MICE LACKING CANNABINOID CB1-, CB2-RECEPTORS OR BOTH RECEPTORS SHOW INCREASED SUSCEPTIBILITY TO TRINITROBENZENE SULFONIC ACID (TNBS)-INDUCED COLITIS

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This study was performed to assess whether mice lacking the cannabinoid receptor CB1, CB2 or both receptors show increased susceptibility to TNBS colitis in comparison to wildtype mice. Previously, activation of CB1 and CB2 receptors showed attenuation of TNBS colitis in mice. The aim of the study was to investigate the susceptibility of three mouse strains CB1-, CB2- and CB1+2 double knockout mice in the model of TNBS colitis. The different knockout mice were given each a single enema with TNBS 7 mg, volume 150 µl (in 50% ethanol solution) on day 1. Control group (C57BL/6 mice) received the same concentration of TNBS enema and each strain received vehicle application of 150 µl 50% ethanol solution. After a 3-day period, the animals were sacrificed and their colon excised. A scoring system was used to describe macroscopical and histological changes. Messenger RNA-expression of TNF-α and IL-1β as pro-inflammatory markers was measured by RT-PCR. All three knockout strains showed increased susceptibility to TNBS colitis quantified by macroscopical and histological scoring systems and pro-inflammatory cytokine expression in comparison to the TNBS control group (wild type C57BL/6 animals). Mice lacking the CB1-, CB2-receptor or both receptors showed aggravation of inflammation in the model of TNBS colitis. Lacking of both cannabinoid receptors did not result in potentiation of colitis severity compared to lacking of each CB1 or CB2, respectively. These results suggest that the endocannabinoid system may have tonic inhibitory effects on inflammatory responses in the colon.

Key words: endocannabinoid system, trinitrobenzene sulfonic acid colitis, proinflammatory cytokines, CB1 receptors, anandamide

INTRODUCTION

A variety of natural and synthetic cannabinoids have been shown to possess anti-nociceptive and anti-inflammatory activity also in the gut (1-5). The endocannabinoid system (ECS) which is expressed throughout the gastrointestinal tract consists of endocannabinoids, cannabinoid receptors CB1 and CB2, carrier proteins in the cell membrane (endocannabinoid membrane transporter, EMT) and microsomal enzymes of degradation (fatty acid amide hydrolase, FAAH; monoacylglycerol lipase, MAGL) (1, 5-11). The ECS exerts multiple effects on the gastrointestinal tract. In vivo pharmacological studies have shown that activation of CB1 receptors reduces emesis (12, 13), produces inhibition of gastric acid secretion (14) and relaxation of the lower oesophageal sphincter (15) and inhibits intestinal motility and secretion (16). CB1 receptors are located on intrinsic (enteric) (17) and extrinsic neurons (18) such as cell bodies of sensory neurons in the dorsal root ganglia, nodose ganglion and vagal efferenttrons (19-24) and CB2 receptors are expressed mainly on immune cells, although there is also recent evidence for neuronal expression (25). Recently, CB2 receptor expression has been observed in the enteric nervous system in rodents and human ileum (18, 26). The inflammatory process in inflammatory bowel disease (IBD) consists of a complex network of interactions between the nervous system and the immune system. Mast cells, lymphocytes, granulocytes and macrophages are under the permanent influence of peptidergic neurons in the gut (27, 28). Increased epithelial CB1 and CB2 immunoreactivity was observed at sites of mucosal ulceration in inflammatory bowel diseases (29-33). Elevation of anandamide levels and an increase in CB1 and CB2 receptor expression was reported in the colon of tri- (TNBS) and dinitrobenzene sulfonic acid (DNBS) -treated mice and in biopsies of patients with ulcerative colitis (26, 29, 33). Furthermore FAAH activity was found to be reduced in the inflamed mouse colon (26). Taken together, this suggests a reactive overactivity of the ECS in inflammatory states. ECS overactivity subsequently results in anti-inflammatory effects, which is supported by a large body of evidence. Our group recently showed that intraperitoneal application of anandamide attenuated TNBS colitis and decreased levels of the pro-inflammatory cytokines TNF-α and IL-1β (34). Systemical administration of a CB2-selective receptor agonist improved dextrane sulphate sodium (DSS) and mustard oil colitis, however was less effective than treatment with a CB1-selective agonist (32). Genetic ablation or pharmacological inhibition of FAAH as well as systemic administration of an inhibitor of anandamide cellular reuptake caused amelioration of experimental DNBS and TNBS colitis (26, 33).
Interestingly, to date data on inhibition of the ECS in inflammatory states is rare. Massa et al. (31) showed that genetic or pharmacological blockade of CB1 receptors causes worsening of DNBS and DSS colitis. To identify whether the CB1 or CB2 receptor plays the predominant role in experimental colitis we used double knockout mice CB1+2(-/-). This study was aimed to elucidate whether mice lacking both cannabinoid receptors demonstrated a potentiation of increased susceptibility to TNBS colitis in comparison to mice lacking only one of both receptors.

