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Cannabinoids and gastrointestinal motility: animal and human studies

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Abstract. – The plant *Cannabis* has been known for centuries to be beneficial in a variety of gastrointestinal diseases, including emesis, diarrhea, inflammatory bowel disease and intestinal pain. Δ^9-tetrahydrocannabinol, the main psychotropic component of *Cannabis*, acts via at least two types of cannabinoid receptors, named CB_1 and CB_2 receptors. CB_1 receptors are located primarily on central and peripheral neurons (including the enteric nervous system) where they modulate neurotransmitter release, whereas CB_2 receptors are concerned with immune function, inflammation and pain. The discovery of endogenous ligands [i.e. anandamide and 2-arachidonoyl glycerol (2-AG)] for these receptors indicates the presence of a functional endogenous cannabinoid system in the gastrointestinal tract.

Anatomical and functional evidence suggests the presence of CB_1 receptors in the myenteric plexus, which are associated with cholinergic neurons in a variety of species, including in humans. Activation of prejunctional CB_1 receptors reduces excitatory enteric transmission (mainly cholinergic transmission) in different regions of the gastrointestinal tract. Consistently, in vivo studies have shown that cannabinoids reduce gastrointestinal transit in rodents through activation of CB_1, but not CB_2, receptors. However, in pathophysiological states, both CB_1 and CB_2 receptors could reduce the increase of intestinal motility induced by inflammatory stimuli. Cannabinoids also reduce gastrointestinal motility in randomized clinical trials. Overall, modulation of the gut endogenous cannabinoid system may provide a useful therapeutic target for disorders of gastrointestinal motility.

Key Words:

Cannabinoid receptors, Anandamide, Intestinal motility, Myenteric plexus, Gastric emptying, Fatty acid amide hydrolase, Irritable bowel syndrome, Inflammatory bowel disease.

Introduction

The marijuana plant *Cannabis* has been used for the treatment of a number of diseases including those affecting gastrointestinal motility[1-4]. Δ^9-tetrahydrocannabinol (Δ^9-THC), the main active ingredient of *Cannabis*, activates two $G_{i/o}$-coupled membrane receptors, named CB_1 and CB_2 receptors. Cannabinoid CB_1 receptors are located primarily on central and peripheral neurons, including those innervating the gut, whereas CB_2 receptors are mainly expressed by inflammatory/immune cells[5].

Endogenous ligands that activate cannabinoid receptors [(i.e. the endocannabinoids, anandamide and 2-arachydonylglycerol (2-AG)] have been identified in mammalian tissues, and their levels increase after exposure of animals to noxious stimuli as well as in intestinal biopsies of patients with colon cancer, diverticulitis and celiac disease[6-10]. Endocannabinoids are biosynthesized 'on demand' from membrane phospholipids and released from cells immediately after their production. Following receptor activation and induction of a biological response, endocannabinoids are inactivated through a two-step process: (i) first through a reuptake process facilitated by a putative membrane transporter whereby endocannabinoids are transported into cells, and (ii) subsequent enzymatic degradation by the fatty acid amide hydrolase (FAAH)[5,10]. There is pharmacological and biochemical evidence that proteins involved in endocannabinoid biosynthesis and inactivation are present in the gut. Both the anandamide-biosynthesizing N-acyl-phosphatidyl-ethanolamine-selective phospholipase D (NAPE-PLD) and the metabolizing enzyme FAAH have been identified in the rodent gut, although their precise localization is still elusive. NAPE mRNA has been found in the rodent stom-

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ach[11] and in enterocytes and lamina propria cells within the small intestine[12]. FAAH has been detected in different regions of the rodent gastrointestinal tract, and its inhibition results in reduction of gastrointestinal motility[13,14], protection against experimental colitis[7,15] and antitumoral effects[16]. There is at present no direct evidence for the existence of a membrane transporter for endocannabinoid reuptake in the gut; however, functional studies suggest that this process might be involved in some experimental pathophysiological states such as inflammation[7] and diarrhea[17].

Although endocannabinoids have been implicated in the control of a number of important physiological and pathophysiological functions in the digestive tract, including emesis[18], gastroprotection[19,20], intestinal ion transport[21], inflammation[7,9], visceral sensation[22] cell proliferation and cancer[16,23,24], this review will deal with the role of the endogenous cannabinoid system in the control of gastrointestinal motility.

Cannabinoid Receptors in the Digestive Tract: Focus on the Myenteric Pplexus

There is evidence for the presence of both CB_1 and CB_2 receptors in the gut. While CB_2 receptors have been mostly identified in inflammatory/immune cells[25,26], CB_1 receptors have been found on epithelial cells, smooth muscle cells, inflammatory/immune cells and in neurons, which include extrinsic afferent sensory neurons, vagal efferents and the neurons of the enteric nervous system (Figure 1)[3].

