British Journal of Pharmacology (2007) 152, 663–670

0007–1188/07 $30.00
www.brjpharmacol.org

REVIEW

Endocannabinoids and the gastrointestinal tract: what are the key questions?

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Cannabinoid (CB_1) receptor activation acts neuronally, reducing GI motility, diarrhoea, pain, transient lower oesophageal sphincter relaxations (TLESRs) and emesis, and promoting eating. CB_2 receptor activation acts mostly via immune cells to reduce inflammation. What are the key questions which now need answering to further understand endocannabinoid pathophysiology? GPR55. Does this receptor have a GI role? Satiety, Nausea, Vomiting, Gastro-Oesophageal Reflux, Gastric Emptying. Endocannabinoids acting at CB_1 receptors can increase food intake and body weight, exert anti-emetic activity, reduce gastric acid secretion and TLESRs; CB_2 receptors may have a small role in emesis. *Question 1:* CB_1 receptor activation reduces emesis and gastric emptying but the latter is associated with nausea. How is the paradox explained? *Q2:* Do non-CB receptor actions of endocannabinoids (for example TRPV1) also modulate emesis? *Q3:* Is pathology necessary (gastritis, gastro-oesophageal reflux) to observe CB_2 receptor function? Intestinal Transit and Secretion. Reduced by endocannabinoids at CB_1 receptors, but not by CB_2 receptor agonists. *Q1:* Do the effects of endocannabinoids rapidly diminish with repeat-dosing? *Q2:* Do CB_2 receptors need to be pathologically upregulated before they are active? Inflammation. CB_1, CB_2 and TRPV1 receptors may mediate an ability of endocannabinoids to reduce GI inflammation or its consequences. *Q1:* Are CB_2 receptors upregulated by inflammatory or other pathology? Pain. Colonic bacterial flora may upregulate CB_2 receptor expression and thereby increase intestinal sensitivity to noxious stimuli. *Q1:* Are CB_2 receptors the interface between colonic bacteria and enteric- or extrinsic nerve sensitivity? Relevance of endocannabinoids to humans. Perhaps apart from appetite, this is largely unknown.
British Journal of Pharmacology (2007) **152,** 663–670; doi:10.1038/sj.bjp.0707422; published online 3 September 2007

Keywords: endocannabinoid; CB_1; CB_2; emesis; satiety; reflux; secretion; inflammation; pain; bacteria

Abbreviations: 2-AG, 2-arachidononyl glycerol; AM1241, (*R,S*)-(2-iodo-5-nitro-phenyl)-[l-(l-methyl-piperidin-2-ylmethyl)-l*H*-ubdik-3-yl]-methanone; AM251, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; AM630, 6-iodo-r-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)-methanone; AP, area postrema; CB, cannabinoid; CCK, cholecystokinin; D_2, dopamine-2 receptor; DMVN, dorsal motor vagal nucleus; FAAH, fatty acid amide hydrolase; GLP-1, glucagon-like peptide-1; HU210, 3-(1, 1-dimethylheptyl)-(−)-11-hydroxy-Δ^8-tetrahydrocannabinol; 5-HT, 5-hydroxytryptamine; JWH 015, (2-methyl-1-propyl1*H*-indol-3-yl)-1-cyclohexanol; JWH 133, (6a*R*,10a*R*)-3-(1,1-dimethyl-butyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b,d*]pyran; LES, lower oesophageal sphincter; LPS, lipopolysaccharide; M6G, morphine-6-glucuronide; NTS, nucleus tractus solitarius; SR 144528, *N*-(1*S*)-*endo*-1,2,3,-trimethyl bicycle [2.2.1]heptan-2yl-5-(4-chloro-3-methyl-phenyl)-1(4-methylbenzyl)-pyrazole-3-carboxamide; Δ^9-THC, (9)-tetrahydrocannabinol; TLESR, transient lower oesophageal sphincter relaxation; TNBS, 2,4,6-trinitrobenzenesulphonic acid; TRPV1, transient receptor potential vanilloid subtype 1; URB 597, cyclohexylcarbamic acid 3′-carbamoyl-biphenyl-3-yl ester; VDM11, (all *Z*) *N*-(2-methyl-3-hydroxy-phenyl)-5,8,11,14-eicosa-tetraenamide; WIN 55212-2, *R*-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)-methyl]pyrrolo [1,2,3-*de*]-1,4-benzoazinyl]-(1-naphtalenyl)methanone mesylate

