Differential Expression of Cannabinoid Receptors in the Human Colon: Cannabinoids Promote Epithelial Wound Healing

KAREN WRIGHT,* NICHOLAS ROONEY,† MARK FEENEY,§ JEREMY TATE,§ DUNCAN ROBERTSON,¶ MELANIE WELHAM,* and STEPHEN WARD*

*Department of Pharmacy and Pharmacology, University of Bath, Bath; †Department of Cellular Pathology, Southmead Hospital, Bristol; §Department of Gastroenterology, Royal United Hospital, Bath; and ¶Department of Surgery, Royal United Hospital, Bath, United Kingdom

Background & Aims: **Two G-protein–coupled cannabinoid receptors, termed *CB1* and *CB2*, have been identified and several mammalian enteric nervous systems express CB1 receptors and produce endocannabinoids. An immunomodulatory role for the endocannabinoid system in gastrointestinal inflammatory disorders has been proposed and this study sought to determine the location of both cannabinoid receptors in human colon and to investigate epithelial receptor function.** ***Methods:*** **The location of CB1 and CB2 receptors in human colonic tissue was determined by immunohistochemistry. Primary colonic epithelial cells were treated with both synthetic and endogenous cannabinoids in vitro, and biochemical coupling of the receptors to known signaling events was determined by immunoblotting. Human colonic epithelial cell lines were used in cannabinoid-binding studies and as a model for in vitro wound-healing experiments.** ***Results:*** **CB1-receptor immunoreactivity was evident in normal colonic epithelium, smooth muscle, and the submucosal myenteric plexus. CB1- and CB2-receptor expression was present on plasma cells in the lamina propria, whereas only CB2 was present on macrophages. CB2 immunoreactivity was seen in the epithelium of colonic tissue characteristic of inflammatory bowel disease. Cannabinoids enhanced epithelial wound closure either alone or in combination with lysophosphatidic acid through a CB1–lysophosphatidic acid 1 heteromeric receptor complex.** ***Conclusions:*** **CB1 receptors are expressed in normal human colon and colonic epithelium is responsive biochemically and functionally to cannabinoids. Increased epithelial CB2-receptor expression in human inflammatory bowel disease tissue implies an immunomodulatory role that may impact on mucosal immunity.**

The plant *Cannabis sativa* contains more than 60 aromatic hydrocarbon compounds called cannabinoids, of which $\triangle^9$-tetrahydrocannabinol ($\triangle^9$-THC) is the most abundant and is the main psychotropic constituent.[1] The effects of $\triangle^9$-THC are mediated primarily by G-protein–coupled cannabinoid receptors, of which 2 so far have been identified: *CB1*[2,3] and *CB2*.[4] Endogenous lipid ligands for these receptors have been isolated and identified. Anandamide[2] (AEA) and 2-arachidonoyl glycerol[5,6] can bind both receptors at physiologically relevant concentrations and noladin ether[7] (NE) is CB1-specific at low nanomolar concentrations. Recently, an endogenous antagonist, virodhamine, was characterized.[8] Cellular uptake of endocannabinoids may be through facilitated diffusion or transport by a specific, as yet unknown, protein[9] and inactivation[10] by fatty acid amide hydrolase (FAAH) or monoacylglyceride lipase. Other metabolic pathways exist for endocannabinoids,[11] with some metabolites having cannabimimetic activities.[12] Cannabinoid receptors, their endogenous ligands, and the enzymes involved in their inactivation constitute the so-called *endocannabinoid system*.

It has been known for some time that the medicinal use of *Cannabis* can be beneficial for digestive disorders such as pain and diarrhea.[13,14] Plant-derived $\triangle^9$-THC has been shown previously to inhibit peristalsis, reduce gastric and intestinal secretions, and protect against ulcers in rodents.[15–18] More recent studies have provided good functional evidence that the enteric nervous system of several mammals, including the mouse, rat, guinea pig, and human beings, express CB1 receptors.[19–25] The endocannabinoids AEA and 2-arachidonoyl glycerol are present in the digestive tracts of several mammalian species,[26,27] including human beings,[28] and functions largely relate to retardation of intestinal motility through CB1 receptors,[25,29–31] but include control of secretion,[32] proliferation,[28] and neurotransmitter release.[22,23,33,34]

Abbreviations used in this paper: AEA, anandamide; DNBS, dinitrobenzene sulphonic acid; ERK, extracellular-regulated kinase; FAAH, fatty acid amide hydrolase; GSK, glycogen synthase kinase; LPA, lysophosphatidic acid; MAPK, mitogen-activated protein kinase; NE, noladin ether; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; TBS, Tris-buffered saline; THC, tetrahydrocannabinol.