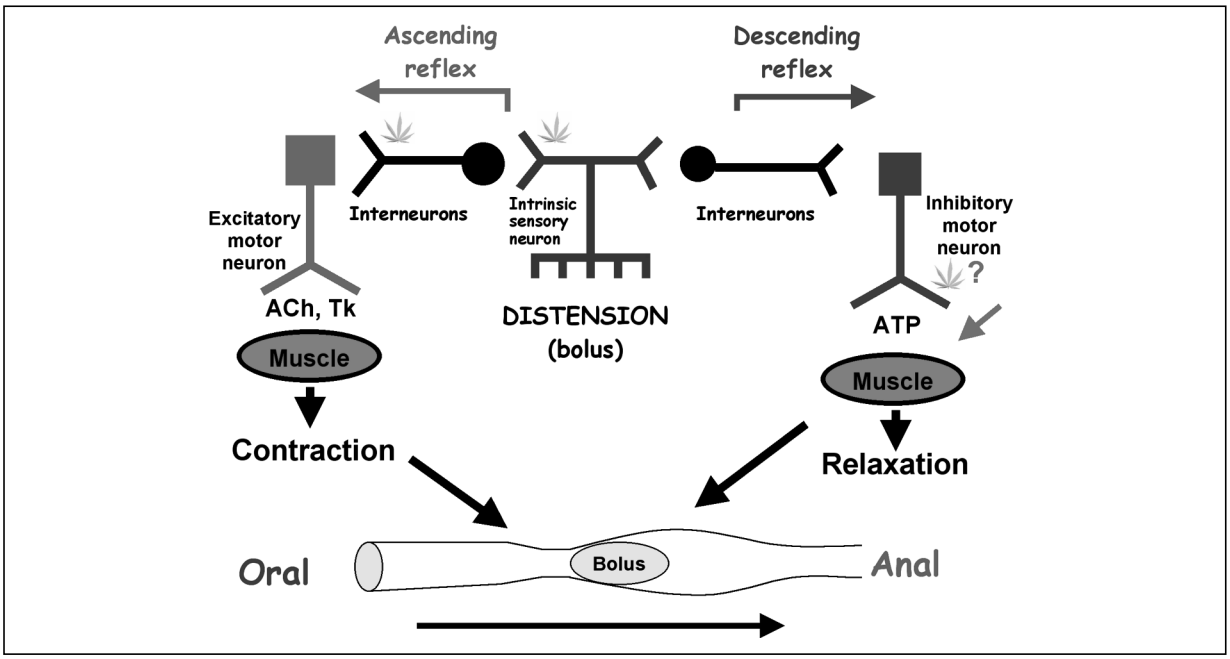

Figure 1. Polarized enteric motor pathways involved in control of intestinal motor activity and localization of CB_1 receptors. Peristalsis occurs in response to radial distension of the intestinal wall. Distention activates enteric reflexes, which include an ascending excitatory reflex leading to contraction at the oral end via the release of excitatory transmitters such as acetylcholine (ACh) and tachykinins (Tk), and a descending inhibitory reflex which causes relaxation of the smooth muscle at the aboral end via the release of inhibitory transmitters such as NO and ATP. The coordinated oral contraction and anal relaxation enables the intestinal bolus to be transported in the anal direction. Neurons in this pathway include intrinsic sensory neurons, which respond to mechanical or chemical stimuli, motor neurons, which may cause either contraction (excitatory motor neurons) or relaxation (inhibitory motor neurons) and interneurons (orally and anally projecting), which connect sensory neurons to motor neurons. CB_1 receptor (indicated with the marijuana leaf) immunoreactivity has been identified on intrinsic sensory neurons, ascending interneurons and final excitatory motor neurons (cholinergic and possibly tachykinergic neurons) projecting to the longitudinal and circular muscles. The presence of CB_1 receptors on inhibitory motor neurons (possibly purinergic neurons), mediating relaxant effects via the fast (apamin-sensitive) inhibitory junction potential has been hypothesized on the basis of functional studies. No colocalization between CB_1 receptor immunoreactivity and NO synthase has been observed. Pharmacological evidence suggests that the main site of action of cannabinoid drugs is the CB_1 receptor located on the final excitatory cholinergic motor neurons whose activation leads to a reduction of acetylcholine release.

