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Cannabinoids and the gut: New developments and emerging concepts

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ABSTRACT

Cannabis has been used to treat gastrointestinal (GI) conditions that range from enteric infections and inflammatory conditions to disorders of motility, emesis and abdominal pain. The mechanistic basis of these treatments emerged after the discovery of Δ^9-tetrahydrocannabinol as the major constituent of *Cannabis*. Further progress was made when the receptors for Δ^9-tetrahydrocannabinol were identified as part of an endocannabinoid system, that consists of specific cannabinoid receptors, endogenous ligands and their biosynthetic and degradative enzymes. Anatomical, physiological and pharmacological studies have shown that the endocannabinoid system is widely distributed throughout the gut, with regional variation and organ-specific actions. It is involved in the regulation of food intake, nausea and emesis, gastric secretion and gastroprotection, GI motility, ion transport, visceral sensation, intestinal inflammation and cell proliferation in the gut. Cellular targets have been defined that include the enteric nervous system, epithelial and immune cells. Molecular targets of the endocannabinoid system include, in addition to the cannabinoid receptors, transient receptor potential vanilloid 1 receptors, peroxisome proliferator-activated receptor alpha receptors and the orphan G-protein coupled receptors, GPR55 and GPR119. Pharmacological agents that act on these targets have been shown in preclinical models to have therapeutic potential. Here, we discuss cannabinoid receptors and their localization in the gut, the proteins involved in endocannabinoid synthesis and degradation and the presence of endocannabinoids in the gut in health and disease. We focus on the pharmacological actions of cannabinoids in relation to GI disorders, highlighting recent data on genetic mutations in the endocannabinoid system in GI disease.

Contents

Abbreviations: 2-AG, 2-arachidonoyl glycerol; AE, acylethanolamide; ACEA, *N*-(2-chloroethyl)5,8,11,14-eicosaetraenamide; ACh, acetylcholine; AP, area postrema; CB, cannabinoid; CBD, cannabidiol; CBDA, cannabidiolic acid; CBDV, cannabidivarin; CBDA, cannabidiolic acid; CBG, cannabigerol; CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; DAGL, diacylglycerol lipase; DNBS, dinitrobenzene sulphonic acid; ENS, enteric nervous system; FAAH, fatty acid amide hydroxylase; GI, gastrointestinal; IBD, inflammatory bowel disease; IL, interleukin; LPS, lipopolysaccharide; MGL, monoacylglycerol lipase; NADA, *N*-arachidonoyldopamine; NAPE-PLD, *N*-acyl-phosphatidylethanolamine-selective phospholipase D; NGF, nerve growth factor; NO, nitric oxide; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; PPAR, peroxisome proliferator-activated receptor; SP, substance P; THC, Δ^9-THC; Δ^9-THCA, Δ^9-tetrahydrocannabinolic acid; Δ^9-THCV, Δ^9-tetrahydrocannabivarin; TNBS, trinitrobenzene sulphonic acid; TNF-α, tumour necrosis factor-α; TRPV1, transient receptor potential vanilloid 1; VIP, vasoactive intestinal peptide.

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1. Introduction

Disorders of the gastrointestinal (GI) tract have been treated with herbal and plant-based remedies for centuries (Di Carlo & Izzo, 2003; Comar & Kirby, 2005). Prominent amongst these therapeutics are preparations derived from the marijuana plant *Cannabis* sp. (Di Carlo & Izzo, 2003). *Cannabis* has been used to treat a variety of GI conditions that range from enteric infections and inflammatory conditions, including inflammatory bowel disease (IBD) to disorders of motility, emesis and abdominal pain (Grinspoon & Bakalar, 1993; Izzo & Coutts, 2005). The mechanistic basis of these treatments gradually emerged after the discovery of Δ^9-tetrahydrocannabinol (Δ^9-THC) as the major psychoactive constituent of *Cannabis*. Even before a specific receptor for Δ^9-THC was cloned in 1990, progress had been made in identifying the site and mechanism of action of THC in the GI tract (Pertwee, 2001; Izzo & Coutts, 2005). For example, Gill et al. (1970) and then Roth (Roth, 1978) demonstrated that Δ^9-THC inhibited cholinergic contractions of the ileum evoked by electrical stimulation of enteric nerves. Since this occurred in the absence of an effect on contractions produced by acetylcholine, it implied a presynaptic or prejunctional locus of action on acetylcholine release. These observations were confirmed and extended using isolated intestinal preparations and in whole animal studies. Cannabinoids (CBs) inhibit peristalsis and GI motility throughout the gut (Pertwee, 2001; Coutts & Izzo, 2004). Whilst these findings helped explain some of the therapeutic properties of *Cannabis*, they did not provide adequate explanations for the anti-inflammatory, anti-emetic and anti-secretory properties of *Cannabis*, as well as more recently described anti-proliferative actions.

