for the antagonism by SR141716A of CP 55,940 and anandamide (Mang et al. 2001). Whether this difference can be explained by the concomitant effects on ACh release via a VR1-mediated process and/or is due to anandamide metabolism remains to be resolved. There is evidence that VR1 receptor activation by anandamide increases ethylene diamine-induced $\gamma$-aminobutyric acid (GABA) release from guinea-pig myenteric plexus by a capsazepine-sensitive mechanism (Begg et al. 2002b). However, it should be noted that no evidence for an activation of capsaicin-sensitive receptors by anandamide has been observed in the human sigmoid colon (Bartho et al. 2002).

Finally, 2-AG has been found to induce contractions in the longitudinal smooth muscle from the guinea-pig distal colon in vitro in a tetrodotoxin-sensitive manner. This response was mimicked by anandamide, but not by the cannabinoid receptor agonist WIN 55,212-2 or the vanilloid receptor agonist AM404 and was not inhibited by antagonists of cannabinoid or vanilloid receptors (Kojima et al. 2002). Since the response to 2-AG was partially reduced by the lipoxygenase inhibitor nordihydroguaiaretic acid, it is possible that leukotrienes may contribute to the neurogenic contractile action of 2-AG.

## 8 Conclusion

There is now substantial evidence for the presence of endocannabinoid and endovanilloid systems in the GI tract. The anti-inflammatory, anticancer, antiulcerogenic and antiemetic responses to $CB_1$ receptor activation holds promise for the future management of gastrointestinal diseases. Thus, exploitation of the endocannabinoid system by facilitation at sites of endocannabinoid activity by preventing cellular re-uptake or reducing EC degradation may enhance beneficial endocannabinoid effects without the psychotropic side-effects found with systemic administration of exogenous cannabinoids. Manipulation of the endocannabinoid

system, rather than the administration of exogenous cannabinoids, would also lessen the possibility of adverse pharmacokinetic effects or the development of tolerance to or dependence on exogenous cannabinoids. The upregulation of VR1 receptor expression and increased tissue levels of endocannabinoids in inflammatory conditions may have implications for possible therapeutic applications of endovanilloid modulation in a variety of inflammatory gastric (ulceration and oesophageal reflux) and bowel conditions in the future. Clearly, further exploration of the gastrointestinal EC system is likely to produce worthwhile results.

## References

Adami M, Frati P, Bertini S, Kulkarni-Narla A, Brown DR, de Caro G, Coruzzi G, Soldani G (2002) Gastric antisecretory role and immunohistochemical localization of cannabinoid receptors in the rat stomach. Br J Pharmacol 135:1598–1606

Ahluwalia J, Urban L, Capogna M, Bevan S, Nagy I (2000) Cannabinoid 1 receptors are expressed in nociceptive primary sensory neurons. Neuroscience 100:685–688

Akiba Y, Nakamura M, Ishii H (2001) Immunolocalization of vanilloid receptor-1 (VR-1) in CGRP-positive neurons and interstitial cells of Cajal in the myenteric plexus of the rat gastrointestinal tract. Gastroenterology 120:1721

Anavi-Goffer S, Coutts AA (2003) Cellular distribution of vanilloid VR1 receptor immunoreactivity in the guinea-pig myenteric plexus. Eur J Pharmacol 458:61–71

Anavi-Goffer S, McKay MG, Ashford MLJ, Coutts AA (2002) Vanilloid receptor type 1-immunoreactivity is expressed by intrinsic afferent neurones in the guinea-pig myenteric plexus. Neurosci Lett 319:53–57

Anderson PF, Jackson DM, Chesher GB, Malor R (1975) Tolerance to the effects of delta-9-tetrahydrocannabinol in mice on intestinal motility, temperature and locomotor activity. Psychopharmacologia 43:31–36

Bartho L, Benko R, Lazar Z, Illenyi L, Horvath OP (2002) Nitric oxide is involved in the relaxant effect of capsaicin in the human sigmoid colon circular muscle. Naunyn Schmiedebergs Arch Pharmacol 366:496–500

Basilico L, Parolaro D, Colleoni M, Costa B, Giagnoni G (1999) Cross-tolerance and convergent dependence between morphine and cannabimimetic agent WIN 55,212-2 in the guinea-pig ileum myenteric plexus. Eur J Pharmacol 376:265–271