Introduction

The effects of cannabinoid (CB) receptor activation and roles for endocannabinoids in the GI tract have been extensively reviewed (Di Carlo and Izzi, 2003; Vigna, 2003; Coutts and Izzo, 2004; Hornby and Prouty, 2004; Darmani, 2006; Massa and Monory, 2006). At its simplest, CB_1 receptor activation acts mostly via enteric, vagal, brainstem and spinal nerves to reduce GI motility, diarrhoea, pain or hyperalgesia, transient lower oesophageal sphincter relaxations (TLESRs), emesis and gastric acid secretion, as well as promote eating. CB_2 receptor activation acts mostly via immune cells to reduce inflammation.

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Received 24 May 2007; revised 26 June 2007; accepted 23 July 2007; published online 3 September 2007

In summary, the principal endocannabinoids are the endogenous lipids arachidonoyl ethanolamide (anandamide) and 2-arachidononyl glycerol (2-AG), which are selective agonists at the CB_1 and CB_2 receptors, respectively (see Palmer *et al*., 2002); smaller amounts of others are suggested (for example, noladin ether and virodhamine), but their presence and functions within the gut have not been studied. Both anandamide and 2-AG can function as neurotransmitters or neuromodulators. They are hydrolysed by the fatty acid amide hydrolase (FAAH) in the case of anandamide or monoglyceride lipase in the case of 2-AG (see Palmer *et al*., 2002, and also Capasso *et al*., 2005 for how FAAH can also catalyse the hydrolysis of other bioactive amides). High levels of 2-AG and anandamide have been shown in mouse colon, together with high activity of anandamide amidohydrolase (Pinto *et al*., 2002). FAAH mRNA is found throughout the small and large intestine of mice (Capasso *et al*., 2005).

To understand the biology of the endocannabinoids it is essential to have an appreciation of the selectivity and non-selectivity of the natural and synthetic ligands active at the CB receptors and at the mechanisms controlling reuptake and breakdown of the endocannabinoids. For a review of the pharmacology of compounds which mimic, block or modulate the reuptake and metabolism of endocannabinoids, see Palmer *et al*. (2002), Fowler *et al*. (2005) and Mackie (2006). Areas those deserve highlighting are:

(1) Anandamide and 2-AG may also activate transient receptor potential vanilloid subtype 1 (TRPV1) receptors and cannabinoid ligands may inhibit 5-hydroxytryptamine (5-HT)-induced depolarization of rat nodose ganglia and allosterically modulate the $5\text{-}HT_{3A}$ receptor (see Townsend *et al*., 2002). The ability to activate both CB_1 and TRPV1 receptors is illustrated by experiments with anandamide, in which relatively low concentrations reduce electrically evoked cholinergically mediated contractions of guinea-pig isolated ileum (longitudinal muscle-myenteric plexus preparation) via CB_1 receptors sensitive to the selective CB_1 r-antagonist rimonabant (SR141716), whereas at higher concentrations, anandamide increased basal ACh release in a manner reversible by the TRPV1 r-antagonist capsazepine but not by rimonabant (Mang *et al*., 2001).

(2) The mechanism of compounds reported as inverse agonists at the CB_1 receptor also needs to be considered. In an excellent introduction to the relevant arguments, Hornby and Prouty (2004) point out that while such compounds do indeed act as inverse agonists in host cells expressing the recombinant receptor (at a density that cannot be correlated to the density of receptors expressed in the native, therapeutic target cell), the translation of such activity to a native tissue has not been equivocally demonstrated. Accordingly, in the absence of such translation it may be safer to regard the actions of such compounds in native tissues (*in vitro* or *in vivo*) as operating via simple antagonists. A final note of caution about the need to correctly translate activity obtained using a recombinant receptor system to that obtained using a native tissue is illustrated by the finding that partial agonism has been reported for rimonabant in rat heart (Krylatov *et al*., 2005).

