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RESEARCH PAPER

Cannabidiol, extracted from *Cannabis sativa*, selectively inhibits inflammatory hypermotility in mice

R Capasso, F Borrelli, G Aviello, B Romano, C Scalisi[1], F Capasso and AA Izzo

Department of Experimental Pharmacology, University of Naples Federico II and Endocannabinoid Research Group, Naples, Italy

Background and purpose: Cannabidiol is a *Cannabis*-derived non-psychotropic compound that exerts a plethora of pharmacological actions, including anti-inflammatory, neuroprotective and antitumour effects, with potential therapeutic interest. However, the actions of cannabidiol in the digestive tract are largely unexplored. In the present study, we investigated the effect of cannabidiol on intestinal motility in normal (control) mice and in mice with intestinal inflammation.
Experimental approach: Motility *in vivo* was measured by evaluating the distribution of an orally administered fluorescent marker along the small intestine; intestinal inflammation was induced by the irritant croton oil; contractility *in vitro* was evaluated by stimulating the isolated ileum, in an organ bath, with ACh.
Key results: *In vivo*, cannabidiol did not affect motility in control mice, but normalized croton oil-induced hypermotility. The inhibitory effect of cannabidiol was counteracted by the cannabinoid CB_1 receptor antagonist rimonabant, but not by the cannabinoid CB_2 receptor antagonist SR144528 (*N*-[-1*S*-*endo*-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide), by the opioid receptor antagonist naloxone or by the α_2-adrenergic antagonist yohimbine. Cannabidiol did not reduce motility in animals treated with the fatty acid amide hydrolase (FAAH) inhibitor *N*-arachidonoyl-5-hydroxytryptamine, whereas loperamide was still effective. *In vitro*, cannabidiol inhibited ACh-induced contractions in the isolated ileum from both control and croton oil-treated mice.
Conclusions and implications: Cannabidiol selectively reduces croton oil-induced hypermotility in mice *in vivo* and this effect involves cannabinoid CB_1 receptors and FAAH. In view of its low toxicity in humans, cannabidiol may represent a good candidate to normalize motility in patients with inflammatory bowel disease.
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Keywords: cannabidiol; cannabinoid receptors; fatty acid amide hydrolase; inflammatory bowel disease; intestinal transit; intestinal motility; intestine; phytocannabinoids

Abbreviations: AA-5-HT, *N*-arachidonoyl-5-hydroxytryptamine; CBD, cannabidiol; FAAH, fatty acid amide hydrolase; GC, geometric centre; JWH 015, 2-methyl-1-propyl-1*H* indol-3-yl)-1-naphthalenymethanone; SR144528, *N*-[-1*S*-*endo*-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide

Introduction

The plant *Cannabis sativa* contains more than 60 terpenophenolic compounds, named phytocannabinoids. The best-studied phytocannabinoid is Δ^9-tetrahydrocannabinol, which binds specific G-protein-coupled receptors, named cannabinoid (CB_1 and CB_2) receptors (Mechoulam *et al*., 2002; Russo and Guy, 2006; Pertwee, 2007; Alexander *et al*., 2008). The well-known psychotropic effects of Δ^9-tetrahydrocannabinol, which are largely mediated by activation of brain cannabinoid CB_1 receptors, have always raised a number of clinical and ethical problems. Therefore, a valid therapeutic alternative may be the use of non-psychotropic phytocannabinoids, including cannabidiol (CBD). CBD, unlike Δ^9-tetrahydrocannabinol, has very low affinity for both cannabinoid CB_1 and CB_2 receptors (McPartland *et al*., 2007), although it has been proposed that CBD may modulate endocannabinoid function through its ability to inhibit the hydrolysis of anandamide and to act as a transient receptor potential vanilloid 1 agonist (Watanabe *et al*., 1998; Bisogno *et al*., 2001). CBD is a major component of Sativex, a preparation of cannabinoids, which has been approved by Health Canada for the treatment of neuropathic pain in multiple sclerosis.

