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Effect of cannabidiol on sepsis-induced motility disturbances in mice: involvement of CB_1 receptors and fatty acid amide hydrolase

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Abstract *Sepsis is an inflammatory condition that is associated with reduced propulsive gastrointestinal motility (ileus). A therapeutic option to treat sepsis is to promote intestinal propulsion preventing bacterial stasis, overgrowth and translocation. Recent evidence suggests that anti-oxidants improve sepsis-induced ileus. Cannabidiol, a non-psychotropic component of Cannabis sativa, exerts strong anti-oxidant and anti-inflammatory effects without binding to cannabinoid CB_1 or CB_2 receptors. Cannabidiol also regulates the activity of fatty acid amide hydrolase (FAAH) which is the main enzyme involved in endocannabinoid breakdown and which modulates gastrointestinal motility. Because of the therapeutic potential of cannabidiol in several pathologies, we investigated its effect on sepsis-induced ileus and on cannabinoid receptor and FAAH expression in the mouse intestine. Sepsis was induced by treating mice with lipopolysaccharides for 18 h. Sepsis led to a decrease in gastric emptying and intestinal transit. Cannabidiol further reduced gastrointestinal motility in septic mice but did not affect gastrointestinal motility in control mice. A low concentration of the CB_1 antagonist AM251 did not affect gastrointestinal motility in control mice but reversed the effect of cannabidiol in septic mice. Sepsis was associated with a selective upregulation of intestinal CB_1 receptors without affecting CB_2 receptor expression and with increased FAAH expression. The increase in FAAH expression was completely reversed by cannabidiol but not affected by AM251. Our results show that sepsis leads to an imbalance of the endocannabinoid system in the mouse intestine. Despite its proven anti-oxidant and anti-inflammatory properties, cannabidiol may be of limited use for the treatment of sepsis-induced ileus.*

Keywords *cannabidiol, endocannabinoid system, fatty acid amide hydrolase, septic ileus.*

INTRODUCTION

Marijuana from *Cannabis sativa* L. and its derivatives have been used in medicine for many centuries. The interest in the therapeutic effects of cannabinoids was renewed by the discovery of cannabinoid CB_1 and CB_2 receptors and their endogenous ligands anandamide and 2-arachidonoylglycerol respectively. However, the well-known psychotropic effects of cannabis have always raised clinical and ethical questions and a valid therapeutic alternative may be represented by the use of non-psychotropic cannabinoids, such as cannabidiol. Cannabidiol, a bioactive constituent of marijuana, exerts a wide range of effects including antiepileptic, anxyolitic, antinauseous, neuroprotective and antitumoural activities[1–9] without having significant activity on CB_1 and CB_2 receptors.[5,10–13] Because of its beneficial effect in several pathologies, cannabidiol is regarded as a promising therapeutic compound (recently reviewed in Refs 3,14).

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The pharmacological actions of cannabidiol can be attributed to its anti-oxidative properties[5,15] and its interaction with the endocannabinoid system.[11,16] Cannabidiol enhances the level of anandamide by reducing its degradation and inhibiting its uptake.[11] The exact pathways involved in this effect still have to be elucidated but may include the enzyme fatty acid amide hydrolase (FAAH). Fatty acid amide hydrolase metabolizes endogenous fatty acids, including the endocannabinoids anandamide and 2-arachidonoylglycerol.[17] Through its inhibitory effect on the hydrolysis of FAAH,[18,19] cannabidiol has the potency to modulate the endocannabinoid system.