The enteric nervous system is formed by two major plexuses, called the submucosal plexus, which is primarily involved in the control of intestinal ion transport, and the myenteric plexus, which plays an important role in the control of intestinal motility. The myenteric plexus lies between the longitudinal and circular layers of muscle and extends over the entire length of the gut[27-29]. The neurons that make up the enteric nervous system can be classified as intrinsic primary afferent neurons, interneurons, and motor neurons. Intrinsic primary afferent neurons, which form the sensory limb of all intrinsic motor reflexes, are all cholinergic and may or may not contain substance P. CB_1 receptor immunoreactivity colocalizes with cholinergic nerves containing calbindin, a marker for intrinsic primary afferent neurons in the guinea pig ileum[30]. Interneurons are interposed between the primary afferent neurons and the motor neurons. Interneurons involved in motor reflexes are directed orally or anally and are designated as ascending or descending, respectively. Ascending excitatory cholinergic neurons of the guinea pig ileum are calretinin immunoreactive and express CB_1 receptors[30]. The motor neurons can be classified as excitatory neurons, which contract smooth muscles through the release of acetylcholine and tachykinins, and inhibitory motor neurons, which relax smooth muscles through the release of nitric oxide (NO), adenosine triphosphate (ATP) or vasoactive intestinal polypeptide (VIP). CB_1 immunoreactivity completely colocalizes with the marker of cholinergic neurons choline acetyltransferase in the guinea pig[30], rat[30], porcine[31] and human[32,33] intestine. Colocalization of CB_1 receptors with substance P immunoreactive neurons (or unidentified noncholinergic excitatory motor neurons) has been also reported[30,31,34]. These data suggest that CB_1 receptors are associated with cholinergic and noncholinergic excitatory motor neurons. Finally, the presence of CB_1 receptors on inhibitory motor neurons (possibly purinergic neurons), mediating depressant effects on the fast (apamin-sensitive) inhibitory junction potential has been hypothesized on the basis of functional studies only. On the other hand, several studies have reported that in the myenteric plexus there is no overlap between NO synthase and CB_1 receptor immunoreactivity[31,35].

Animal Studies on Gastrointestinal Motility

Cannabinoids have been shown to reduce gastric, small intestinal and colonic motility both in isolated segments and in vivo studies in rodents. This effect is largely mediated by CB_1 receptor activation, although CB_2 receptors may be involved in some pathophysiological states.

Animal Studies in vitro

It is generally accepted that cannabinoid agonists act on prejunctional CB_1 receptors to reduce smooth muscle contractility, ascending neural contractions and peristalsis in different regions of the gastrointestinal tract. The mechanisms by which CB_1 activation reduces contractility are mainly related to reduction of acetylcholine release from enteric nerves, although other mechanisms, such as inhibition of nonadrenergic noncholinergic (NANC) excitatory transmission have been proposed[2,8,36].

Excitatory Transmission

Transmission from cholinergic and NANC excitatory neurons has been evaluated in various intestinal segments and in a number of animal species. Results suggest that activation of CB_1 receptors reduce cholinergic and possibly NANC excitatory transmission. The action resembles the putative effects of μ-opioid receptor and α_2-adrenoceptor agonists in intestinal tissues.

Cholinergic Transmission

Cholinergic excitatory neurons supply both the longitudinal and the circular muscle of the stomach, small intestine and large intestine. The receptor for acetylcholine on the muscle has been shown to be the M_3 subtype muscarinic receptor[37]. Cannabinoid receptor agonists, including the plant-derived Δ^9-THC and the endogenous cannabinoid agonist anandamide have been shown to reduce electrically-induced contractions in different regions of the gastrointestinal tract, including the rodent stomach[38,39], small intestine[40,41] and colon[42]. Both the longitudinal and the circular muscle are generally responsive to the inhibitory action of cannabinoid receptor agonists. Detailed studies in the guinea pig ileum (Table I) have shown that cannabinoid-induced inhibition of contractility was competitively and reversibly antagonized by CB_1 receptor antagonists, without any effect on the inhibitory responses to morphine (μ-opioid receptor agonist) or clonidine (α_2-adrenoceptor agonist)[40,41]. It is likely that cannabinoids reduce (via CB_1 receptor activation) electrically-induced contractions by

Table I. Potency (ED_{50}) of cannabinoid receptor agonists in reducing electrically-induced contractions (EFS) in the guinea pig ileum, upper gastrointestinal transit (UGT) and colonic motility in the mouse in vivo (intraperitoneal administration).

Cannabinoid	Guinea pig ileum EFS-induced contractions IC_{50} (nM)	Mouse UGT ED_{50} (mg/kg)	Mouse colonic motility ED_{50} (mg/kg)
Anandamide	8823*	4.24	4.73
WIN 55,212-2	5.54	0.262	0.375
ACEA	Not studied	0.203	0.174
Cannabinol	3913	7.784	11.203
D^9-THC	214	1.3**	Not studied
CP55,940	3.46	0.047***	Not studied

*The IC_{50} was 289 nM in the presence of a FAAH inhibitor; **after i.v. administration; ***data refer to the rat (from Di Carlo and Izzo, 2003, reference 1).

decreasing acetylcholine release from enteric nerves; indeed, cannabinoid agonists (i) did not modify the contractions produced by exogenous acetylcholine which evokes contractions by a direct action on muscarinic receptors located on intestinal smooth muscle)[40,41], but (ii) reduced electrically-evoked acetylcholine release from myenteric nerves[43].