Inevitably, simplifications hide controversies. The intention of this paper is not to regurgitate the information about the roles of cannabinoids and endocannabinoids that have been reviewed by others (see above). Instead, brief summaries are provided of the known effects of exogenous and endogenous cannabinoids on different GI functions, for the purpose of then identifying the key questions, which need to be answered to achieve a greater understanding of the pathophysiological role of endocannabinoids and the possibility that new medicines might be developed as a consequence. The output from these summaries is presented diagrammatically in Figure 1. Hopefully, these can be said to be the current 'hot areas' of research into the GI functions of endocannabinoids.

Satiety, nausea, vomiting, gastro-oesophageal reflux and gastric emptying

These are grouped together because they may share certain common pathways (the gastric—vagal–brainstem link) and because a change in one function can exert a profound influence on another. Delta (9)-tetrahydrocannabinol (Δ^9-THC), for example, is used both as an anti-emetic drug for cancer chemotherapy and also as an appetite-enhancing agent (see Mechoulam and Hanu, 2001 for review). These two actions of a single drug immediately imply a common link between their mechanisms of action (see Sanger and Andrews, 2006 for further discussion).

The mechanisms by which cannabinoids affect appetite, nausea and gastric emptying are species-dependent and at least partly linked together via a common neuronal pathway, requiring care in the interpretation of data

Most feeding studies use rats and mice. As rodents, these animals are unable to vomit (Borison *et al*., 1981) and instead, they have evolved different ways to protect themselves against the accidental ingestion of noxious materials. The latter include an ability to develop taste aversions and engage in pica consumption, as well as the loss of appetite and/or the development of gastric stasis to limit the ingestion of further noxious material; these different behaviours are, therefore, thought to be equivalent to nausea and/or vomiting (see Liu *et al*., 2005). The nature of the rodent responses to emetogenic agents means that care has to be taken when interpreting data in which a substance is said to reduce feeding—is this a genuine reduction in the desire to eat, for example, or an aversive response caused by an unpleasant association with the compound, perhaps at higher doses than those required to reduce the desire to eat? The need for this caution is exemplified by studies in humans where the administration of higher doses of several satiety-inducing gut hormones, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), exenatide and oxyntomodulin, have been shown to induce nausea (see Murphy *et al*., 2006 for references). In humans and other

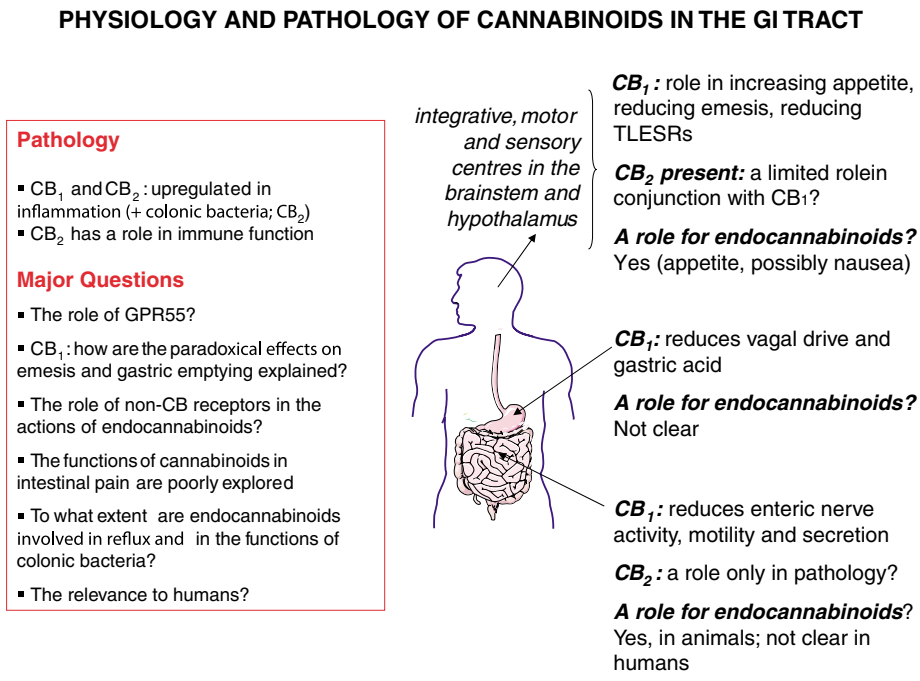

Figure 1 Physiology and pathology of cannabinoids in the GI tract. GI, gastrointestinal.

species which are capable of emesis, the sensations of hunger, satiety and nausea may therefore, be simply points on the same physiological spectrum, operating through similar pathways (see also Greenough *et al*., 1998 for further discussion).