0016-5085/05/$30.00
doi:10.1053/j.gastro.2005.05.026

CB2 receptors are found mainly on immune cells including neutrophils, macrophages, and subtypes of B and T cells,[35] and, until recently, the only evidence for their expression in the digestive tract was the detection of CB2 messenger RNA in guinea-pig whole gut.[36] A new study has provided functional evidence that CB2 receptors in the rat intestine could contribute to reducing the increase of intestinal motility induced by an endotoxic inflammation.[37] Cannabinoids exhibit immunomodulatory properties and influence almost every component of the immune-response machinery, although their role in normal immune homeostasis and development of immune system disorder is unresolved.[38,39]

Many of the functions attributed to cannabinoids are the result of receptor-mediated activation of common G-protein–coupled signal transduction pathways. For example, the extracellular-regulated kinases (ERK) 1/2 mitogen-activated protein kinase (MAPK) pathway results from stimulation of G-protein–coupled receptors[40] such as the CB1 receptor.[41] The cannabinoid-induced stimulation of the mitogen-activated protein kinase cascade has been shown to be prevented by an inhibitor of phosphatidylinositol 3-kinase[42] (PI3K), and cannabinoid-induced PI3K signaling via protein kinase B (PKB) also has been proposed.[43,44] Activated ERK and PKB phosphorylate many intracellular substrates and a target common to both effectors, namely glycogen synthase kinase[45–47] (GSK)-3α/β, has been shown to be phosphorylated in response to cannabinoids.[43,44]

Apart from anecdotal evidence for the therapeutic benefit of *Cannabis* in gastrointestinal pathologies such as Crohn's disease,[14] the extent to which endocannabinoids impact on the immune system in the gut is not well understood. The distribution of cannabinoid receptors has not been investigated in the human large intestine, although some functional studies have been performed in human ileum and colon[24,29] and the presence of both cannabinoid receptors have been found in normal human mucosa at the level of messenger RNA.[28] If we are to exploit the endocannabinoid system for other therapeutic uses, then it is important to know how these drugs might affect gut homeostasis. The purpose of the present study was to determine the location of both CB1 and CB2 receptors in both normal and inflammatory bowel disease (IBD) human colonic tissue and to confirm the functionality of these receptors by establishing whether both endogenous and synthetic cannabinoids couple to known biochemical effector pathways in colonic epithelial cells. In addition, evidence for a cannabinoid role in epithelial wound healing is presented.

Materials and Methods

Subjects

Human colonic biopsy specimens from routine colonoscopy, histopathologically assessed to exclude microscopic inflammation, were retrieved from files at the Royal United Hospital, Bath, United Kingdom. In addition, colonic tissue, which included normal colon at least 20–25 cm from the tumor, was removed from patients undergoing colonic resections. This procedure had the approval of the Bath local research ethics committee, Royal United Hospital Bath National Health Service Trust, United Kingdom. Tissue blocks were fixed in 4% (wt/vol) formaldehyde and embedded in paraffin.

Reagents and Drugs

Cell culture media and plastic ware were purchased from Invitrogen (Paisley, UK). Synthetic (arachidonylcyclopropylamide, JWH 133, and WIN 55,212-2,) and endogenous (AEA, methanadamide, and NE) cannabinoids and the cannabinoid receptor antagonist (AM251) were from Tocris (Bristol, UK). Enzyme inhibitors LY294002 and PD98059 were from Merck Biosciences (Nottingham, UK) and all other chemicals came from Sigma-Aldrich (Dorset, UK). Antibodies to components of the endogenous cannabinoid system were purchased for immunohistochemistry and immunoblotting as follows: anti-CB1 (PA1-743) and anti-CB2 (PA1-744) were from Affinity BioReagents (Cambridge BioScience, Cambridge, UK); anti-CB1 (sc20754) and anti-CB2 (sc25494) were from Santa Cruz (Autogen Bioclear, Wiltshire, UK); anti-CB1 (101500) and anti-CB2 (101550) were from Cayman Chemical (IDS Ltd, Tyne and Wear, UK), and anti-FAAH (11-A) was from Alpha Diagnostic (San Antonio, TX). Other antibodies used in this study included anti–lysophosphatidic acid (LPA)1 (Edg2) from Upstate (Dundee, UK), and anti-ERK, anti–phospho-ERK1/2, anti-PKB, anti–phospho-PKB, anti–phospho-GSK3α/β, and anti-GSK3β from Cell Signaling Technology, New England Biolabs (Hertfordshire, UK).

Immunohistochemical Analysis of Human Colon

The DAKO ChemMate System kit (Cambridgeshire, UK) was used for the immunohistochemical staining of the sections. Briefly, tissue sections (3–5 μm) were mounted on slides. After the sections were deparaffinized with xylene and rehydrated through a series of graded alcohol, antigen retrieval was achieved through boiling in .01 mol/L sodium citrate buffer, pH 6.0, at high pressure for 2 minutes. Sections were blocked in 5% bovine serum albumin in Tris-buffered saline (TBS), pH 9.0, for 1 hour before application of primary antibodies. CB1 and CB2 antibodies (Cayman Chemical) at a 1:1000 dilution in TBS, pH 9.0, were incubated overnight at 4°C. For control slides, primary antibodies were omitted or a 10-fold excess of blocking peptide was used as suggested by the manufacturer (Cayman Chemical). Sections then were incubated in rabbit-specific secondary antibody for 25 minutes