In a single study involving intracellular recordings from neurons of the guinea pig myenteric plexus-longitudinal muscle preparation, cannabinoid receptor agonists were found to inhibit fast and slow excitatory synaptic transmission. In a subset of the neurons tested, this effect was reversed by the CB_1 receptor antagonist rimonabant[44].

Nonadrenergic Noncholinergic (NANC) Excitatory Transmission

The release of enteric excitatory substances other than acetylcholine has been assumed because residual responses to nerve stimulation are detected after the actions of cholinergic nerves have been blocked by atropine[28]. In the circular muscle of guinea pig ileum, in the presence of atropine and guanethidine, high frequency electrical field stimulation of enteric nerves produces a contractile response which is mediated by the release of tachykinins from postganglionic neurons. In this preparation anandamide and the synthetic cannabinoid agonist WIN55,212-2 reduced the NANC excitatory response produced by electrical stimulation in a CB_1 receptor-sensitive manner[40]. As both cannabinoid receptor agonists did not inhibit the contractions produced by exogenous substance P, it was assumed that cannabinoids inhibit the NANC contraction by acting prejunctionally rather than through a direct action on intestinal smooth muscle. Consistent with these results, immunohistochemical studies reported localization of CB_1 receptors to substance P-immunoreactive neurons[30,31,34].

Inhibitory Neurotransmission

In the gastrointestinal tract, a major part of the inhibitory autonomic innervation appears to be provided by NANC nerves, which have two or more primary transmitters, including NO and ATP (or a related purine). The nonnitrergic component of the inhibitory transmission is blocked by apamin, an inhibitor of the small-conductance Ca^{2+}-activated K^+ channels. NANC inhibitory nerves are present throughout the gastrointestinal tract in all species and their blockade results in disruption of peristalsis[28]. Although there is no immunohistochemical evidence for the presence of CB_1 receptors on NANC inhibitory nerves, functional studies indicate that cannabinoids may affect enteric inhibitory transmission in rodents. Focal electrical stimulation of intrinsic neurons of isolated strips of the mouse proximal colon induces 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 the mouse inhibitory transmission, and a slow (sustained) inhibitory junction potential (sIJP) which represents the NO-dependent component of the mouse inhibitory transmission. WIN55,212-2 (in a CB_1 receptor antagonist-sensitive manner) significantly reduced the EJP and fIJP, but not sIJP, suggesting that CB_1 receptor

activation reduces the apamin-sensitive component of the inhibitory transmission in the mouse colon[45]. However, by using a pharmacological approach (i.e. EFS-induced relaxation of isolated intestinal tissues), it has been recently shown that cannabinoids do not modulate NANC inhibitory transmission in the mouse stomach and colon[38,42].

Ascending Excitatory Reflex

The ascending excitatory reflex (AER) is a neurogenic orally-directed motor response of the circular musculature which is triggered by gut wall distention. The reflex involves intrinsic sensory neurons, interneurons and motor neurons. Transmission between these neurons and to the muscle involves nicotinic and muscarinic acetylcholine receptors as well as tachykinin receptors[28]. Heinemann et al showed that methanandamide inhibited the AER contraction elicited by balloon distension[46]. Since methanandamide also depressed hexamethonium-resistant AER (which depends on tachykinergic neuroneural transmission), it was suggested that methanandamide interrupts both cholinergic and noncholinergic junctions within ascending enteric neural pathways[46]. To establish the site of action of cannabinoids on specific enteric neural pathways, Yuece et al used a mouse small intestine preparation set up in a partitioned bath in which ascending nerve pathways were stimulated electrically to activate synaptically excitatory motor neurons to the circular muscle. Thus, the action of cannabinoid drugs on the ascending enteric nerve pathway was studied without interfering with the recording of the smooth muscle contractions (Figure 2)[47]. It was found that anandamide inhibited ascending neural contractions evoked by electrical stimulation only when added to the oral compartment (where contractions were recorded) and not when added to the aboral compartment, where only neurons but not the neuromuscular site was in contact with anandamide. These results suggest that the main site of action of anandamide is the CB_1 receptor located on the neuromuscular unit[47].