Hornby and Prouty (2004) point out an additional complexity. CB_1 receptor activation reduces emesis but will also delay gastric emptying in both rodents and in emetogenic species such as humans. Delayed gastric emptying is often linked to the sensation of nausea so it would seem paradoxical for an endogenous ligand to affect the stomach in this way as well as reduce emesis. The explanation for this apparent conundrum is not clear, but as with the previous comparisons between the doses of certain peptides required to reduce feeding or induce nausea, perhaps the answer again lies in the doses used. Unfortunately, current literature does not yet provide answers to this suggestion, as studies to look at both behaviours induced by the same ligand in the same species, have not been conducted. In separate studies with Δ^9-THC, for example, Parker *et al*. (2003) demonstrated the potential for anti-emetic activity in a rat model of nausea (lithium-induced taste aversion) using a dose of 0.5 mg kg^{-1} intraperitoneally (i.p.), but Krowicki *et al*. (1999) were able to delay rat gastric emptying with 0.02–2 mg kg^{-1} intravenous bolus doses.

CB_1 receptor agonists and endocannabinoids acting at CB_1 receptors, but not CB_2 receptor agonists, increase food intake and body weight in rodents and in emetogenic species, including humans

The likely sites of action involve hypothalamic and other brain areas, although peripheral CB_1 receptors—located in adipose tissues and on GI vagal afferent nerves—should not be ignored. Similar activity has generally been observed using endocannabinoids such as anandamide or 2-AG and a physiological role for endocannabinoids is strengthened by the observations that CB_1 receptor antagonists may reduce both feeding and body weight during repeated compound administration; these reductions in body weight appear greater in obese animals and may be the result of a dual effect on both food intake and metabolic processes (see Vickers and Kennett, 2005 for review). From this discovery, several CB_1 receptor antagonists are now in clinical development for the treatment of obesity (Vickers and Kennett, 2005), including the antagonist or inverse agonist rimonabant (see Carai *et al*., 2005).

CB_1 receptor agonists and endocannabinoids acting at CB_1 receptors have anti-emetic activity; the CB_2 receptor may have a small role to play

The anti-emetic activity of cannabinoids has been well documented (Parker *et al*., 2005; but see Soderpalm *et al*., 2001, for comments on the possibility that efficacy versus nausea may be lower than that versus vomiting). In summary, CB_1 receptors are thought to mediate the anti-emetic action of cannabinoids in ferrets by acting within the dorsal vagal complex (DVC) (see Hornby, 2001), a region which includes the nuclei which receives sensory input from the vagus and the blood circulation (area postrema (AP), nucleus tractus solitarius (NTS)) as well as the motor nuclei (dorsal motor vagal nucleus (DMVN)) involved in the induction of emesis; each of these regions express CB_1 receptors (Van Sickle *et al*., 2003) and FAAH (Van Sickle *et al*., 2001). Further, data obtained using CB receptor antagonists

suggest a role for endocannabinoids acting via the CB_1 receptor. For example:

- In one study with obese patients, a possible dose-dependent incidence of nausea was reported with rimonabant, observed using the 20 mg dose (11.2% incidence) but not with the 5 mg dose, compared with placebo (5.8%) (Pi-Sunyer *et al*., 2006).
- In ferrets, selective CB_1 receptor antagonism by *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251), which alone had no effect, prevented the anti-emetic activity of Δ^9-THC but potentiated vomiting in response to morphine-6-glucuronide (M6G) (Van Sickle *et al*., 2001).
- In the least shrew (an emetic species often demonstrating low potency to agents which evoke or inhibit emesis), vomiting has been induced by rimonabant but not by the CB_2 receptor antagonist *N*-(1*S*)-*endo*-1,2,3,-trimethyl bicycle [2,2,1]heptan-2yl-5-(4-chloro-3-methyl-phenyl)-1(4-methylbenzyl)-pyrazole-3-carboxamide (SR 144528) (Darmani, 2001).
- In models of emesis-like behaviours in rats, the strength of toxin-induced conditioned gaping (Parker and Mechoulam, 2003) or lithium-induced conditioned rejection (Parker *et al*., 2003) may be facilitated by rimonabant.