Correspondence: Professor AA Izzo, Department of Experimental Pharmacology, University of Naples Federico II and Endocannabinoid Research Group, via D Montesano 49, 80131 Naples, Italy.
E-mail: aaizzo@unina.it
[1]Current address: Human Physiology Section, Department of Experimental Medicine, University of Palermo, corso Tukory 129, 90134 Palermo, Italy.
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The pharmacological profile of CBD has been recently reviewed (Mechoulam *et al*., 2007). Briefly stated, CBD has been shown to exert (1) antioxidant (Hampson *et al*., 1998), neuroprotective (Iuvone *et al*., 2004; Esposito *et al*., 2006) and antiproliferative actions (Ligresti *et al*., 2006; Massi *et al*., 2008) in cultured cells and (2) anti-anxiety (Guimarães *et al*., 1990; Moreira *et al*., 2006), hypnotic (Carlini and Cunha, 1981), anticonvulsant (Carlini and Cunha, 1981), neuroprotective (Dirikoc *et al*., 2007; Esposito *et al*., 2007; Hayakawa *et al*., 2007), antinausea (Rock *et al*., 2008), anti-ischaemic (Durst *et al*., 2007), anticancer (Massi *et al*., 2008) and notably anti-inflammatory effects in rodents *in vivo*. The anti-inflammatory effects of CBD have been demonstrated in both acute (Costa *et al*., 2004) and chronic (Malfait *et al*., 2000; Costa *et al*., 2007) experimental models of inflammation, that is, paw oedema and arthritis.

Although oxidative stress plays an important role in the pathogenesis of a number of gastrointestinal diseases (Rezaie *et al*., 2007) and also may alter intestinal motility (Van der Vliet *et al*., 1989; Peluso *et al*., 2002), the effects of CBD (which is a well-known antioxidant compound) (Hampson *et al*., 1998) in the digestive tract are largely unexplored. Early studies showed that CBD did not modify gastric emptying and small intestinal transit in mice and rats (Chesher *et al*., 1973; Shook and Burks, 1989). These results are in agreement with more recent studies showing the lack of effect of CBD on defecation in mice (Fride *et al*., 2005). In the present study, we have evaluated the effect (and the mode of action) of CBD on intestinal hypermotility induced by the irritant croton oil. Croton oil-induced ileitis is characterized by increased intestinal expression of CB_1 receptors and fatty acid amide hydrolase (FAAH) activity (Izzo *et al*., 2001b).

Methods

Animals

All animal procedures and experiments complied with the Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985) and the Italian DL no. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC). Male ICR mice (Harlan Italy, S Pietro al Natisone, UD, Italy) (24–26 g) were used after 1 week of acclimation. Food was withheld 6 h before transit measurement and 18 h before the induction of intestinal inflammation.

Intestinal inflammation

Inflammation was induced as previously described (Puig and Pol, 1998; Borrelli *et al*., 2006). Mice received orally two doses of croton oil (20 μl per mouse) in two consecutive days. Motility was measured 4 days after the first administration of croton oil. This time was selected on the basis of a previous work (Puig and Pol, 1998; Izzo *et al*., 2001b), in which maximal inflammatory response occurred 4 days after the first treatment.

In vivo *transit*

Transit was measured by evaluating the intestinal location of rhodamine-B-labeled dextran (Capasso *et al*., 2005, 2008). Animals were given fluorescent-labeled dextran (100 μL of 25 mg mL^{-1} stock solution) via a gastric tube into the stomach. At 20 min after administration, the animals were killed by asphyxiation with CO_2 and the entire small intestine with its content was divided into 10 equal parts. The intestinal contents of each bowel segment were vigorously mixed with 2 mL of saline solution to obtain a supernatant containing the rhodamine. The supernatant was centrifuged at 35 *g* to precipitate the intestinal chyme. The fluorescence in duplicate aliquots of the cleared supernatant was read in a multi-well fluorescence plate reader (LS55 Luminescence spectrometer, Perkin Elmer Instruments, Waltham, MA, USA; excitation 530 ± 5 nm and emission 590 ± 10 nm) for quantification of the fluorescent signal in each intestinal segment. From the distribution of the fluorescent marker along the intestine, we calculated the geometric centre (GC) of small intestinal transit as follows:

$GC = \Sigma$ (fraction of fluorescence per segment $\times$ segment number)

GC ranged from 1 (minimal motility) to 10 (maximal motility). This procedure has yielded an accurate, non-radioactive measurement of intestinal transit (Capasso *et al*., 2005).