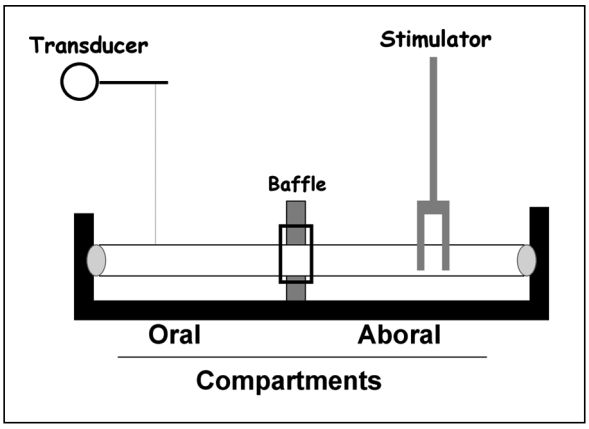

Figure 2. Partitioned bath for the separate application of cannabinoid drugs to enteric nerve pathways and the recording of the resulting contraction of the circular muscle. The partitioned bath enables drugs to be applied to enteric nerve pathways (i.e. in the aboral compartment) without interfering with the recording of the smooth muscle contraction (which occurs in the oral compartment). By using this partitioned bath, it has been demonstrated that anandamide reduced (via CB_1 receptor activation) the ascending neural contraction evoked by electrical stimulation only when added to the oral compartment. Adapted from Yuece et al. 2007, reference 47.

Peristalsis

Peristalsis is a coordinated pattern of motor behavior which occurs in the gastrointestinal tract and allows the contents to be propelled in the anal direction. Because peristalsis is controlled primarily by the enteric nervous system and does not depend on inputs from extrinsic neurons, it can be studied in isolated segments of intestine[27,28]. In the isolated guinea pig small intestine, two phases of peristalsis have been described in response to the slow infusion of liquid which radially stretches the intestinal wall: a preparatory phase, in which the intestine gradually distends until a threshold distension and an emptying phase, in which the circular muscle at the oral end of the intestine contracts, an effect followed by a wave of contraction that propagates aborally along the intestine. The pathways mediating peristalsis involve intrinsic sensory neurons and interneurons, as well as excitatory and inhibitory motor neurons. Acetylcholine acting through both muscarinic and nicotinic receptors and tachykinins are excitatory neurotransmitters participating in the peristaltic activity, whereas VIP, NO and an apamin-sensitive inhibitory transmitter (possibly ATP or a related purine) act as inhibitory mediators[28,29].

The cannabinoid agonists WIN55,212-2 and CP55,940 inhibited peristalsis in the guinea pig ileum as deduced from (i) the decreased longitudinal smooth muscle reflex contraction and the increased threshold pressure and volume required to elicit peristalsis during the preparatory phases of peristalsis and (ii) the reduction of maximal ejection pressure during the emptying phase of

peristalsis[48]. These effects were antagonized by the CB_1 receptor antagonist rimonabant, which, in absence of any agonist, increased maximal ejection pressure during the emptying phase of peristalsis. In a different work, it was found that methanandamide, a metabolically stable analogue of the endocannabinoid anandamide, increased (via CB_1 receptor activation) the peristaltic pressure threshold, which is suggestive of inhibitory effect on peristalsis[46]. The antiperistaltic effect of methanandamide was reduced by the NO synthase inhibitor L-NAME and inhibited by apamin which blocks neuromuscular transmission from motor neurons mediated by ATP (or a related purine). Moreover, when cholinergic transmission was blocked by atropine or hexamethonium (in which case peristalsis is mediated by the release of endogenous tachykinins), the activity of methanandamide to depress peristalsis was preserved. These findings further confirm that cannabinoids inhibit NANC excitatory transmission in the guinea pig ileum.

Mancinelli et al elicited peristaltic activity by distention of the isolated mouse distal colon[49]. Intraluminal pressure, longitudinal displacement, ejected fluid volume and changes in the morphology of the external intestinal wall were recorded. In the pre-drug (control) period, peristaltic activity was characterized by regular, monophasic waves, and the intraluminal content was propelled in an oro-aboral direction. The cannabinoid receptor agonist WIN55,212-2 (in a CB_1 receptor antagonist-sensitive manner) caused a dose-related attenuation of peristaltic activity consequent to the decrease of circular and longitudinal muscle strength. The decrease of contractile activity was followed by a dose-dependent decrease in the amount of fluid ejected during peristalsis. The CB_1 receptor antagonist rimonabant, administered alone, enhanced both tonic and phasic motor activities in the colonic longitudinal smooth muscle[49].

Spontaneous Motility

The effect of cannabinoid drugs has been recently evaluated on the spontaneous contractile activity of the longitudinal muscle in the mouse isolated ileum which displays spontaneous contractions[50]. The endocannabinoid anandamide, the selective cannabinoid CB_1 receptor agonist, ACEA, but not the selective cannabinoid CB_2 receptor agonist, JWH 133 reduced the spontaneous mechanical activity. The inhibitory effect of ACEA, which consisted in a decrease of the mean amplitude of longitudinal spontaneous contractions (without changes in the resting tone), was significantly antagonized by the selective cannabinoid CB_1 receptor antagonist, rimonabant, but not by the selective cannabinoid CB_2 receptor antagonist, AM630. Furthermore, the ACEA-induced reduction of spontaneous contractions was nearly abolished by tetrodotoxin and atropine. The authors concluded that activation of neural CB_1 receptors may play a role in the control of spontaneous mechanical activity through inhibition of acetylcholine release from cholinergic nerve[50].