It is possible that the relationship between the CB_1 receptor and the anti-emetic activity of cannabinoids may not be as simple as that described above (Parker *et al*., 2005). In the least shrew, 2-AG may induce emesis via a mechanism sensitive to inhibition by indomethacin or CB_1 receptor antagonism, whereas anandamide exerts anti-emetic activity, including an ability to prevent the activity of 2-AG (Darmani, 2002). Together, these data suggest that under certain circumstances, CB_1 receptor activation can exert both emetic and anti-emetic activity. However, further experiments are required to support this suggestion, including the need to reproduce the above studies in a different species, as well as determine the intrinsic activity of 2-AG at the native CB_1 receptor in the least shrew.

In spite of all the evidence linking the CB_1 receptor to the mechanisms of cannabinoid-induced emesis, it remains a possibility that the CB_2 receptor can also play at least some role in the mechanisms of emesis. This receptor is present within the brainstem of the rat and ferret, specifically within the DMVN, the nucleus ambiguous and the spinal trigeminal nucleus (Van Sickle *et al*., 2005). In ferrets, the selective endocannabinoid reuptake inhibitor (all *Z*) *N*-(2-methyl-3-hydroxy-phenyl)-5,8,11,14-eicosa-tetraenamide (VDM11), the FAAH inhibitor cyclohexylcarbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB 597) and anandamide were each demonstrated to block emesis evoked by M6G; the response to anandamide was prevented by CB_1 receptor antagonism. Similar activity was also observed using 2-AG but this was prevented by the selective CB_1 receptor antagonist AM251 and also by the CB_2 receptor antagonist 6-iodo-r-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)-methanone (AM630); a possible involvement of the CB_2 receptor in the activity of 2-AG is consistent with the fact that in contrast to anandamide, this molecule can activate the CB_2 receptor. Interestingly, the CB_2 receptor agonists (*R*,*S*)-(2-iodo-5-nitro-phenyl)-[l-(l-methyl-piperidin-2-ylmethyl)-l*H*-ubdik-3-yl]-methanone (AM1241) or (6a*R*,10a*R*)-3-(1,1-dimethyl-butyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran (JWH 133) did not reduce M6G-induced emesis, suggesting that endocannabinoids must exert a more powerful anti-emetic activity and/or there is a requirement to activate both the CB_1 and CB_2 receptors before an influence of the latter receptor can be demonstrated (Van Sickle *et al*., 2005).

Finally, a highly speculative possibility is raised by the findings that in the striatum of rats the release of anandamide can be induced by dopamine-2 receptor (D_2) agonists and antagonism at the CB_1 receptor may facilitate D_2-mediated hyperactivity (reviewed by Self, 1999). Since the D_2 in the AP plays a critical role in certain mechanisms of emesis (see Sanger and Andrews, 2006 for references) it is tempting to speculate that a similar association between anandamide and D_2 function may play a role in the mechanisms of emesis. Thus, it may be hypothesized that when D_2 are activated in the AP, anandamide would be released in an attempt to reduce the emetic consequence.

CB_1, but not CB_2 receptor agonists reduce gastric acid secretion and mechanisms associated with gastro-oesophageal reflux; endocannabinoids acting at CB_1 receptors may be involved in the mechanisms of reflux

The involvement of endocannabinoids in the control of gastric acid secretion is not known. CB_1 (but not CB_2) receptor activation may suppress vagal drive to the stomach and thereby decrease rat gastric acid secretion (for example, Adami *et al*., 2002). In these studies, CB_1 receptor antagonism did not affect the increase in gastric acid caused by pentagastrin, but the nature of the model used (anaesthetized, not conscious rats) does not prove an absence of endocannabinoid regulation of gastric acid secretion in this species.