The endocannabinoid system is upregulated during inflammation of the gastrointestinal tract.[20–23] An important inflammatory condition affecting the gastrointestinal tract is sepsis, which is associated with inhibition of propulsive intestinal motility (ileus) and mucosal barrier dysfunction. Ileus promotes stasis, overgrowth and translocation of bacteria leading to secondary infections and eventually multiple organ failure.[24] The pathogenesis of sepsis is incompletely understood and the therapeutic options for the treatment of sepsis are scarce. The gastrointestinal tract is a therapeutic target to treat sepsis because promotion of propulsive gastrointestinal motility prevents intestinal bacterial stasis and reduces bacterial overgrowth and translocation. We previously reported that sepsis increases inducible nitric oxide synthase (iNOS)-positive residential macrophages in the gastrointestinal tract and this was prevented after anti-oxidant treatment.[25] We recently also showed a beneficial effect of the anti-oxidant melatonin on sepsis-induced motility disturbances in mice.[26] This suggests that reduction of oxidative stress may be a novel adjunct treatment for sepsis. Because cannabidiol possesses important anti-oxidant and anti-inflammatory properties[5,15,27–29] and because the endocannabinoid system is activated during sepsis,[30–33] the aim of the present study was to investigate the effect of cannabidiol on the sepsis-induced motility disturbances in mice.

METHODS

Experimental procedure

All procedures received approval from the Medical Ethical Committee of the University of Antwerp. Male Swiss OF1 mice (30–40 g) were fasted from 9.00 AM by removing food pellets but with free access to tap water. At 4.00 PM, the mice were weighed and received the first i.p. injection of cannabidiol (10 mg kg^{-1}), AM251 (a selective CB_1 antagonist; 1 mg kg^{-1}), AM251 (1 mg kg^{-1}) plus cannabidiol (10 mg kg^{-1}) or the cannabidiol vehicle (10% ethanol, 10% Tween-80 and 80% saline). Thirty minutes later, the mice were divided into a control and lipopolysaccharides (LPS) group receiving, respectively, an i.p. injection of vehicle or LPS (*Escherichia coli* 055:B5; 20 mg kg^{-1}). Six hours after injection of vehicle or LPS, mice received a second i.p. injection of the drug under study (cannabidiol 10 mg kg^{-1}, AM251 1 mg kg^{-1}, the combination or the vehicle). All drugs were injected in a volume ratio of 10 μL g^{-1} body-weight. Eighteen hours after LPS or vehicle injection, mice were gavaged with 25 green glass beads (0.4–0.5 mm in diameter) in 0.5 mL water.[34] The mice were then transferred to a wired bottom cage and after 120 min, mice were anaesthetized with diethyl ether and killed by exsanguination.

Measurement of gastric emptying and geometrical centre

The abdomen was opened and the stomach was rapidly clamped above the lower oesophageal sphincter and beneath the pylorus to prevent further passage of the beads. The complete gastrointestinal tract was gently resected. The small intestine was divided into five segments of equal length and the colon was divided into two segments of equal length. Subsequently, the stomach (Segment 1), the five intestinal segments (Segments 2–6), the caecum (Segment 7) and the two colonic segments (Segments 8 and 9) were cut open and the number of glass beads in each segment was counted under a stereomicroscope. The faeces of each mouse, collected during 120 min after injection of the glass beads, was also examined for the presence of beads and considered as 'Segment 10'.

Gastric emptying (GE) was calculated from the equation:

$$GE = \left[1 - \frac{\text{number of beads in the stomach}}{\text{total number of beads}}\right] \times 100.$$

The geometric centre (GC), which expresses the relative distribution of the glass beads throughout the gastrointestinal tract was calculated[34] from the equation:

$$GC = \Sigma(\%\text{beads per segment} \times \text{segment number})/100.$$

After counting the glass beads, small intestinal tissues were collected for Western blot analysis.