Bateman DN (1983) Delta-9-tetrahydrocannabinol and gastric emptying. Br J Clin Pharmacol 15:749–751

Begg M, Dale N, Llaudet E, Molleman A, Parsons ME (2002a) Modulation of the release of endogenous adenosine by cannabinoids in the myenteric preparation of the guinea-pig plexus-longitudinal muscle ileum. Br J Pharmacol 137:1298–1304

Begg M, Molleman A, Parsons M (2002b) Modulation of the release of endogenous gamma-aminobutyric acid by cannabinoids in the guinea pig ileum. Eur J Pharmacol 434:87–94

Bifulco M, Di Marzo V (2002) Targeting the endocannabinoid system in cancer therapy: a call for further research. Nat Med 8:547–550

Brown DR, Poonyachoti S, Osinski MA, Kowalski TR, Pampusch MS, Elde RP, Murtaugh MP (1998) Delta-opioid receptor mRNA expression and immunohistochemical localization in porcine ileum. Dig Dis Sci 43:1402–1410

Burdyga G, Lal S, Varro A, Dimaline R, Thompson DG, Dockray GJ (2004) Expression of cannabinoid CB1 receptors by vagal afferent neurons is inhibited by cholecystokinin. J Neurosci 24:2708–2715

Calignano A, La Rana G, Makriyannis A, Lin SY, Beltramo M, Piomelli D (1997) Inhibition of intestinal motility by anandamide, an endogenous cannabinoid. Eur J Pharmacol 340:R7–R8

Capasso R, Izzo AA, Fezza F, Pinto A, Capasso F, Mascolo N, Di Marzo V (2001) Inhibitory effect of palmitoylethanolamide on gastrointestinal motility in mice. Br J Pharmacol 134:945–950

Casu MA, Porcella A, Ruiu S, Saba P, Marchese G, Carai MA M, Reali R, Gessa GL, Pani L (2003) Differential distribution of functional cannabinoid CB1 receptors in the mouse gastroenteric tract. Eur J Pharmacol 459:97–105

Chesher GB, Dahl CJ, Everingham M, Jackson DM, Marchant Williams H, Starmer GA (1973) The effect of cannabinoids on intestinal motility and their antinociceptive effect in mice. Br J Pharmacol 49:588–594

Colombo G, Agabio R, Lobina C, Reali R, Gessa GL (1998) Cannabinoid modulation of intestinal propulsion in mice. Eur J Pharmacol 344:67–69

Coruzzi G, Adami M, Coppelli G, Frati P, Soldani G (1999) Inhibitory effect of the cannabinoid receptor agonist WIN 55,212–2 on pentagastrin-induced gastric acid secretion in the anaesthetized rat. Naunyn Schmiedebergs Arch Pharmacol 360:715–718

Coutts AA (2004) Cannabinoid receptor activation and the endocannabinoid system in the gastrointestinal tract. Curr Neuropharmacol 2:91–102

Coutts AA, Pertwee RG (1997) Inhibition by cannabinoid receptor agonists of acetylcholine release from the guinea-pig myenteric plexus. Br J Pharmacol 121:1557–1566

Coutts AA, Brewster N, Ingram T, Razdan RK, Pertwee RG (2000) Comparison of novel cannabinoid partial agonists and SR141716A in the guinea-pig small intestine. Br J Pharmacol 129:645–652

Coutts AA, Irving AJ, Mackie K, Pertwee RG, Anavi-Goffer S (2002) Localisation of cannabinoid CB1 receptor immunoreactivity in the guinea pig and rat myenteric plexus. J Comp Neurol 448:410–422

Croci T, Manara L, Aureggi, Guagnini F, Rinaldi-Carmona M, Maffrand J-P, Le Fur G, Mukenge S, Ferla G (1998) In vitro functional evidence of neuronal cannabinoid CB1 receptors in human ileum. Br J Pharmacol 125:1393–1395

Croci T, Landi M, Galzin AM, Marini P (2003) Role of cannabinoid CB1 receptors and tumor necrosis factor-alpha in the gut and systemic anti-inflammatory activity of SR 141716 (Rimonabant) in rodents. Br J Pharmacol 140:115–122

Darmani NA (2001a) Delta(9)-tetrahydrocannabinol and synthetic cannabinoids prevent emesis produced by the cannabinoid CB1 receptor antagonist/inverse agonist SR 141716A. Neuropsychopharmacology 24:198–203