In vivo *drug administration*

CBD (1–10 mg kg^{-1}), JWH 015 (2-methyl-1-propyl-1*H* indol-3-yl)-1-naphthalenymethanone) (10 mg kg^{-1}), loperamide (0.075 mg kg^{-1}), clonidine (0.075 mg kg^{-1}), *N*-arachidonoyl-5-hydroxytryptamine (AA-5-HT, 7.5 mg kg^{-1}) or vehicles were given intraperitoneally 30 min before rhodamine administration to mice with inflammation. In some experiments, naloxone (2 mg kg^{-1}, to block opioid receptors), rimonabant (0.1 mg kg^{-1}, to block cannabinoid CB_1 receptors), SR144528 (1 mg kg^{-1}, to block cannabinoid CB_2 receptors) or yohimbine (1 mg kg^{-1}, to block α_2-adrenoceptors) were given 10 min before CBD (5 mg kg^{-1}) or before the corresponding receptor agonists, that is, loperamide 0.075 mg kg^{-1}, clonidine 0.075 mg kg^{-1} or JWH 015 10 mg kg^{-1}. In preliminary experiments, CBD (5 and 10 mg kg^{-1}) was given 30 min before rhodamine administration to control mice (that is, mice not treated with croton oil). The doses of antagonists used in the present study (that is, rimonabant, SR144528, naloxone, yohimbine) were selected on the basis of previous work (Capasso *et al*., 2001, 2008); the doses of loperamide and clonidine were selected on the basis of preliminary experiments that showed that these agonists, both at the 0.075 mg kg^{-1} dose, had an inhibitory effect on motility which was similar to that produced by CBD 5 mg kg^{-1}.

In vitro *experiments*

Segments (1–1.5 cm) of the terminal ileum from both control and croton oil-treated mice (killed by asphyxiation with CO_2) were removed, flushed free of luminal contents and placed in Krebs' solution (composition in mM: NaCl 119, KCl 4.75, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.5, $CaCl_2$ 2.5 and glucose 11). The isolated organ was set up to record contractions from the longitudinal axis in an organ bath filled with warm (37 °C) aerated (95% O_2/5% CO_2) Krebs'

solution (Capasso *et al*., 2006). The tissues were connected to an isotonic transducer (load 0.5 g) connected to a 'Gemini' recording apparatus (Ugo Basile, Comerio, Italy). At the beginning of each experiment, the ileum was stimulated with ACh (1 mM) to obtain a maximal contraction (100% contraction). After at least 1 h for equilibration, the tissues were stimulated with ACh (1 μM) (Borrelli *et al*., 2006). ACh was added to the bath and left in contact with the tissue for 30 s and then washed out. The interval between each stimulation was 20 min. After at least three stable control contractions, the contractile responses were repeated in the presence of increasing (non-cumulative) concentrations of CBD (0.01–100 μM) added 20 min before ACh (that is, after washing the tissue). Preliminary experiments showed that a 20 min contraction time was sufficient for CBD to achieve the maximal inhibitory response. In some experiments, control tissues were stimulated with prostaglandin $F_{2\alpha}$ (0.2 μM, added to the bath and left in contact with the tissue for 60 s and then washed out) and the effect of CBD (0.01–100 μM) was evaluated as described above for the contractions evoked by ACh.