Organ bath experiments

Muscle strips of the mouse jejunum were prepared as described previously.[35] Briefly, a jejunal segment of the small intestine was gently flushed with Krebs–Ringer solution (118.3 mmol L^{-1} NaCl, 4.7 mmol L^{-1} KCl, 1.2 mmol L^{-1} $MgSO_4$, 1.2 mmol L^{-1} KH_2PO_4, 2.5 mmol L^{-1} $CaCl_2$, 25 mmol L^{-1} $NaCHO_3$, 0.026 mmol L^{-1} CaEDTA and 11.1 mmol L^{-1} glucose) and opened along the mesenteric border. The mucosa was removed and muscle strips were cut in the longitudinal direction. Muscle strips were mounted in organ baths (volume 5 mL) filled with Krebs–Ringer solution (37 °C, aerated with 5% CO_2/95% O_2) and connected to a strain gauge transducer (Scaime transducers, Annemasse, France) for recording of isometric tension. After a 30-min equilibration period, strips were contracted with 0.1 μmol L^{-1} carbachol. Carbachol was subsequently washed away from the organ bath, strips were stretched (increments of 2.5 mN) and carbachol was added again. This procedure was repeated until the contraction to 0.1 μmol L^{-1} carbachol was maximal. This point was taken as the point of optimal length–tension relationship.[35] The tissues were allowed to equilibrate for 60 min before starting the experimentation. During the equilibration period, the preparations were washed every 15 min with fresh Krebs–Ringer solution.

Preparation of cytosolic extracts

For homogenization, intestinal tissue was placed in ice-cold hypotonic lysis buffer (20 mmol L^{-1} HEPES, 100 mmol L^{-1} MgCl2, 0.4 mol L^{-1} NaCl, 0.5 mmol L^{-1} phenylmethylsulphonylfluoride, 15 μg mL^{-1} soybean trypsin inhibitor, 3 μg mL^{-1} pepstatin A, 2 μg mL^{-1} leupeptin, 40 μmol L^{-1} benzamidine, 1 mmol L^{-1} dithiothreitol, 1% Nonidet P40, 20% glycerol) in a ratio of 0.4 mL per 100 μg of tissue and homogenized at the highest setting for 2–5 min in Polytron PT300 tissue homogenizer (Kinematica AG, Luzern, Switzerland). Protein concentration was determined using the BioRad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

Western blot Analysis

Immunoblotting analysis of CB_1, CB_2, FAAH and tubulin protein was performed on cytosolic and nuclear extracts. Cytosolic or nuclear extract fraction proteins were mixed with gel loading buffer (50 mmol L^{-1} Tris, 10% SDS, 10% glycerol 2-mercaptoethanol, 2 mg bromophenol mL^{-1}) in a ratio of 1 : 1, boiled for 5 min and centrifuged at 10 000 ***g*** for 10 min. Protein concentration was determined and equivalent amounts (50 μg) of each sample were separated under reducing conditions in 12% SDS-polyacrylamide mini-gel. The proteins were transferred onto nitrocellulose membrane according to the manufacturer's instructions (Bio-Rad Laboratories). The membranes were blocked by incubation at 4 °C overnight in high salt buffer (50 mmol L^{-1} Trizma base, 500 mmol L^{-1} NaCl, 0.05% Tween-20) containing 5% bovine serum albumin and incubated with either anti-CB_1 (1 : 250) (cat no.: PA1-745; ABR Affinity BioReagents, Golden, CO, USA), anti-CB_2 antibody (1 : 200) (cat no.: 101550; Cayman Chemical, Ann Arbor, MI, USA), anti-β-tubulin (1 : 1000) (cat no.: T2200; Sigma-Aldrich, St Louis, MO, USA) or anti-FAAH (1 : 250 v/v) (cat no.: 101600; Cayman Chemical) for 1 h at room temperature, followed by incubation with specific horseradish peroxidase (HRP)-conjugate secondary antibody (1 : 2000 v/v) (Dako, Golstrup, Denmark). In preliminary experiments, the blocking peptide for the different antibodies was mixed with the specific antibody in a ratio of 1 : 1 (v/v) as recommended by the manufacturer. The immune complexes were developed using enhanced chemiluminescence detection reagents (Amersham, Milan, Italy) according to the manufacturer's instructions and exposed to Kodak X-Omat film (Kodak Eastman Company, Milan, Italy). The protein bands on X-ray film were scanned and densitometrically analysed with a GS-700 imaging densitometer (Bio-Rad Laboratories).