Darmani NA (2001b) The cannabinoid CB1 receptor antagonist SR 141716A reverses the antiemetic and motor depressant actions of WIN 55, 212–2. Eur J Pharmacol 430:49–58

Darmani NA (2002) The potent emetogenic effects of the endocannabinoid, 2-AG (2- arachidonoylglycerol) are blocked by delta(9)- tetrahydrocannabinol and other cannabinoids. J Pharmacol Exp Ther 300:34–42

Darmani NA, Janoyan JJ, Kumar N, Crim JL (2003a) Behaviorally active doses of the CB1 receptor antagonist SR 141716A increase brain serotonin and dopamine levels and turnover. Pharmacol Biochem Behav 75:777–787

Darmani NA, Sim-Selley LJ, Martin BR, Janoyan JJ, Crim JL, Parekh B, Breivogel CS (2003b) Antiemetic and motor-depressive actions of CP55,940: cannabinoid CB1 receptor characterization, distribution, and G- protein activation. Eur J Pharmacol 459:83–95

De Petrocellis L, Cascio MG, Di Marzo V (2004) The endocannabinoid system: a general view and latest additions. Br J Pharmacol 141:765–774

Dewey WL, Harris LS, Kennedy JS (1972) Some pharmacological and toxicological effects of l-trans-delta-8- and l-trans-delta-9-tetrahydrocannabinol in laboratory rodents. Arch Int Pharmacodyn Ther 196:133–145

Di Carlo G, Izzo AA (2003) Cannabinoids for gastrointestinal diseases: potential therapeutic applications. Expert Opin Investig Drugs 12:39–49

Facer P, Knowles CH, Tam PK H, Ford AP, Dyer N, Baecker PA, Anand P (2001) Novel capsaicin (VR1) and purinergic (P2X(3)) receptors in Hirschsprung's intestine. J Pediatr Surg 36:1679–1684

Feher E, Vajda J (1982) Effect of capsaicin on the nerve elements of the small intestine. Acta Morphol Acad Sci Hung 30:57–63

Frederickson RC A, Hewes CR, Aiken JW (1976) Correlation between the in vivo and in vitro expression of opiate withdrawal precipitated by naloxone: their antagonism by l-(–)-delta- 9-tetrahydrocannabinol. J Pharmacol Exp Ther 199:375–384

Germanò MP, D'Angelo V, Mondello R, Pergolizzi S, Capasso F, Capasso R, Izzo AA, Mascolo N, De Pasquale R (2001) Cannabinoid CB1-mediated inhibition of stress-induced gastric ulcers in rats. Naunyn Schmiedebergs Arch Pharmacol 363:241–244

Gomez R, Navarro M, Ferrer B, Trigo JM, Bilbao A, Del Arco I, Cippitelli A, Nava F, Piomelli D, Rodriguez de Fonseca F (2002) A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. J Neurosci 22:9612–9617

Griffin G, Fernando SR, Ross RA, McKay NG, Ashford MLJ, Shire D, Huffman JW, Yu S, Lainton JA H, Pertwee RG (1997) Evidence for the presence of CB2-like cannabinoid receptors on peripheral nerve terminals. Eur J Pharmacol 339:53–61

Guanini F, Croci T, Aureggi G, Manara L, Rinaldi-Carmona M, Mukenge S, Aldrighetti L, Ferla G, Maffrand J-P, Le Fur G (2000) Tolerance to (+)WIN55,212–2 inhibitory effect and withdrawal by the cannabinoid CB1 receptor antagonist SR 141716 in isolated strips of small intestine. International Cannabinoid Research Society symposium on the Cannabinoids, Burlington, Vermont

Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE, Kustanovich I, Mechoulam R (2001) 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. Proc Natl Acad Sci USA 98:3662–3665

Heinemann A, Shahbazian A, Holzer P (1999) Cannabinoid inhibition of guinea-pig intestinal peristalsis via inhibition of excitatory and activation of inhibitory neural pathways. Neuropharmacology 38:1289–1297

Heshmati H, Caplain H, Bellisle F, Mosse M,Fauveau C, Le Fur G (2001) SR141716, a selective CB1 receptor cannabinoid receptor antagonist reduces hunger, caloric intake, and body weight in overweight or obese men. Obes Res 9:70S