MATERIALS AND METHODS

Animals

All experiments were performed in accordance with the local standing committee for ethical experimentation on animals approved by the Animal Protection Authority of the District Government (Regierung von Mittelfranken, Wurzburg, Germany). We used wild type (WT) C57BL/6 mice (20-30 g) and colonies of CB1(-/-), CB2(-/-) and CB1+2(-/-) mice (15-30 g) (generously provided by Prof. Dr. A. Zimmer, University of Bonn, Germany). All mice were conventionally genotyped using commercially available primers (Metabion).

Induction of colitis and dosing regimens

All mouse strains were housed under standard conditions and supplied with drinking water and food ad libitum. After 24 hours of starving, colitis was induced in mice by application of a single enema with 7 mg TNBS (2,4,6-trinitrobenzene sulfonic acid; Sigma-Aldrich laboratories, Seelze, Germany) via a polyethylene catheter (outer diameter 2 mm) 4 cm from the anus. In pilot experiments, this dose of TNBS was found to induce reproducible colitis in C57BL/6 mice. Noteworthy, our previous study showed that C57BL/6 WT mice were less susceptible towards TNBS-induced colitis than AKR mice (34). Interestingly, inflammation severity was increased in the middle and distal parts of the colon in all mouse strains. Animals were lightly anesthetized with ketamine (40 mg/kg, i.p.) immediately before the procedure. TNBS was dissolved in 50% ethanol solution and the total volume of the enema was 150 µl. Ethanol is required to break the mucosal barrier, whereas TNBS is believed to haptenize colonic autologous or microbiotic proteins rendering them immunogenic to the host immune system (35). Similarly, as observed in inflammatory bowel diseases in humans, this experimental model involves the immune and neuroendocrine systems and leads to long-lasting ulcerative damages of the colonic mucosa (36). Vehicle control (50% ethanol solution 150 µl) was performed in all strains (each n=4). For 3 days mice were monitored for colitis and then were killed in a 100% carbon dioxide atmosphere and their colons excised and collected for further analysis. This time point was chosen because maximal acute TNBS-induced inflammation has been reported in mice after 3 days (37). At 3 cm ab ano colonic segments were dissected (slice thickness 1-3 mm) and placed in 10% neutral-buffered formalin for subsequent histological analysis.

Scoring systems

Different scoring systems were used to describe clinical and macroscopical changes before tissue samples were collected. All scoring systems were used previously with slight modifications and are accepted scoring systems in colitis models (36, 39). Among the macroscopical parameters analysed were length of the colon starting from 5 mm above the anus to the top of the caecum, colon weight and the consistency of any stools found within the colon. The summation of these parameters resulted in the Macroscopical Score (MS) with a maximum of 12 points (Table 1). A semiquantitative score was used to evaluate severity of histological changes in the inflamed colon (Histopathological Colitis Score, HCS). The HCS featured the parameters inflammation extent, crypt architecture, hyperemia/edema and infiltration with inflammatory cells, with a maximum of 11 points (Table 2). The scoring system used to describe the changes for each of these parameters is detailed in Table 2. The two scores MS and HCS were added together to provide the Major Colitis Score (MCS) with a maximum of 23 points graded in mild, moderate and severe colitis (Table 3).

Histology evaluations

Cross-sectioned segments from the colon of each animal were removed, rinsed in saline and then fixed in 10% neutral-buffered formalin. They were embedded in paraffin, sectioned and stained with haematoxylin/eosin. The sections were examined by light microscopy (Olympus Model BX-50 microscope, Olympus Instruments, Melville, NY, USA) and were scored by an investigator; blinded to the experimental groups tested. Scoring method was described before (Table 2). Photomicrographs were taken with a Leitz Laborlux S microscope (Wild Leitz, Wetzlar, Germany) using a 4x and 10x

Table 1. Macroscopical score (MS).