Drugs used

NaCl 0.9% (Plurule®; Baxter, Lessines, Belgium), LPS (*E. coli* serotype 055:B5; Sigma-Aldrich); cannabidiol (also referred to as '(−)-cannabidiol') and AM251 (Tocris Bioscience, Bristol, UK). Cannabidiol and AM251 were dissolved in 10% ethanol, 10% Tween-80 and 80% saline. Glass beads (0.40–0.52 mm diameter) were purchased from VWR International, Leuven, Belgium.

Presentation of results and statistical analysis

For the functional *in vivo* experiments on gastric emptying and geometric centre, values are shown as mean ± SEM for *n* indicating the number of mice used. For statistical analysis, we used two-way ANOVA. The first factor concerned the presence or absence of LPS, the second parameter the drug under study. For *post hoc* testing, we used a one-way ANOVA followed by a Bonferroni *post hoc* test or a non-paired Student's *t*-test as appropriate. *P*-values < 0.05 were considered to be

significant. For the *in vitro* organ bath experiments, values are expressed in mN contraction and shown as mean ± SEM for *n* indicating the number of mice used. These data were analysed with the SPSS for Windows software (SPSS Inc., Chicago, IL, USA).

For Western blot analysis, results were expressed as the mean ± SEM of *n* animals where each value is the average of responses in duplicate sites. Statistical comparisons were made by one-way ANOVA followed by a Bonferroni *post hoc* test for multiple comparisons or unpaired Student's *t*-test. *P*-values of <0.05 were considered to be significant. These data were analysed with GraphPad Instat (Graphpad Software, San Diego, CA, USA).

RESULTS

Gastrointestinal motility

Gastric emptying in control (vehicle-treated) mice was not significantly affected by cannabidiol or the selective CB_1 antagonist AM251 but slightly inhibited by the combination of cannabidiol plus AM251 (Fig. 1A). Treatment of mice with LPS significantly reduced gastric emptying in vehicle-treated mice. Cannabidiol further inhibited gastric emptying in LPS-treated mice (Fig. 1A). AM251 *per se* had no effect on the LPS-induced inhibition of gastric emptying but completely blocked the additional cannabidiol-induced reduction of gastric emptying in LPS-treated mice (Fig. 1A).

The geometric centre represents the relative distribution of the beads throughout the gastrointestinal tract. The geometric centre in control mice was not significantly affected when mice were treated with cannabidiol, AM251 or AM251 plus cannabidiol (Fig. 1B). Lipopolysaccharides significantly reduced the geometric centre in vehicle-treated mice (Fig. 1B). This effect was more pronounced when mice were treated with cannabidiol whereas AM251 *per se* had no effect on the geometric centre (Fig. 1B). However, AM251 completely prevented the cannabidiol-induced reduction of the geometric centre in LPS-treated mice (Fig. 1B).