Animals Studies in vivo

Animal studies *in vivo* have shown that endocannabinoids (via CB_1 receptor activation) constitute a physiological "brake" along the gastrointestinal tract. This concept is based on the findings of (i) a high amount of endocannabinoids in intestinal tissues[50], (ii) the strategic location of CB_1 receptors on neurons of the myenteric plexus[34,35], (iii) inhibitory actions of cannabinoid agonists, including the endocannabinoid anandamide, on gastric and intestinal motility[13,51], (iv) prokinetic effects of the CB_1 receptor antagonist rimonabant[13,35,51], (v) increased gastrointestinal transit in CB_1 receptor-deficient mice[47], and (vi) a depressant action on motility of inhibitors of anandamide inactivation[13,35,52]. Cannabinoids also reduce motility in the inflammed gut and this effect may be mediated by both CB_2 receptors and hyper-expressed myenteric CB_1 receptors[25].

Gastric Motility

A number of cannabinoid receptor agonists, including anandamide, ^{9}-THC, WIN55,212-2, CP 55,940 and cannabinol reduce gastric motility in mice and rats and this effect is antagonized by the CB_1 receptor antagonist rimonabant, but not by the CB_2 receptor antagonist SR144528[13,53,54,55]. Most notably, intravenous ^{9}-THC inhibited gastric motility and decreased intragastric pressure in anesthetized rats. Also, the application of ^{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 ^{9}-THC[54]. Taken together, these data indicate that the gastric effects of systemically administered ^{9}-THC depend on an intact vagal circuitry.

Intestinal Motility

The ability of cannabinoids to reduce intestinal motility has been known before the discovery of cannabinoid receptors. In 1972, Dewey et al first reported the effect of a cannabinoid on the rate of passage of a charcoal meal along the small intestine[56]. These authors demonstrated that ^{9}-THC was at least ten times less potent than morphine in mice in delaying intestinal motility. These results were confirmed by Chesher et al (1973), who also showed that ^{8}-THC and three different *Cannabis* extracts dose-dependently reduced the passage of a charcoal meal in mice[57]. ^{8}-THC and ^{9}-THC were shown to be equipotent, while cannabidiol was inactive[57]. In a more complete study, Shook and Burks (1989) reported that ^{9}-THC and cannabinol slowed small intestinal transit when injected intravenously in mice and rats. It was noteworthy that when injected intravenously, ^{9}-THC was found to be equipotent to morphine in delaying intestinal motility[58].

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 endogenous, synthetic and plant-derived cannabinoids inhibited intestinal transit and colonic propulsion in mice (Table I) and rats[35,59-63], an effect counteracted by the selective CB_1 receptor antagonist rimonabant, but not by the CB_2 receptor antagonist SR144528 or by the opioid receptor antagonist naloxone. Notably, the inhibitory effect of anandamide was not reduced by the transient receptor potential vanilloid type-1 (TRPV1) channel antagonist capsazepine or by a chronic treatment with capsaicin (a treatment which ablates capsaicin-sensitive afferent neurons)[60], thus implying that the effect of anandamide on intestinal transit is independent of TRPV1 activation.

Cannabinoids were significantly more effective when administered intracerebroventricularly (i.c.v.) than when administered intraperitoneally[35,63] 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 intraperitoneally injected cannabinoid receptor agonists was not modified by the ganglion blocker hexamethonium[35,63]. The primary role of peripheral (enteric) CB_1 receptors was emphasized by the observation that i.c.v. administered rimonabant did not significantly reduce the effect of intraperitoneal WIN55,212-2[55].

Carai et al investigated whether tolerance develops after repeated administration of the cannabinoid receptor antagonist rimonabant. Mice were treated intraperitoneally twice a day for up to 8 consecutive days with rimonabant. On day 1, rimonabant markedly activated intestinal peristalsis, but complete tolerance to this effect developed within the third day of treatment[64].

Motility Under Pathophysiological States

The presence of dysmotility in inflammatory diseases of the small and large intestine is a well-recognized and clinically accepted phenomenon. Changes in the endogenous cannabinoid system during inflammation may alter and/or contribute to these motility changes. Depending of the inflammatory insult, both CB_1 and CB_2 receptor activation may reduce hypermotility associated with gut inflammation. In the model of ileitis induced in mice by the irritant croton oil, intestinal CB_1 receptors are hyper-expressed and cannabinoid agonists are consequently more active in reducing transit compared to control mice[51]). Moreover, using CB_1-deficient mice, Sibaev et al showed that such receptors are involved in early protective mechanisms against electrophysiological disturbances (i.e. disturbances in the neuromuscular unit) initiated in the distal colon by dinitrobenzene sulphonic acid administration[65]. On the other hand, in the lipopolysaccharide model of intestinal hypermotility in the rat, the CB_1 receptor-mediated control of intestinal motility was completely replaced by a CB_2 receptor-mediated mechanism. Indeed, in these animals, hypermotility was normalized by a CB_2, but not by a CB_1 receptor agonist[66].