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
<th>Score</th>
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<tr>
<td>Inflammation extent</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>mucosa</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>mucosa + submucosa</td>
<td>2</td>
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<tr>
<td>Damage in crypt architecture</td>
<td>none</td>
<td>0</td>
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<tr>
<td></td>
<td>regeneration</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>destruction</td>
<td>2</td>
</tr>
<tr>
<td>Hyperemia / Edema</td>
<td>without</td>
<td>0</td>
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<tr>
<td></td>
<td>mild</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>2</td>
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<td></td>
<td>severe</td>
<td>3</td>
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<tr>
<td>Infiltration with inflammatory cells</td>
<td>without</td>
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<tr>
<td></td>
<td>mild</td>
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Table 3. Major colitis score MCS (= MS + HCS). The major colitis score (MCS) is derived from the macroscopical and microscopical (histological) features listed above with a total maximum of 23 points. Severity is graded according to the following table:

| Major colitis score MCS | 0 (no signs of colitis) | ≤7 (mild colitis) | ≤14 (moderate colitis) | >14 (severe colitis) |

objective and a SPOT RT digital camera (Diagnostic Instruments, Sterling Heights, MI, USA).

Reverse transcription polymerase chain reaction (RT-PCR)

The expression of TNF-α and IL-1β was analysed by means of RT-PCR. Mucosal specimens were scraped off on ice using slide glass and immediately snap frozen in liquid nitrogen and stored at -80°C until analysis. Total RNA was extracted from mucosal samples using a guanidium isothiocyanate phenol chloroform single step extraction kit from Stratagene (Heidelberg, Germany) based on the method described by Chomczynski and Sacchi (40). Following precipitation, RNA was resuspended in RNAse-free TE buffer and its concentration was estimated by absorbance at 260 nm wavelength. Furthermore, the quality of each RNA sample was determined by running the agarose-formaldehyde electrophoresis. RNA samples were stored at -80°C until analysis. Single stranded cDNA was generated from 5 µg of total cellular RNA using Moloney murine leukaemia virus reverse transcriptase (MMLV-RT) (Stratagene, Heidelberg, Germany) and oligo-(dT)-primers (Stratagene, Heidelberg, Germany). Briefly, 5 µg of total RNA was uncoiled by heating (65°C for 5 min) and then reverse transcribed into complementary DNA (cDNA) in a 50 µl reaction mixture that contained 50 U MMLV-RT, 0.3 µg oligo-(dT)-primer, 1 µl RNase block ribonuclease inhibitor (40 U/µl), 2 µl of a 100 mM/l mixture of deoxyadenosine triphosphate (dATP), deoxythymydine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP) and deoxycytidine triphosphate (dCTP), 10 mM/l Tris-HCl (pH=8.3), 50 mM KCl, 5 mM MgCl₂. The resultant cDNA (2 µl) was amplified in a 50 µl reaction volume containing 2 U Taq polymerase, dNTP (200 µM each) (Pharmacia, Germany), 1.5 mM MgCl₂, 5 µl 10x polymerase chain reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH=8.3) and specific primers for β-actin, TNF-α and IL-1β used at final concentration of 1 mM (all reagents from Takara, Shiga, Japan).

The mixture was overlaid with 25 µl of mineral oil to prevent evaporation. The polymerase chain reaction products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Location of predicted products was confirmed by using a 100-bp ladder (Takara, Shiga, Japan) as a standard size marker. The gel was then photographed under UV-transillumination. The intensity of PCR products was measured using the video image analysis system (Kodak Digital Science) as described earlier (41). The signal for investigated mRNA was standardised against that of the β-actin mRNA from each sample and the results were expressed as analysed mRNA/β-actin mRNA ratio as described earlier (42).

Statistical analysis

Results are presented as means±standard error of the mean (SEM). The number (n) that is quoted throughout the manuscript refers to the number of animals. Accordingly for inter-individual statistical comparison the Mann-Whitney U test was used (Statistica®, StatSoft, USA). Asterisks in the figures indicate significance: *p<0.05.