Control of gastric acid secretion is necessary to help alleviate the symptoms associated with gastro-oesophageal reflux. There are several reasons why reflux may occur, including a reduction in the barrier function provided by the lower oesophageal sphincter (LES) during hiatus hernia. In addition, at least some reflux may occur because of an inappropriate increase in the number or duration of TLESRs (for example, Tougas and Banemai, 2001). These relaxations are part of a vago–vagal reflex, originating from the stomach, to relieve gastric intraluminal pressure via the release of gas into the oesophagus and mouth. In dogs, where TLESRs were evoked by gastric nutrient infusion and air insufflation, the relaxations were prevented by the CB_1 receptor agonist *R*-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)-methyl]pyrrolo[1,2,3-*de*]-1,4-benzoazinyl]-(1-naphtalenyl)methanone mesylate (WIN 55212-2) acting within the DVC, but not by the CB_2 receptor agonist SR144528 (Lehmann *et al*., 2002). Further, rimonabant alone increased the occurrence of TLESRs suggesting that endocannabinoids mediate a pro-reflux event. Finally, Partosoedarso *et al*. (2003) showed that CB_1 receptor agonists, again acting within the DVC, reduced a sustained relaxation of the LES evoked by gastric distension in ferrets; rimonabant prevented this activity but when

tested alone, had no effects on the gastric distension-induced relaxation of the LES. Together, these data indicate a role for cannabinoids in the control of LES function, but the exact role of the endocannabinoids in these models and species and in healthy versus diseased conditions, requires further clarification.

What are the key questions?

- CB_1 receptor activation reduces emesis and delays gastric emptying: how is this apparent paradox explained?
- To what extent do the non-CB receptor activities of endocannabinoids (for example, TRPV1 activation) modulate the anti-emetic and other upper-gut activities of endocannabinoids mediated via the CB receptors (for example, see Yamakuni *et al.* (2002) for the emetic and anti-emetic activities of TRPV1 agonists)?
- Is anandamide released in an attempt to reduce the emetic consequence of D_2 activation?
- What is the role of the CB_2 receptor? The receptor is present within the brainstem of the rat and ferret (see above), but apart from a possible involvement in the mechanisms of vomiting, seems to have little function. Is it necessary to introduce pathology, such as gastritis, gastro-oesophageal reflux or another inflammatory stimulus, in order to observe the true function of this receptor? The latter becomes a possibility in view of a suggested involvement of the CB_2 receptor in the increased intestinal motility and secretion associated with lipopolysaccharide treatment and the upregulation of the receptor by specific colonic bacteria (see below).
- What is the relevance of any of the endocannabinoid findings to humans? Cannabinoid receptor binding is present within the DMN and NTS of human brain (Glass *et al.*, 1997) and some nausea is apparent with high doses of rimonabant in patients with nausea (see above). These data suggest some relevance, but rigorous translational studies have not been undertaken.

Intestinal transit and secretion

CB_1 receptor agonists and endocannabinoids acting at CB_1 receptors, but not CB_2 receptor agonists, reduce intestinal motility and secretion

CB_1 receptors are located within the myenteric plexus and their activation can reduce excitatory cholinergic neurotransmission in the intestine (for example, Storr *et al.*, 2004) of various species including humans (see Hinds *et al.*, 2006), leading to reduced peristalsis and reduced gastrointestinal (GI) motility and transit *in vivo* (see Izzo *et al.*, 2001, for references). In normal rats, CB_2 receptor agonists may have no effects on intestinal transit (Mathison *et al.*, 2004). In a mouse model of colonic propulsion and defecation (measuring the time taken to expel a glass bead, artificially inserted into the colon), a similar inhibitory activity of the endocannabinoid anandamide has been reported (Pinto *et al.*, 2002).

Rimonabant has been reported to increase electrically evoked, cholinergically mediated contractions of guinea-pig isolated ileum, a behaviour which suggests a tonic involvement of endocannabinoids in the regulation of cholinergic function and hence, intestinal motility in this species (for example, Guagnini *et al.*, 2006). This excitatory activity finds consistency with an ability of rimonabant to increase tonic and phasic activity in a model of peristalsis of mouse isolated colonic propulsion (Mancinelli *et al.*, 2001) and increase intestinal motility and defecation in rodents (for example, Izzo *et al.*, 1999a, b). Conversely, the inhibitor of anandamide cellular reuptake, VDM11, decreased mouse colonic propulsion and defecation (measuring the time taken to expel an artificially inserted glass bead; Pinto *et al.*, 2002) and again in mice, intestinal motility (measured using a fluorescent marker) was reduced by FAAH inhibition via a mechanism prevented by rimonabant (Capasso *et al.*, 2005). Finally, paralytic ileus induced by i.p. administration of acetic acid was alleviated by rimonabant and worsened by VDM11 (Mascolo *et al.*, 2002).