Hine B, Friedman E, Torrelio M, Gershon S (1975) Morphine-dependent rats: blockade of precipitated abstinence by tetrahydrocannabinol. Science 187:443–445

Holzer P (2001) Gastrointestinal afferents as targets of novel drugs for the treatment of functional bowel disorders and visceral pain. Eur J Pharmacol 429:177–193

Holzer P (2003) Acid-sensitive ion channels in gastrointestinal function. Curr Opin Pharmacol 3:618–625

Ihenetu K, Molleman A, Parsons ME, Whelan CJ (2003) Inhibition of interleukin-8 release in the human colonic epithelial cell line HT-29 by cannabinoids. Eur J Pharmacol 458:207–215

Izzo AA, Mascolo N, Borrelli F, Capasso F (1998) Excitatory transmission to the circular muscle of the guinea-pig ileum: evidence for the involvement of cannabinoid CB1 receptors. Br J Pharmacol 124:1363–1368

Izzo AA, Mascolo N, Capasso R, Germano MP, DePasquale R, Capasso F (1999a) Inhibitory effect of cannabinoid agonists on gastric emptying in the rat. Naunyn Schmiedebergs Arch Pharmacol 360:221–223

Izzo AA, Mascolo N, Borrelli F, Capasso F (1999b) Defaecation, intestinal fluid accumulation and motility in rodents: implications of cannabinoid CB1 receptors. Naunyn Schmiedebergs Arch Pharmacol 359:65–70

Izzo AA, Mascolo N, Tonini M, Capasso F (2000) Modulation of peristalsis by cannabinoid CB1 ligands in the isolated guinea-pig ileum. Br J Pharmacol 129:984–990

Izzo AA, Pinto L, Borrelli F, Capasso R, Mascolo N, Capasso F (2000b) Central and peripheral cannabinoid modulation of gastrointestinal transit in physiological states or during the diarrhoea induced by croton oil. Br J Pharmacol 129:1627–1632

Izzo AA, Capasso R, Pinto L, Di Carlo G, Mascolo N, Capasso F (2001a) Effect of vanilloid drugs on gastrointestinal transit in mice. Br J Pharmacol 132:1411–1416

Izzo AA, Fezza F, Capasso R, Bisogno T, Pinto L, Iuvone T, Esposito G, Mascolo N, Di Marzo V, Capasso F (2001b) Cannabinoid CB1-receptor mediated regulation of gastrointestinal motility in mice in a model of intestinal inflammation. Br J Pharmacol 134:563–570

Izzo AA, Mascolo N, Capasso F (2001c) The gastrointestinal pharmacology of cannabinoids. Curr Opin Pharmacol 1:597–603

Izzo AA, Capasso F, Costagliola A, Bisogno T, Marsicano G, Ligresti A, Matias I, Capasso R, Pinto L, Borrelli F, Cecio A, Lutz B, Mascolo N, Di Marzo V (2003) An endogenous cannabinoid tone attenuates cholera toxin-induced fluid accumulation in mice. Gastroenterology 125:765–774

Joseph J, Niggemann B, Zaenker KS, Entschladen F (2004) Anandamide is an endogenous inhibitor for the migration of tumor cells and T lymphocytes. Cancer Immunol Immunother 53:723–728

Katayama K, Ueda N, Kurahashi Y, Suzuki H, Yamamoto S, Kato I (1997) Distribution of anandamide amidohydrolase in rat tissues with special reference to small intestine. Biochim Biophys Acta 1347:212–218

Kojima S, Sugiura T, Waku K, Kamikawa Y (2002) Contractile response to a cannabimimetic eicosanoid, 2-arachidonoylglycerol, of longitudinal smooth muscle from the guinea-pig distal colon in vitro. Eur J Pharmacol 444:203–207

Krowicki ZK, Moerschbaecher JM, Winsauer PJ, Digavalli SV, Hornby PJ (1999) Delta9-tetrahydrocannabinol inhibits gastric motility in the rat through cannabinoid CB1 receptors. Eur J Pharmacol 371:187–196

Kulkarni-Narla A, Brown DR (2000) Localization of CB1-cannabinoid receptor immunoreactivity in the porcine enteric nervous system. Cell Tissue Res 302:73–80