RESULTS

Intracolon administration of 150 µl of 50% ethanol did not induce detectable inflammation in all vehicle groups (each n=4) after 3 days. Colons of these mice macroscopically and histologically had only minor changes (Fig.1-3 show vehicle-treated C57BL/6 MS 0.3±0.3, HCS 0.8±0.8, MCS 1±1; n=4). There were no significant differences between vehicle-treated C57BL/6, CB1(-/-), CB2(-/-) and CB1+2(-/-) mice (data not shown). After intracolon administration of TNBS (7 mg/150 µl), macroscopical and histopathological evaluation of all knockout strains colons revealed stronger inflammation as compared to the C57BL/6 control group (Fig. 1-3). All CB knockout strains demonstrated higher stool score, increased colon weight gain and a shorter colon which resulted in a higher MS in comparison to control C57BL/6 treated with TNBS. Interestingly, double knockout mice showed a significant higher MS than did CB1(-/-) or CB2(-/-) respectively (C57BL/6 3.9±0.5, n=9; CB1(-/-) 6.4±0.6, n=9; CB2(-/-) 6.7±0.6, n=11; CB1+2(-/-) 8.6±0.5, n=10, p<0.05) (Fig. 1). However, summed up with the HCS (Fig. 2) the MCS (Fig. 3) of CB double knockout mice did not reach statistical significance compared to CB1(-/-) or CB2(-/-). Histopathological colitis score (HCS) consisted of five criteria as described in methods. All three knockout strains compared to WT (C57BL/6 8.6±0.9, n=9; CB1(-/-) 6.9±0.8, n=9, p<0.05, CB2(-/-) 8.2±0.5, n=11, p<0.05) and CB1 + 2 (-/-) 7.4±0.8, n=10, p<0.05) compared to C57BL/6 (4.7±0.6, n=9) (Fig. 2). However, the HCS showed no differences between CB1(-/-), CB2(-/-) or CB1+2 double knockout mice. All strains of null mutant animals revealed severe transmural colitis with severe loss of epithelium, thickening of the bowel wall and inflammatory infiltrates compared to C57BL/6 mice (Fig. 4A-J). Summed up the Major Colitis Score (MCS) was increased in all three CB knockout strains compared to WT (C57BL/6 8.6±0.9, n=9; CB1(-/-) 13.6±1.4, n=9; CB2(-/-) 14.6±0.8, n=11; CB1+2(-/-) 16.0±1.0, n=10, p<0.05) (Fig. 3). According to our scoring system, all three knockout strains showed severe colitis, whereas in the wild type group colitis had a significant lower extent and severity with a moderate degree.

RT-PCR analysis

No differences between all vehicle-treated strains were observed for both cytokines (data not shown). Particularly,
knockout animals (vehicle group) did not show increased levels of mRNA for the pro-inflammatory cytokines IL-1β and TNF-α per se (data not shown). The exposure to TNBS and induction of colitis was associated with a significant increase in the mRNA expression for IL-1β and TNF-α in colonic tissue of all mice strains. TNBS treatment of either CB1(-/-) (n=9), CB2(-/-) (n=11) or double knockout mice (n=10) resulted in a significant increase in the mRNA expression for IL-1β and TNF-α compared to the TNBS control group (Fig. 5). However, there was no difference in IL-1β and TNF-α mRNA expression between each CB knockout strain in an interindividual comparison.

DISCUSSION

Lacking of cannabinoid receptors results in increased susceptibility to experimental colitis

Here we demonstrate for the first time that lacking of either CB1, CB2 or both receptors results in aggravation of experimental colitis in mice. This increase in inflammatory activity resulted in higher levels of mRNA expression of the pro-inflammatory mediators TNF-α and IL-1β and in increased macro- and microscopical parameters.

To date, inhibition of the ECS in experimental colitis has not been as extensively studied as activation. One reason for that might be that therapeutic effects correlate with activation of the ECS. However, for our understanding of the pathophysiology of colitis and the role of the ECS under normal and pathologic conditions it is of importance to evaluate whether perturbance of the ECS aggravates/disinhibits colonic inflammation. Previously, Massa et al. reported stronger inflammation in CB1(-/-) littersmates and in mice treated with a selective CB1 receptor antagonist in DNBS and DSS colitis (31). In addition to their findings we report aggravation of colitis in the model of TNBS-colitis in CB1(-/-) littersmates. For the first time we demonstrate here that deletion of the CB2 receptor or both CB1 and CB2 results in increased susceptibility to TNBS colitis to a degree that is comparable to mice lacking only the CB1 receptor. Worsening of macroscopic and microscopic scores was mirrored in increased expression of the pro-inflammatory cytokines TNF-α and IL-1β in all three KO strains to a comparable degree.