CB_1 receptor agonists also reduce stimulated ion transport across the mucosa of the intestine, reducing water accumulation. This action may involve intrinsic and extrinsic nerves, rather than a direct action at the epithelium (see Hornby and Prouty, 2004 review). In mice given cholera toxin (CT) to increase fluid accumulation in the small intestine, an increased level of anandamide and CB_1 mRNA has been reported (Izzo *et al.*, 2003). CB_1 receptor activation and VDM11 each prevented CT-enhanced fluid accumulation. Rimonabant, but not the $TRPV_1$ r-antagonist capsazepine, increased fluid accumulation (Izzo *et al.*, 2003).

What are the key questions?

- Do the effects of the endocannabinoids rapidly diminish with repeat dosing or continual presence and if so, is this because of an upregulation of another mechanism? In one study, the ability of rimonabant to increase intestinal transit in mice was found to rapidly diminish with repeat dosing (Carai *et al.*, 2004).
- Are there cross-talk between CB and other receptors, such as the opiate receptors? Currently, the evidence does not suggest this (reviewed by Massa and Monory, 2006).
- What is the role of the CB_2 receptor? In one study, CB_2 receptor agonism may reduce lipopolysaccharide-induced stimulation of intestinal motility without affecting normal transit (Mathison *et al.*, 2004). In another, the receptor may be upregulated by specific colonic bacteria, increasing the sensitivity of the intestine to a noxious stimulus (see below). Does there need to be upregulation of the function of this receptor, via inflammatory stimuli or by colonic bacteria, to detect its activity?
- What is the relevance of any of the endocannabinoid findings to humans? In one laboratory, rimonabant has been shown to enhance neuronally mediated contractions of guinea-pig isolated ileum, confirming studies by others and suggesting a role for endocannabinoids in the motility of this region of the gut. However, rimonabant did not have a similar excitatory activity in similar preparations of human isolated ileum unless the preparations were made tolerant to the inhibitory effect of the CB receptor agonist (+) WIN 55212-2 (Guagnini *et al.*, 2006).

Inflammation

CB_1, CB_2 and TRPV1 receptors are implicated in the mechanisms by which endocannabinoids influence GI inflammation or the consequences of inflammation on intestinal motility and secretion

In two different models of colonic inflammation (oral administration of dextrane suphate sodium and intrarectal infusion of dinitrobenzene sulfonic acid (DNBS)), higher levels of inflammation were apparent in CB_1 receptor knockout mice, compared to their wild-type littermates. Similarly, rimonabant induced similar elevations in inflammation, whereas the CB_1 receptor agonist 3-(1,1-dimethylheptyl)-(−)-11-hydroxy-Δ^8-tetrahydrocannabinol (HU210) reduced inflammation scores (Massa *et al*., 2004). In patients with ulcerative colitis, and in DNBS-treated mice, increased levels of anandamide but not 2-AG were demonstrated (D'argenio *et al*., 2006). Genetic deletion of FAAH decreased levels of inflammation (Massa *et al*., 2004). Cannabinoids promote epithelial wound healing either alone or in combination with lysophosphatidic acid. Further CB_2 receptor expression was increased in the diseased colon tissue (Wright *et al*., 2005).

The studies summarized above, suggest an involvement of endocannabinoids in GI inflammation. Additional studies, in which the measured end points were nociception (see below) or intestinal motility and secretion are consistent with this view. For example, in mice given croton oil, CB_1 receptor activation was more effective in delaying intestinal motility than in control mice (Izzo *et al*., 2001), a finding associated with an increase in CB_1 receptor expression and higher levels of anandamide amidohydrolase activity. In this study the amounts of anandamide and 2-AG detected in the intestine were unchanged by the croton oil treatment, suggesting that turnover of endocannabinoids may be increased to reduce motility. Rimonabant increased motility approximately equally in both normal and croton oil-treated animals. In another study, however, the CB_2 receptors are argued to play a role in the mechanisms, which try to re-establish normal GI transit after an inflammatory stimulus. Thus, although CB_2 receptor agonists had no effects on transit in normal rats (transit shown to be reduced by CB_1 receptor agonists) they did reduce transit in lipopolysaccharide (LPS)-treated animals (but CB_1 receptor agonists did not); this action may involve the activation of cyclooxygenase (Mathison *et al*., 2004). Interestingly, CB_2 receptor antagonism had no effects in the LPS-treated rats suggesting no involvement of endocannabinoids at least in this model of inflammation.