Lower Esophageal Sphincter Relaxation

Gastroesophageal reflux disease refers to reflux of gastric contents into the esophagus leading to esophagitis, reflux symptoms sufficient to impair quality of life, or long-term complications. Transient relaxation of the lower esophageal sphincter (LES) is believed to be the primary mechanism of the disease, although the underlying cause remains uncertain[67]. The principal anatomical components of LES relaxation are afferent gastric pathways, an integrative center in the brainstem, and efferent inhibitory pathways to the LES. Cannabinoid receptor agonists inhibited (via CB_1 receptor activation) LES relaxation in dogs[68] and ferrets[69], the effect being associated, at least in the dog, with inhibition of gastroesophageal reflux[68]. Central and peripheral vagal

mechanisms are involved in these functional changes. This is in agreement with the observation that CB_1 receptor staining is present in cell bodies within the dorsal vagal complex (i.e. the area postrema, nucleus of the solitary tract and nodose ganglion)[68].

Human Studies on Gastrointestinal Motility

In Vitro Studies on Cholinergic Transmission

Immunohistochemical and pharmacological evidence suggests that CB_1 receptors are functionally present in the human ileum and colon; their activation results in inhibition of cholinergic transmission subserving contraction of both the circular and longitudinal muscle[32,70-72]. A number of cannabinoid receptor agonists, including the nonselective cannabinoid agonist WIN55,212-2 and the selective CB_1 receptor agonist ACEA, inhibited the contractions evoked by electrical stimulation without affecting the contractions induced by endogenous agonists such as carbachol or acetylcholine. Immunohistochemical studies localized CB_1 receptors to cholinergic neurons in the intestine (particularly in the circular muscle) based on colabelling with choline acetyltransferase[32]. By contrast, the CB_1 receptor agonist ACEA did not significantly modify electrically evoked relaxation of both circular and longitudinal muscle preparations, thus suggesting a negligible effect on human colonic inhibitory transmission[32].

Diverticular disease is often associated with motor abnormalities of the affected colonic segment[73]. Guagnini et al compared the *in vitro* contractile response to electrical field stimulation of longitudinal smooth muscle strips from colonic segments close to diverticula with that of anatomically healthy segments from patients with nonobstructive colon cancer, which served as controls[6]. In the healthy colon, the cannabinoid agonist WIN55,212-2 inhibited electrically evoked contractions and this effect was competitively antagonized by rimonabant; moreover, the CB_1 receptor antagonist rimonabant had no intrinsic effects, being unable to inhibit electrical twitches[6]. Unlike the healthy colon strips, those from diverticular disease patients were markedly less sensitive to the inhibitory action of WIN55,212-2. In contrast to control tissues, rimonabant had a marked intrinsic action, as it increased twitch contractions in colonic tissues from diverticular disease patients. These results suggest that diverticular strips are tolerant to the inhibitory action of endogenous cannabinoids[6]. A possible interpretation of these results is that in diverticular – but not in the healthy – colon there is a strong tonic inhibitory drive sustained by endocannabinoids which reduce the release of excitatory neurotransmitters through an action at the prejunctional cannabinoid CB_1 receptor. Consistent with this hypothesis, colonic levels of anandamide were increased in diverticulitis specimens compared to healthy controls[6].

Finally, it has recently been demonstrated that the endocannabinoids anandamide and 2-AG can exert myogenic inhibitory effects (i.e. inhibition of acetylcholine-induced contractions) in strips of human colonic longitudinal and circular muscle and that this effect is independent from cannabinoid receptor activation[74].

Clinical Studies

Although not all studies yielded similar conclusions, data on humans suggest that cannabinoid agonists reduce gastric emptying. In a randomized double blind trial, McCallum et al showed that oral ^{9}-THC, at a dose used for preventing chemotherapy-induced nausea and vomiting, delayed gastric emptying of a radiolabelled solid meal in nine male and four female healthy subjects who were experienced cannabis users[75]. Bateman found that gastric emptying of liquid, measured by real time ultrasound, was unaffected by intravenous ^{9}-THC (0.5 and 1 mg/kg, a dose which produced cannabis-like psychomotor and psychological effects in humans) in seven fasted cannabis-naïve male volunteers[76]. Apart from the different doses and techniques to measure motility in the two studies, as well as the choice of human volunteers (*Cannabis* users *vs Cannabis* naïve subjects), it should be noted that there are differences in the patterns and the motor mechanisms involved in the gastric emptying of solid and liquid foods. Gastric emptying of liquid food is driven mainly by the tone of the gastric fundus.