Kulkarni-Narla A, Brown DR (2001) Opioid, cannabinoid and vanilloid receptor localization on porcine cultured myenteric neurons. Neurosci Lett 308:153–156

Kwiatkowska M, Parker LA, Burton P, Mechoulam R (2004) A comparative analysis of the potential of cannabinoids and ondansetron to supress cisplatin-induced emesis in the Suncus murinus (house musk shrew). Psychopharmacology (Berl). 174:254–259

Lambert DM, Vandevoorde S, Jonsson KO, Fowler CJ (2002) The palmitoylethanolamide family: A new class of anti-inflammatory agents? Curr Med Chem 9:663–674

Landi M, Croci T, Rinaldi-Carmona M, Maffrand JP, Le Fur G, Manara L (2002) Modulation of gastric emptying and gastrointestinal transit in rats through intestinal cannabinoid CB1 receptors. Eur J Pharmacol 450:77–83

Lee MC, Smith FL, Stevens DL, Welch SP (2003) The role of several kinases in mice tolerant to Delta(9)-tetrahydrocannabinol. J Pharmacol Exp Ther 305:593–599

Lehmann A, Blackshaw LA, Branden L, Carlsson A, Jensen J, Nygren E, Smid SD (2002) Cannabinoid receptor agonism inhibits transient lower esophageal sphincter relaxations and reflux in dogs. Gastroenterology 123:1129–1134

Ligresti A, Bisogno T, Matias I, De Petrocellis L, Cascio MG, Cosenza V, D'Argenio G, Scaglione G, Bifulco M, Sorrentini I, Di Marzo V (2003) Possible endocannabinoid control of colorectal cancer growth. Gastroenterology 125:677–687

López-Redondo F, Lees GM, Pertwee RG (1997) Effects of cannabinoid receptor ligands on electrophysiological properties of myenteric neurones of the guinea-pig ileum. Br J Pharmacol 122:330–334

Lundgren O (2002) Enteric nerves and diarrhoea. Pharmacol Toxicol 90:109–120

Lynn AB, Herkenham M (1994) Localization of cannabinoid receptors and nonsaturable high-density cannabinoid binding sites in peripheral tissues of the rat: implications for receptor-mediated immune modulation by cannabinoids. J Pharmacol Exp Ther 268:1612–1623

MacNaughton WK, Cushing K, Van Sickle MD, Keenan CM, Mackie K, Sharkey KA (2003) Cannabinoid CB1 receptor distribution and function in neurally mediated chloride secretion in the guinea pig ileum. Gastroenterology 124:A342

MacNaughton WK, Van Sickle MD, Keenan CM, Cushing K, Mackie K, Sharkey KA (2004) Distribution and function of the cannabinoid-1 receptor in the modulation of ion transport in the guinea pig ileum: relationship to capsaicin-sensitive nerves. Am J Physiol Gastrointest Liver Physiol (in press)

Manara L, Croci T, Guagnini F, Rinaldi-Carmona M, Maffrand JP, Le Fur G, Mukenge S, Ferla G (2002) Functional assessment of neuronal cannabinoid receptors in the muscular layers of human ileum and colon. Dig Liver Dis 34:262–269

Mancinelli R, Fabrizi A, Del Monaco S, Azzena GB, Vargiu R, Colombo GC, Gessa GL (2001) Inhibition of peristaltic activity by cannabinoids in the isolated distal colon of mouse. Life Sci 69:101–111

Mang CF, Erbelding D, Kilbinger H (2001) Differential effects of anandamide on acetylcholine release in the guinea-pig ileum mediated via vanilloid and non-CB1 cannabinoid receptors. Br J Pharmacol 134:161–167

Mascolo N, Izzo AA, Ligresti A, Costagliola A, Pinto L, Cascio MG, Maffia P, Cecio A, Capasso F, Di Marzo V (2002) The endocannabinoid system and the molecular basis of paralytic ileus in mice. Faseb J 16:1973–1975

Massa F, Marsicano G, Hermann H, Cannich A, Krisztina M, Cravatt BF, Ferri G–L, Sibaev A, Lutz B (2004) The endogenous cannabinoid system protects against colonic inflammation. J Clin Invest 113:1202–1209

McCallum RW, Soykan I, Sridhar KR, Ricci DA, Lange RC, Plankey MW (1999) Delta-9-tetrahydrocannabinol delays the gastric emptying of solid food in humans: a double-blind, randomized study. Aliment Pharmacol Ther 13:77–80