Cannabinoid receptor activation inhibits experimental colitis

Previously, a multiplicity of studies focused on ECS activation and its role in inflammation. Activation of both cannabinoid receptors showed inhibition of inflammation in the gut, although different colitis models produced discrepant results with regard to the question which receptor CB1 or CB2 played the predominant role.
Vehicle group (C57BL/6) showed minor changes in epithelial structure (Fig. 4A). TNBS-treated C57BL/6 control animals showed moderate disruption of the epithelial structure and infiltration of neutrophils with acute inflammation extending into the colonic submucosa (Fig. 4C, D). CB1(-/-) (Fig. 4E, F), CB2(-/-) (Fig. 4G, H) and CB1+2(-/-) (Fig. 4I, J) knockout mice showed severe colonic inflammation with necrosis and increased infiltration by inflammatory cells in comparison to TNBS-treated C57BL/6. HCS did not reach statistical significance in an interindividual comparison between the knockout strains (see Fig. 2). M, mucosa; SM, submucosa; ML, muscle layer.
It was shown that endocannabinoids might regulate response to gut inflammation at different levels (47). Synthetic and endogenous cannabinoids were found to reduce release of neurotransmitters that affect intestinal motility and secretion at inflammatory states and by that reduced diarrhea. Systemic application of anandamide reduced colonic levels of TNF-$\alpha$ and IL-1$\beta$ and improved macroscopical and microscopical scores of colitis (34). Additionally, it has been demonstrated that CB1 receptors promoted colonic epithelial wound healing (28).

Recently, Storr et al. showed that protective effects of FAAH or EMT blockade were mediated by both CB1 and CB2 receptors in an acute model of TNBS colitis (25). There is substantial evidence that under different in vitro and in vivo inflammatory conditions, both endogenous and synthetic cannabinoid receptor agonists downregulate mast cells and granulocytes, and reduce cytokine release by acting mainly at CB2 receptors (43-46). CB2 receptor activation in TNBS- and DSS-induced colitis limited immune cell recruitment, decreased cytokine and chemokine production and improved macroscopic and histological scores (47). Previously it was found that a CB2-selective agonist was unable to protect mice from acute DSS colitis, but was able to protect animals from an immune-mediated colitis (Gst2$^{-/-}$ T-cell transfer model of colitis) (48). Another study from the same group found that CB2 receptor deficient mice had profound deficiencies in splenic marginal zone, peritoneal B1a cells, splenic memory CD4$^+$ T cells, and intestinal natural killer cells and natural killer T cells and it was suggested that these findings indicate that CB2-selective agonists may modulate the development and activity of immunoregulatory cell subsets and that the endocannabinoid system is required for the formation of T- and B-cell subsets involved in immune homeostasis (49).

Recently, data obtained from colonic epithelial cell lines (HT-29) strengthened the role of CB2 receptors in protection against gut inflammation. A selective CB2 receptor but not a selective CB1 receptor agonist inhibited TNF-$\alpha$ induced release of IL-8 in a CB2 antagonist-sensitive manner in HT-29 cells (50).

Non-cannabinoid receptor mediated effects of anandamide in experimental colitis

We assumed that endocannabinoid synthesis and release were still abundant in all knockout strains. Since anti-inflammatory effects in colitis were described before for each CB1 or CB2 mediated pathways one would have expected that colonic inflammation would be increased in double knockout mice in comparison to mice lacking one of both CB receptors - but this was not the case in our experiments. Non-cannabinoid receptor mediated anti-inflammatory effects of anandamide in experimental colitis were described previously and may explain our observation (36, 51-56). Anandamide is an agonist at the transient receptor potential of the vanilloid type 1 (TRPV1), expressed on primary afferent neurons and causing neurogenic inflammation in vivo when activated (57). Massa et al. propagated a protective role of TRPV1 receptors in mouse DNBS colitis (36) as TRPV1(-/-) mice showed aggravation of colitis.
Topical capsaicin administration partly protected against TNBS-induced colitis in the rat (51), was shown to induce hyperaemia in the colon (52) and to protect against colon injury elicited by acetic acid (53). It was furthermore hypothesized that the protective effect was mediated by the local release of vasoactive neuropeptides, such as calcitonin gene-related peptide (CGRP) from nerve endings (54, 55); consistently, Reinschagen et al. demonstrated worsening of TNBS colitis in rats after subcutaneous application of a CGRP but not substance P (SP) receptor antagonist or after intraperitoneal instillation of a CGRP antibody (56). However, neurogenic inflammation in the gut has also been proposed to exert aggressive mechanisms (58-60).