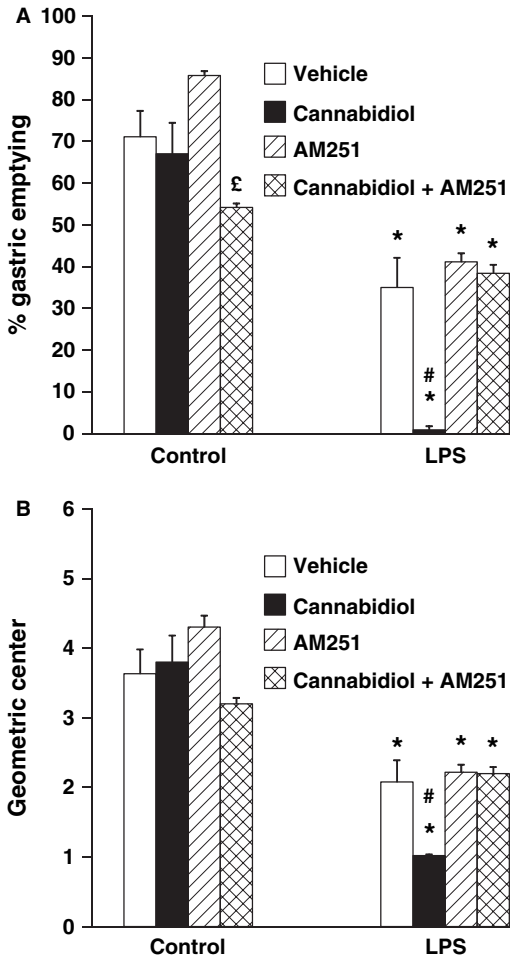

Figure 1 Effect of intraperitoneal lipopolysaccharides (i.p. LPS) in mice treated with vehicle (10% ethanol, 10% Tween-80 and 80% saline, open bars, $n = 15$), 10 mg kg^{-1} cannabidiol (solid bars, $n = 15$), 1 mg kg^{-1} AM251 (hatched bars, $n = 13$) or 1 mg kg^{-1} AM251 plus 10 mg kg^{-1} cannabidiol (cross hatched bars, $n = 10$) on (A) gastric emptying and (B) the geometric centre, representing the relative distribution of the beads throughout the gastrointestinal tract. Results are shown as mean ± SEM. Two-way ANOVA was used followed by *post hoc* testing: one-way ANOVA followed by Bonferroni or unpaired Student's *t*-test. *$P \leq 0.05$, significantly different from control mice treated with the same drug; £$P \leq 0.05$, significantly different from AM251-treated mice; #$P \leq 0.05$, significantly different from other LPS-treated mice.

Cannabinoid receptor expression

The expression of cannabinoid CB_1 and CB_2 receptors was studied in the mouse small intestine. Immunoblotting analysis demonstrated CB_1 and CB_2 protein expression in the small intestine of control mice (Fig. 2). Treatment of mice with LPS significantly enhanced the expression of CB_1 receptors (Fig. 2A) but did not affect the expression of CB_2 receptors (Fig. 2B).

Fatty acid amide hydrolase protein expression

As FAAH can act as a hydrolytic enzyme for endogenous cannabinoids such as anandamide, we investigated the expression of FAAH in the intestine of control mice and mice treated with LPS. Immunoblotting analysis demonstrated the expression of FAAH protein in the intestine of control mice (Fig. 3). This was significantly enhanced in mice treated with LPS

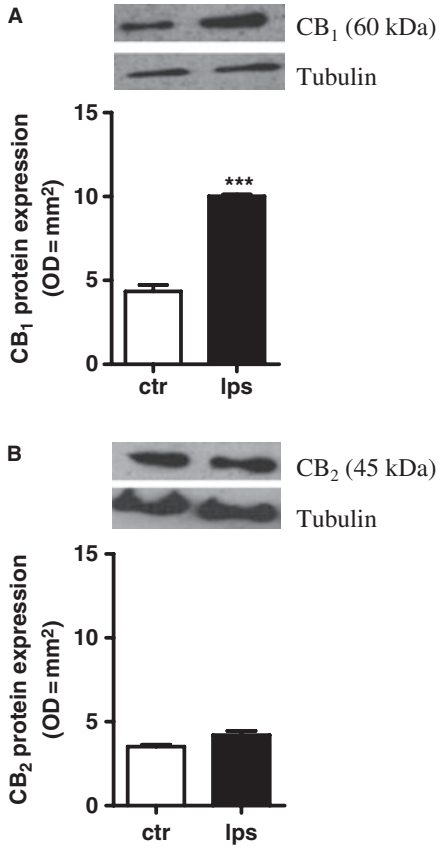

Figure 2 Effects of lipopolysaccharides (LPS) treatment on (A) CB_1 and (B) CB_2 receptor expression in the mouse small intestine. Upper panels show a representative Western blot analysis, graphs show the densitometric analysis for $n = 3$ experiments. Results are the mean values ± SEM. $^{***}P < 0.01$ *vs* control, unpaired Student's *t*-test.