McVey DC, Schmid PC, Schmid HH O, Vigna SR (2003) Endocannabinoids induce ileitis in rats via the capsaicin receptor (VR1). J Pharmacol Exp Ther 304:713–722

Morrone LA, Romanelli L, Mazzanti G, Valeri P, Menichin F (1993) Hashish antagonism on the in vitro development of withdrawal contracture. Pharmacol Res 27 (Suppl 1):63–64

Nye JS, Seltzman HH, Pitt CG, Snyder SH (1985) High affinity cannabinoid binding sites in brain membranes labeled with [H-3]-5/-trimethylammonium delta-8-tetra-hydrocannabinol. J Pharmacol Exp Ther 234:784–791

Oleinik VM (1995) Distribution of digestive enzyme activities along intestine in blue fox, mink, ferret and rat. Comp Biochem Physiol A Physiol 112:55–58

Parker LA, Mechoulam R (2003) Cannabinoid agonists and antagonists modulate lithium-induced conditioned gaping in rats. Integr Physiol Behav Sci 38:134–146

Parker LA, Mechoulam R, Schlievert C (2002) Cannabidiol, a non-psychoactive component of cannabis and its synthetic dimethylheptyl homolog suppress nausea in an experimental model with rats. Neuroreport 13:567–570

Parker LA, Mechoulam R, Schlievert C, Abbott L, Fudge ML, Burton P (2003) Effects of cannabinoids on lithium-induced conditioned rejection reactions in a rat model of nausea. Psychopharmacology (Berl) 166:156–162

Partosoedarso ER, Abrahams TP, Scullion RT, Moerschbaecher JM, Hornby PJ (2003) Cannabinoid1 receptor in the dorsal vagal complex modulates lower oesophageal sphincter relaxation in ferrets. J Physiol 550:149–158

Paton WDM, Zar MA (1968) The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. J Physiol (Lond) 194:13–33

Pertwee RG (1997) Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol Ther 74:129–180

Pertwee RG (2001) Cannabinoids and the gastrointestinal tract. Gut 48:859–867

Pertwee RG, Ross RA (2002) Cannabinoid receptors and their ligands. Prostaglandins Leukot Essent Fatty Acids 66:101–121

Pertwee RG, Stevenson LA, Elrick DB, Mechoulam R, Corbett AD (1992) Inhibitory effects of certain enantiomeric cannabinoids in the mouse vas deferens and the myenteric plexus preparation of guinea-pig small intestine. Br J Pharmacol 105:980–984

Pertwee RG, Fernando SR, Griffin G, Abadji V, Makriyannis A (1995) Effect of phenylmethylsulphonyl fluoride on the potency of anandamide as an inhibitor of electrically evoked contractions in two isolated tissue preparations. Eur J Pharmacol 272:73–78

Pertwee RG, Fernando SR, Nash JE, Coutts AA (1996) Further evidence for the presence of cannabinoid CB1 receptors in guinea-pig small intestine. Br J Pharmacol 118:2199–2205

Pertwee RG, Fernando S, Ritchie JEA (1998) Preliminary validation of a novel experimental model for the study of cannabinoid tolerance. International Cannabinoid Research Society symposium on the Cannabinoids, Burlington, Vermont

Pinto L, Capasso R, Di Carlo G, Izzo AA (2002a) Endocannabinoids and the gut. Prostaglandins Leukot Essent Fatty Acids 66:333–341

Pinto L, Izzo AA, Cascio MG, Bisogno T, Hospodar-Scott K, Brown DR, Mascolo N, Di Marzo VCapasso F (2002b) Endocannabinoids as physiological regulators of colonic propulsion in mice. Gastroenterology 123:227–234

Poonyachoti S, Kulkarni-Narla A, Brown DR (2002) Chemical coding of neurons expressing delta- and kappa-opioid receptor and type I vanilloid receptor immunoreactivities in the porcine ileum. Cell Tissue Res 307:23–33

Rosell S, Agurell S (1975) Effects of 7-hydroxy-D6-tetrahydrocannabinol and some related cannabinoids on the guinea-pig isolated ileum. Acta Physiol Scand 94:142–144

Rosell S, Agurell S, Martin BR (1976) Effects of cannabinoids on isolated smooth muscle preparations. New York, Springer-Verlag