Recently, Esfandyari et al have fond that ^{9}-THC administration was associated with a significant delay in gastric emptying of a standard solid and liquid meal, and there is a suggestion of a gender effect influencing the response to the cannabinoid agonist, as indicated by the borderline gender interaction effect (gastric emptying reduced in females but not in males)[77].

Cannabinoids also modulate colonic motility; specifically, ^{9}-THC administration was associated

with relaxation of the colon and inhibition of the increase in tone after the meal[78]. Increased colonic compliance was predominantly due to a significant effect in female subjects. It was hypothesized that a cannabinoid receptor agonist might be effective in reducing postprandial stimulation of colonic propulsion, as occurs in some patients with diarrhea and urgency associated with irritable bowel syndrome or dysautonomia.

Genetic Variation in IBS Patients

Irritable bowel syndrome (IBS) is a highly prevalent functional gastrointestinal disorder (FGID) affecting up to 3-15% of the general population in Western countries. It is characterized by unexplained abdominal discomfort and bowel habit alterations: constipation (C-IBS), diarrhea (D-IBS), or mixed IBS[79]. In a study of 482 patients with a range of functional gastrointestinal disorders and 252 healthy volunteers, there was a significant association of *FAAH* genotype with FGID phenotype and with specific individual phenotypes (specifically with D-IBS and mixed IBS and with accelerated colonic transit in D-IBS). The association of a genetic variation in metabolism of endocannabinoids with symptom phenotype in D-IBS and mixed IBS and with faster colonic transit in D-IBS supports the hypothesis that cannabinoid mechanisms may play a role in the control of colonic motility[80].

Non-cannabinoid Receptor-Mediated Effects

Endocannabinoids may also exert pharmacological actions through activation of the TRPV1 channel, of orphan G protein-coupled receptors (such as GPR55) or members of the nuclear receptor family (i.e. the peroxisome proliferator-activated receptor subtype α)[81]. The most studied among these potential targets is TRPV1, which is mainly expressed by primary afferent neurons[82]. Activation of TRPV1 channels by anandamide resulted in ileitis in rats[83] and stimulation of acetylcholine release from guinea pig myenteric nerves[84]. In addition, there is evidence that TRPV1 activation by anandamide increases ethylene diamine-induced GABA release from the guinea pig myenteric plexus[85]. However, Bartho et al[86] could find no evidence for anandamide activation of capsaicin-sensitive receptors in the isolated human sigmoid colon.

Finally, there is also *in vitro* evidence that endocannabinoids may act through non-cannabinoid receptor, non-TRPV1 mechanisms. Mang et al showed that anandamide inhibited electrically evoked acetylcholine release in the guinea pig ileum and that this effect was not counteracted by cannabinoid or TRPV1 antagonists[84]. Also, 2-AG contracted the longitudinal smooth muscle from the guinea pig distal colon in a tetrodotoxin-sensitive manner[87]. In human tissues, anandamide and 2-AG inhibited myogenic contractions independent of cannabinoid receptor activation[74].

Conclusions

An increasing number of articles has shown that cannabinoids may reduce intestinal motility in vivo through activation of CB_1 receptors under physiological states and under pathophysiological states involving hyperexpression of CB_1 and/or CB_2 receptors. Conversely, blockade of CB_1 receptors resulted in increased motility, which is consistent with the occurrence of diarrhea in patients which receive the antiobesity drug rimonabant (CB_1 receptor antagonist)[88]. The major role of cannabinoid receptors seems to be that of a "braking" mechanism, which operates when intestinal functions are over-stimulated by excitatory neurotransmitters. Cannabinoid drugs may restore motility when this is pathologically perturbed. Thus, CB_1 receptor agonists or antagonists could represent novel drugs either to decrease gut motility (e.g. cannabinoid agonists to decrease gut motility during diarrhea, inflammation or in IBS patients) or to increase motility (cannabinoid antagonists as prokinetic agents).

The main problem with nonselective cannabinoid receptor agonists is the activation of brain CB_1 receptors, resulting in sedation, cognitive dysfunction and psychotropic effects. The unwanted side effects may be minimized by several approaches, such as the development of selective CB_1 agonists that do not cross the blood-brain barrier, selective targeting of CB_2 receptors since CB_2 receptors are largely absent from the brain, or the use of inhibitors of endocannabinoid inactivation (i.e. FAAH inhibitors and inhibitors of endocannabinoid reuptake) which increase intestinal levels of anandamide to act on local CB_1 receptors, without central effects.

Finally, *Cannabis*-derived non-psychotropic cannabinoids can avoid cannabinoid-associated central effects. The best studied among these compounds is cannabidiol, an antioxidant compound which selectively inhibit intestinal motility in the inflamed – but not in the normal – gut[89].

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