For the protective effects of anandamide on TNBS colitis vasodilatory effects might as well be responsible. Anandamide was shown to increase microcirculation (61) and cause vasodilatation by activation of presynaptic neuronal and postsynaptic vascular CB1 receptors (62) or by induction of CGRP release from sensory nerve endings via TRPV1 receptor activation (63). Furthermore vasodilatation in response to anandamide was demonstrated to be mediated via the EP4 receptor in vascular smooth muscle cells or the induction of nitric oxide (NO) synthesis. However, data on the role of the NO pathway in inflammation remains controversial since numerous animal studies have described an improvement of experimental colitis with inducible NO synthase (iNOS) inhibition (64, 65), but other reports showed ineffectiveness (66, 67) or detrimental effects of those inhibitors (68, 69). Finally anandamide acts on the orphan G-protein-coupled receptor GPR55 (70, 71), the transient receptor potential melastatin type 8; TRPM; TRPV1 receptor activation (72). The role of these receptors in experimental colitis remains to be elucidated in future. Previously, the endocannabinoid system was identified to control food intake and energy balance (73, 74). The role of these receptors in experimental colitis remains controversial since numerous animal studies have described an improvement of experimental colitis with inducible NO synthase (iNOS) inhibition (64, 65), but other reports showed ineffectiveness (66, 67) or detrimental effects of those inhibitors (68, 69). Finally anandamide acts on the orphan G-protein-coupled receptor GPR55 (70, 71), the transient receptor potential ankyrin type 1 channel (TRPA1) and inhibits the transient receptor potential channel of melastatin type 8 (TRPM) receptor (72). The role of these receptors in experimental colitis remains to be elucidated in future. Previously, the endocannabinoid system was identified to control food intake and energy balance (73, 74). Since we did not measure possible alterations in levels of other hormones that take part in control circuits of energy homeostasis in our knockout mice, we cannot exclude hormonal induced changes of colitis susceptibility in these animals. Particularly, it has been suggested that the endocannabinoid tone was linked to ghrelin levels. This inter-relation might have contributed to a disinhibition of colonic inflammation, as we recently showed that exogenous application of ghrelin accelerated healing of colonic lesions in chemically-induced colitis (75).

We conclude that in CB1+2 double knockout mice anti-inflammatory effects of non-cannabinoid receptors or possible alterations in hormonal levels were not sufficient to inhibit colonic inflammation, yet may be responsible for the absence of an exaggeration of the inflammatory response in comparison to mice lacking only CB1 or CB2. Finally, this observation suggests that cannabinoid receptor mediated effects of endocannabinoids play the predominant role in tonic suppression of inflammatory reactions in the gut.

CONCLUSION

Overactivity of the endocannabinoid system has become a well established concept in human intestinal conditions with an inflammatory component (37). This concept can be expanded now as we showed here that deletion of each, respectively both known cannabinoid receptors led to aggravation of colonic inflammation. The inflammatory response to TNBS challenge involved both cannabinoid receptors to a comparable degree. Disintegration of the ECS resulted in a profound disinhibition of an obviously tonic anti-inflammatory effect of the ECS. Interestingly, cannabinoid receptor mediated effects of endocannabinoids play the predominant role in tonic suppression of inflammatory reactions in the gut. However, the net effects of a supposed sustained endocannabinoid action via non-CB1 and non-CB2 receptors (e.g. via TRPV1 or TRPA1) functionally might have impeded the potentiation of an inflammatory reaction in the gut in vivo.

Abbreviations: CB: cannabinoid receptor; CGRP: calcitonin gene-related peptide; DNBS: dinitrobenzene sulfonic acid; DSS: dextran sulphate sodium; EMT: endocannabinoid membrane transporter; EP4: subclass of PGE2 receptors; FAAH: fatty acid amide hydrolase; IBD: inflammatory bowel disease; MAGL: monoacyl glycerol lipase; NO: nitric oxide; SP: substance P; TNBS: trinitrobenzene sulfonic acid; TRPA1: transient receptor potential channel of ankyrin type 1; TRPM 5: transient receptor potential melastatin type 5; TRPV1: transient receptor potential vanilloid 1.

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Conflict of interest: None declared

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