Ross RA, Brockie HC, Fernando SR, Saha B, Razdan RK, Pertwee RG (1998) Comparison of cannabinoid binding sites in guinea-pig forebrain and small intestine. Br J Pharmacol 125:1345–1351

Rumessen JJ, d'Exaerde AD, Mignon S, Bernex F, Timmermans JP, Schiffmann SN, Panthier JJ, Vanderwinden JM (2001) Interstitial cells of Cajal in the striated musculature of the mouse esophagus. Cell Tissue Res 306:1–14

Shire D, Carillon C, Kaghad M, Calandra B, Rinaldi-Carmona M, Le Fur G, Caput D, Ferrara P (1995) An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. J Biol Chem 270:3726–3731

Shook JE, Burks TF (1989) Psychoactive cannabinoids reduce gastrointestinal propulsion and motility in rodents. J Pharmacol Exp Ther 249:444–449

Simoneau II, Hamza MS, Mata HP, Siegel EM, Vanderah, TW, Porreca F, Makriyannis A, Malan TP (2001) The cannabinoid agonist WIN55,212–2 suppresses opioid-induced emesis in ferrets. Anesthesiology 94:882–887

Sofia RD, Diamantis W, Harrison JE, Melton J (1978) Evaluation of antiulcer activity of delta-9-tetrahydrocannabinol in the Shay rat test. Pharmacology 17:173–177

Storr M, Gaffal E, Saur D, Schusdziarra V, Allescher HD (2002) Effect of cannabinoids on neural transmission in rat gastric fundus. Can J Physiol Pharmacol 80:67–76

Storr M, Sibaev A, Marsicano G, Lutz B, Schusdziarra V, Timmermans JP Allescher HD (2004) Cannabinoid receptor type 1 modulates excitatory and inhibitory neurotransmission in mouse colon. Am J Physiol Gastrointest Liver Physiol 286:G110–G117

Sugiura T, Kobayashi Y, Oka S, Waku K (2002) Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological significance. Prostaglandins Leukot Essent Fatty Acids 66:173–192

Todorov S, Pozzoli C, Zamfirova R, Poli E (2003) Prejunctional modulation of non-adrenergic non-cholinergic (NANC) inhibitory responses in the isolated guinea-pig gastric fundus. Neurogastroenterol Motil 15:299–306

Tominaga M, Wada M, Masu M (2001) Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. Proc Natl Acad Sci U S A 98:6951–6956

Tramer MR, Carroll D, Campbell FA, Reynolds DJ M, Moore RA, McQuay HJ (2001) Cannabinoids for control of chemotherapy induced nausea and vomiting: quantitative systematic review. Br Med J 323:16–21

Tyler K, Hillard CJ, Greenwood-Van Meerveld B (2000) Inhibition of small intestinal secretion by cannabinoids is CB1 receptor-mediated in rats. Eur J Pharmacol 409:207–211

Ueda N, Yamamoto S (2000) Anandamide amidohydrolase (fatty acid amide hydrolase). Prostaglandins Other Lipid Mediat 61:19–28

Van Sickle MD, Oland LD, Ho, W, Hillard CJ, Mackie K, Davison JS, Sharkey KA (2001) Cannabinoids inhibit emesis through CB1 receptors in the brainstem of the ferret. Gastroenterology 121:767–774

Van Sickle MD, Oland LD, Mackie K, Davison JS, Sharkey KA (2003) Delta-9–tetrahydrocannabinol selectively acts on cannabinoid 1(CB1) receptors in specific regions of the dorsal vagal complex to inhibit emesis in the ferret. Am J Physiol Gastrointest Liver Physiol 285:G566–G576

Vigano D, Cascio MG, Rubino T, Fezza F, Vaccani A, Di Marzo V, Parolaro D (2003) Chronic morphine modulates the contents of the endocannabinoid, 2-arachidonoyl glycerol, in rat brain. Neuropsychopharmacology 28:1160–1167

Yiangou Y, Facer P, Dyer NH C, Chan CL H, Knowles C, Williams NS, Anand P (2001) Vanilloid receptor 1 immunoreactivity in inflamed human bowel. Lancet 357:1338–1339

Zygmunt PM, Petersson J, Andersson DA, Chuang HH, Sorgard M, Di Marzo V, Julius D, Hogestatt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400:452–457