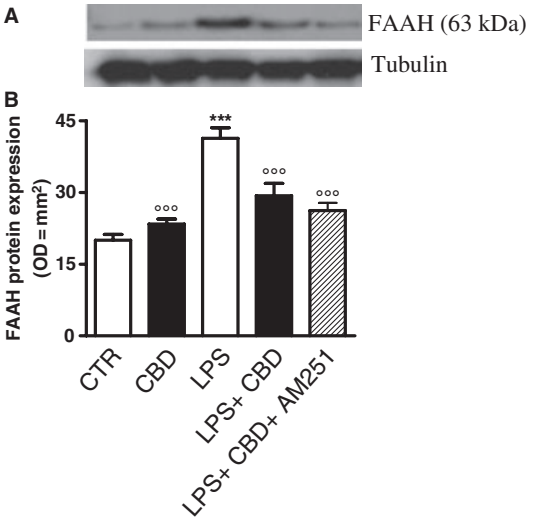

Figure 3 Fatty acid amide hydrogenase (FAAH) expression in the mouse small intestine from control mice treated with vehicle (CTR) and from mice treated with cannabidiol (CBD), lipopolysaccharides (LPS), LPS plus cannabidiol and LPS plus CBD plus AM251. Panel A shows a representative Western blot analysis, panel B shows the densitometric analysis for $n = 3$ experiments. Results are the mean values ± SEM. $^{***}P < 0.001$ *vs* control; $^{°°°}P < 0.001$ *vs* LPS, one-way ANOVA followed by Bonferroni.

(Fig. 3). Cannabidiol *per se* did not affect FAAH expression in control mice but reversed the upregulation of FAAH in LPS-treated mice (Fig. 3). Blockade of CB_1 receptors did not further influence this effect of cannabidiol (Fig. 3).

Organ bath experiments on isolated muscle strips of the small intestine

Because recent evidence suggests that cannabidiol may activate transient receptor potential of the vanilloid type I (TRPV1), we compared the functional effect of cannabidiol in the mouse intestine with that of the TRPV1 agonist capsaicin. Capsaicin (1 μmol L^{-1}) induced sustained contractions of 5.0 ± 0.8 mN ($n = 4$) of isolated muscle strips of the mouse small intestine (Fig. 4A). Cannabidiol at a wide range of concentrations (0.01, 0.1, 1 and 10 μmol L^{-1}, $n = 4$ each) failed to induce any contraction in mouse small intestinal muscle strips (Fig. 4B, C show the effect of cannabidiol 1 and 10 μmol L^{-1}).

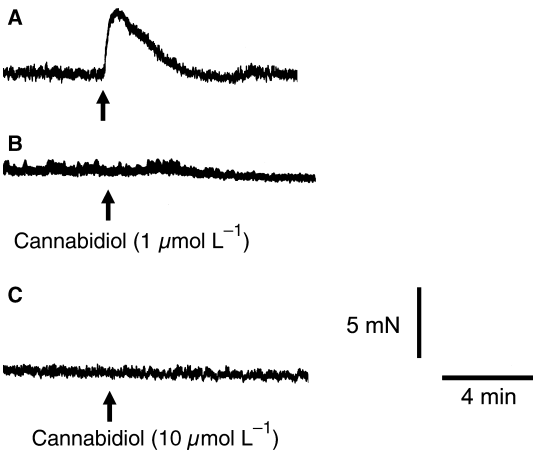

Figure 4 Representative tracings of three different muscle strips of the mouse small intestine showing the effect of (A) the TRPV1 agonist capsaicin (1 μmol L^{-1}) and (B, C) cannabidiol (1 and 10 μmol L^{-1}).