Finally, at least one study has now implicated the ability of endocannabinoids to activate TRPV1 receptors in the mechanisms of intestinal inflammation. Thus, in segments of rat ileum given toxin A from *Clostridium difficile*, there was an increased concentration of anandamide and 2-AG, and administration of either of the endocannabinoids mimicked the inflammatory effects of toxin A. However, pretreatment with CB receptor antagonists did not prevent the effects of these agents whereas capsazepine was effective (McVey *et al*., 2003).

What are the key questions?

- To what extent are GI functions of the CB_2 receptors revealed by inflammatory stimuli (or by colonic bacteria; see below)?

Pain

CB receptors are involved in intestinal pain and colonic bacterial flora may upregulate CB_2 receptor expression and increase intestinal sensitivity to a noxious stimulus

In spite of the strong association between endocannabinoids, CB_1 and CB_2 receptor agonists in different forms of somatic and visceral pain (see Rice *et al*., 2002), very little of this research has been extended to the gut. One study (Sanson *et al*., 2006) has shown that at least one dose of the CB_1 and CB_2 receptor agonists WIN 55212-2 and (2-methyl-1-propyl1*H*-indol-3-yl)-1-cyclohexanol (JWH 015), may reduce the number of abdominal contractions evoked by noxious colorectal distension, albeit in an apparent bell-shaped dose–response manner. However, after the induction of inflammation and hypersensitivity to colorectal distension, following intra-rectal administration of 2,4,6-trinitrobenzenesulphonic acid (TNBS), the sensitivity to the effects of these agonists was increased. Conversely, the sensitivity to distension was increased after administration of rimonabant, but not after the CB_2 receptor antagonist SR 144528.

It is sometimes not appreciated that the lumen of the colon plays host to an astonishing collection of microflora. Increasingly, these are being recognized to exert profound activity not just on the metabolism of nutrients, but also on epithelial function, protection from pathogens and the regulation of intestinal functions in general. Recently, the *Lactobacillus acidophilus* bacteria, commonly found in human faeces, has been shown to produce a sustained increase in the expression of both mu opioid receptor gene (OPRM1) and CB_2 receptor mRNA, in both cultured HT-29 epithelial cells and in colonic epithelial cells following oral administration of the live strain of *L. acidophilus* for 15 days to both rats and mice. The authors were then able to demonstrate a reduction in intestinal sensitivity to colorectal distension in both normal rats and in rats made hypersensitive by the pre-administration of butyrate enemas following treatment with *L. acidophilus*; the latter activity was reduced by administration of the CB_2 receptor antagonist AM630 (Rousseaux *et al*., 2007).

What are the key questions?

- Is the CB_2 receptor a key player in regulating the interface between colonic bacteria and enteric or spinal afferent nerve sensitivity?

Summary and conclusions

The many actions of endocannabinoids within the GI tract include their effects on several upper- and lower-gut functions, during both healthy and pathological conditions. These actions continue to widen in scope. For example, the

suggested link between an increased level of anandamide and 2-AG in colon biopsies from patients with carcinomas or adenomatous polyps, and the known ability of these agents to inhibit cancer cell proliferation via CB_1 receptor activation now suggests a potential new role for GI endocannabinoids at non-neuronal CB_1 receptors (reviewed by Vigna, 2003 and Massa and Monory, 2006). However, the function of this review was not to extensively cover all of the literature pertinent to the influence of endocannabinoids on GI biology; but instead, to seek out those areas requiring further study, in the hope that these will represent the 'hot topics' of today and perhaps tomorrow. These are listed individually in each section. In summary, the questions, which seem to dominate are:

(1) What are the roles of endocannabinoids in GI pathophysiology?
(2) What is the relevance of any of the endocannabinoid findings to humans?
(3) What is the pathophysiological role of the CB_2 receptor within the gut?
(4) Is there a key link between the CB receptors, endocannabinoids and the colonic bacterial flora?
(5) Does the G-protein receptor GPR55, now known to be activated by cannabinoids and present as mRNA in the gut (Baker *et al*., 2006), have a role in vascular or other GI functions?

Conflict of interest

The author states no conflict of interest.

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