After the discovery and cloning of the CB_1 and CB_2 receptors in 1990 and 1993, respectively (Matsuda et al., 1990; Munro et al., 1993), there was a renewed interest in the cannabinoid system. This led to the identification of endogenous cannabinoid ligands, anandamide and 2-arachidonylglycerol (2-AG, Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995) and the development of the concept of the endocannabinoid system (Di Marzo & Fontana, 1995). After these important discoveries, pharmacological, biochemical and molecular tools became widely available for investigations into the endocannabinoid system in the GI tract. This has led to considerable progress in describing the sites and mechanisms of actions of CBs in the gut, as described below. Much remains to be determined, but most of the actions of *Cannabis* and its derivatives can be at least partially explained.

One very significant development has been the identification of the biosynthetic and metabolic (degradative) pathways for the endocannabinoids (Piomelli, 2003; Di Marzo, 2009; Pertwee, 2009). Pharmacological tools have been discovered that, in particular, inhibit the enzymes responsible for the degradation of endocannabinoids. This allows for the manipulation of endocannabinoid levels in the gut, which has significant functional consequences and confirms the physiological and pathophysiological importance of the endocannabinoid system in the GI tract. In recent years, it has become clear that the endocannabinoids are part of a larger family of lipid mediators synthesized from common precursors, which act on both CB and other receptors, before having their actions terminated through common degradation pathways. In this focused review, we shall describe the endocannabinoid system in the gut, the pharmacological actions of CBs and recent developments in the therapeutic targets of the endocannabinoid system in the GI tract.

2. Cannabinoid targets and their localization in the gut

CBs by definition act at CB receptors. However, even from early studies it became clear that other receptor systems were involved in the actions of these pleiotropic molecules. The major receptors for CBs and their localization in the gut are described below.

2.1. Cannabinoid receptors

CB_1 and CB_2 receptors are the classical cognate receptors for all types of CB agonist — endocannabinoids, phytocannabinoids and synthetic CBs (Pertwee, 2009). Whilst there are examples of non-CB_1/CB_2 actions of CBs, there are no other molecularly-characterized CB receptors.

CB receptors have a distinct distribution in the GI tract, being largely distributed in the enteric nervous system (ENS, Duncan et al., 2005). Both CB_1 and CB_2 receptors are found by immunohistochemistry on enteric neurons, nerve fibres and terminals in the ENS. The CB_1 receptor is found on nerve fibres throughout the wall of the gut, but with the highest density in the two ganglionated plexuses, the myenteric and submucosal plexus, of the ENS (Duncan et al., 2005; Wright et al., 2008). Enteric ganglia consist of motor neurons, interneurons and intrinsic primary afferent neurons; CB_1 and CB_2 receptors appear to be localized on all of the functional classes of enteric neurons. Double-labelling immunohistochemistry of CB_1 receptor in neurons expressing choline-acetyltransferase, calretinin and substance P suggests that it is present on excitatory motor neurons (Kulkarni-Narla & Brown, 2000; Coutts et al., 2002), some classes of interneurons and intrinsic primary afferent neurons. The presence of CB_1 receptors on interneurons is also suggested by electrophysiological studies using multi-chambered organ baths (Yuece et al., 2007). Neither CB_1 nor CB_2 receptors are found on inhibitory motor neurons containing nitric oxide synthase (Kulkarni-Narla & Brown, 2000; Coutts et al., 2002; Storr et al., 2004; Duncan et al., 2008a). In rodents, immunoreactivity for the calcium binding protein calbindin is a marker for intrinsic primary afferent neurons. The CB_1 receptor is colocalized with calbindin (Coutts et al., 2002), suggesting that the CB_1 receptor is present on intrinsic primary afferent neurons. The presence of message for these receptors in the ENS was confirmed by in situ hybridization and reverse transcription polymerase chain reaction (Buckley et al., 1998; Storr et al., 2002).

Apart from the ENS, the pattern of cannabinoid receptor expression has not been fully elucidated in any species. There is a report of CB_1 receptors on the normal and inflamed human colonic epithelium, as well as in a number of colonic epithelial cell lines (Wright et al., 2005; Marquéz et al., 2009), however, CB_1 receptor expression was not observed in the duodenal epithelium in controls or patients with celiac disease (D'Argenio et al., 2007). CB_1 receptors were also shown on parietal cells of the human stomach by immunohistochemistry and *in situ* hybridization (Pazos et al., 2008). CB_2 receptors appear to be present in the normal murine colonic epithelium, but not to any extent in the rat and human (Wright et al, 2005; Rousseaux et al, 2007; Marquéz et al., 2009). However, there is an induction of CB_2 receptor immunoreactivity in the mouse and the rat GI epithelium after treatment with probiotic bacteria (Rousseaux et al., 2007) and in sections of the colon in patients with IBD (Wright et al., 2005; Marquéz et al., 2009). Receptor binding studies revealed a distinct distribution of specific CB binding in the outer regions of Peyer's patches of the rat ileum (Lynn & Herkenham, 1994). These have not been followed up with immunohistochemical studies. In