DISCUSSION

Interest in the gastrointestinal effects of cannabinoids stems from their ability to control gastrointestinal

motility by interacting with cannabinoid CB_1 or CB_2 receptors. It is well known that CB_1 receptor activation decreases gastrointestinal transit. We here report that cannabidiol, a non-psychotropic component of *Cannabis sativa* that does not directly interact with CB_1 or CB_2 receptors, decreases gastrointestinal transit in mice with LPS-induced sepsis but not in healthy controls.

Sepsis was induced by treating mice with LPS. We previously reported that LPS-induced sepsis leads to decreased gastrointestinal motility in mice.[25,26,36] Because of its beneficial effect in several pathologies,[2–9] we investigated the effect of cannabidiol on sepsis-induced gastrointestinal motility disturbances. We found that cannabidiol had no effect on gastrointestinal motility in healthy control mice which is in line with previous studies on the effect of this compound on gastrointestinal motility.[37,38] Cannabidiol is a stereochemic compound of which only the (−)-enantiomer (also described as '(−)-cannabidiol') is naturally present in *Cannabis sativa.* Therefore, (−)-cannabidiol, which was also used in our study, is generally referred to simply as 'cannabidiol'. The (+)-enantiomer of cannabidiol (or '(+)-cannabidiol') does not occur naturally but can be obtained as a synthetic compound. Fride *et al.*[38] recently reported that mouse gastrointestinal transit was not affected by naturally occurring (−)-cannabidiol but inhibited by several synthetic derivatives of (−)-cannabidiol, including (+)-cannabidiol. This indicates that conformational changes alter the biological activity of cannabidiol. This finding is highly relevant for the development of therapeutic cannabinoids that lack the typical psychoactive side effects.

Although cannabidiol did not affect gastrointestinal motility in control mice, it further reduced gastric emptying and the geometric centre in mice with LPS-induced sepsis. This aggravating effect of cannabidiol was unexpected. We previously reported that antioxidants and inhibitors of iNOS have a beneficial effect on sepsis-induced gastrointestinal motility disturbances.[25,26,36] Because of the reported anti-inflammatory and anti-oxidative actions of cannabidiol and its ability to reduce NO production from iNOS,[27,28] one would expect that cannabidiol ameliorates the LPS-induced gastrointestinal disturbances.

There is firm evidence that cannabinoids modulate intestinal motility through activation of intestinal CB_1 receptors.[39–44] To investigate the involvement of CB_1 receptors in the effect of cannabidiol in septic mice, we studied the effect of a low concentration of the CB_1 antagonist AM251. The low concentration of AM251 was chosen to avoid a significant effect *per se* of this compound on intestinal transit in controls. As expected, AM251 (1 mg kg^{-1}) slightly but not significantly enhanced gastric emptying and gastrointestinal transit in control mice. This tendency to acceleration of gastrointestinal transit after CB_1 receptor blockade agrees with the enhanced intestinal transit observed in CB_1 knock out mice[44] and in mice treated with the CB_1 antagonist SR141716A.[41,42] AM251, at the same low concentration used in controls, did not affect gastric emptying or geometric centre in LPS-treated septic mice. However, AM251 completely reversed the inhibitory effect of cannabidiol on gastrointestinal motility in septic mice. This suggests that the cannabidiol-induced deteriorating effect on gastrointestinal transit during sepsis involved activation of CB_1 receptors. This is not likely to result from a direct effect of cannabidiol on cannabinoid receptors because of the low affinity of cannabidiol for CB_1 and CB_2 receptors.[5,10–13] Also our observation that cannabidiol did not affect gastrointestinal motility in control mice indicates that cannabidiol did not directly activate CB_1 receptors as this would inhibit gastrointestinal transit.[39–44]

We aimed elucidating the mechanism of the cannabidiol-induced inhibition of gastrointestinal motility in septic mice by studying cannabinoid receptor expression in the intestine of control and septic mice. We found that LPS-induced sepsis resulted in an upregulation of CB_1 but not CB_2 receptor expression as previously also reported in the intestine of mice with paralytic ileus[42] or croton oil-induced intestinal inflammation.[41]

Cannabidiol may modulate the endocannabinoid system through an indirect action on cannabinoid receptors. Recent *in vitro* studies showed that cannabidiol antagonized the effect of CB_1 and CB_2 receptor agonists.[45,46] The pathways involved in this effect still have to be identified. Our observation that cannabidiol affected gastrointestinal motility in mice with LPS-induced sepsis but not in healthy controls suggested the involvement of an endotoxin-mediated inflammatory pathway. A key role player in the bioavailability of endocannabinoids is FAAH, an enzyme that is found in several inflammatory cells and especially in mast cells.[47] Fatty acid amide hydrolase is suggested to be the main regulator of endocannabinoid levels in the small intestine and inhibition of FAAH reduces gastrointestinal transit in mice.[48] Plasma FAAH levels are also upregulated during sepsis in humans.[49] We found an increased FAAH expression in the intestine of mice with sepsis. Because inhibition of FAAH reduces mouse intestinal motility,[48] enhanced FAAH expression during sepsis was expected to increase intestinal transit, but we found a decreased transit. Possibly,

endogenous FAAH levels are sufficiently high, masking any effect of enhanced FAAH expression on intestinal transit. Alternatively, LPS also stimulates the synthesis of anandamide[50] and this may counteract the enhanced degradation of endocannabinoids following the upregulated FAAH expression during sepsis.

In LPS-treated mice, cannabidiol reversed the expression of FAAH almost to control levels. This indicates that cannabidiol, by inhibiting FAAH expression, causes an imbalance between synthesis and degradation of endocannabinoids during LPS-induced sepsis. The CB_1 sensitive effect of cannabidiol that we observed in septic mice can be explained by the cannabidiol-induced reduction of FAAH expression, leading to enhanced endocannabinoid levels, increased activation of intestinal CB_1 receptors and hence inhibition of gastrointestinal transit. Our findings agree with those of Mascolo *et al.*,[42] who reported that paralytic ileus in mice enhances endocannabinoid levels which reduce gastrointestinal transit through a CB_1 receptor-dependent mechanism. Altogether, these findings suggest that endocannabinoids may not only play a protective function in gut inflammation but also contribute to its symptoms.[51,52] This is illustrated by the improved survival of patients with septic shock when endogenous anandamide levels are reduced.[53]

Finally, there is recent evidence that cannabidiol may directly interact with TRPV1 receptors.[11,54] We studied a possible direct effect of cannabidiol on TRPV1 receptors in isolated muscle strips from the mouse jejunum. As previously reported,[35] these tissues contract in response to the TRPV1 agonist capsaicin. The pronounced and sustained contractions to capsaicin were however not mimicked by cannabidiol which, over a wide range of concentrations, failed to induce any contractile response. This argues against a major role of cannabidiol as a TRPV1 agonist at the level of the mouse small intestine. However, this does not exclude an effect of cannabidiol on TRPV1 *in vivo* under inflammatory conditions, which have been shown to upregulate TRPV1 expression. More in-depth studies are needed to investigate the involvement of TRPV1 in the aggravating effect of cannabidiol during sepsis.

In conclusion, our results demonstrate that cannabidiol inhibits gastrointestinal motility in septic mice but not in healthy controls. This inhibitory effect of cannabidiol was associated with an inhibition of FAAH expression and activation of CB_1 receptors. Despite its interesting anti-oxidant and anti-inflammatory properties, the therapeutic potential of cannabidiol for the treatment of sepsis-induced ileus may be limited because of its effect on fatty acid metabolism. This illustrates that endocannabinoids may not only play a protective function in gut inflammation but may also aggravate associated gastrointestinal symptoms.

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