Statistics

Data are expressed as the mean ± s.e.mean of experiments in *n* mice. To determine statistical significance, Student's *t* test was used for comparing a single treatment mean with a control mean, and a one-way analysis of variance followed by a Tukey–Kramer multiple comparisons test was used for analysis of multiple treatment means. *P*-values <0.05 were considered significant. The concentrations of CBD that produced 50% inhibition of ACh-induced contractions (IC_{50}) or maximal inhibitory effect (E_{max}) were used to characterize its potency and efficacy, respectively. The IC_{50} and E_{max} values were calculated by nonlinear regression analysis using the equation for a sigmoid concentration–response curve (GraphPad Prism).

Drugs

CBD (purity by HPLC: 99.76%) was kindly supplied by GW Pharmaceuticals (Porton Down, Wiltshire, UK). ACh chloride, prostaglandin $F_{2\alpha}$, naloxone hydrochloride, loperamide hydrochloride, yohimbine hydrochloride and clonidine hydrochloride, were purchased from Sigma (Milan, Italy); JWH 015 was purchased from Tocris (Bristol, UK). AA-5-HT synthesized as previously described (Ortar *et al*., 2003), was a gift from Dr Vincenzo Di Marzo (CNR, Pozzuoli, Italy). Rimonabant and SR144528 (*N*-[-1*S*-*endo*-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide) were a kind gift from Drs Madaleine Mossè and Francis Barth (Sanofi-Aventis, Montpellier, France).

CBD, rimonabant and SR144528 were dissolved in dimethyl sulphoxide (DMSO); AA-5-HT was dissolved in DMSO/Tween 80 (1:4), prostaglandin $F_{2\alpha}$ in ethanol while the other drugs were dissolved in saline. The drug vehicles (DMSO, 4 μL per mouse; 20 μL of DMSO/Tween 80 per mouse; saline 0.1 mL per mouse; DMSO <0.01% *in vitro*) had no significant effect on the responses under study, both *in vitro* and *in vivo*.

Results

In vivo *results*

Oral administration of croton oil produced a significant increase in intestinal transit, shown as an increased value of the GC (Figure 1). Intraperitoneal administration of CBD caused a reduction in intestinal motility in croton oil-treated animals, which was statistically significant at doses of 5 and 10 mg kg^{-1} (Figure 1). However, CBD at these doses (5 and 10 mg kg^{-1}, i.p.) did not modify transit in control mice, that is, in mice not treated with croton oil (GC: control: 5.12 ± 0.24; CBD 5 mg kg^{-1} 4.85 ± 0.28; CBD 10 mg kg^{-1} 5.14 ± 0.30; $n=8$ for each experimental group, $P>0.2$).

The cannabinoid CB_1 receptor antagonist rimonabant, at a dose (0.1 mg kg^{-1}) which *per se* did not modify intestinal motility in croton oil-treated animals (GC: croton oil 6.58 ± 0.42; croton oil + rimonabant 6.89 ± 0.58, $n=8$, $P>0.2$) counteracted the inhibitory effect of CBD (5 mg kg^{-1}) but not that of loperamide (0.075 mg kg^{-1}) on intestinal transit (Figure 2). However, the inhibitory effect of CBD (5 mg kg^{-1}) on motility was not significantly modified by the cannabinoid CB_2 antagonist SR144528 (1 mg kg^{-1}), by the opioid receptor antagonist naloxone (2 mg kg^{-1}) or by the α_2-adrenoceptor antagonist yohimbine (1 mg kg^{-1}) (Table 1). At the doses used, these antagonists significantly ($P<0.05$, $n=8$–10 for each experimental group) counteracted the inhibitory effect on motility of the corresponding agonists, (that is, SR144528 (1 mg kg^{-1}) counteracted the inhibitory effect of JWH 015 10 mg kg^{-1} (GC values in control 4.91 ± 0.43, croton oil 6.65 ± 0.39, croton oil + JWH 015 5.11 ± 0.36, CO + JWH 015 + SR144528 6.60 ± 0.37), naloxone (2 mg kg^{-1}) counteracted the inhibitory effect of loperamide 0.075 mg kg^{-1} (control 4.90 ± 0.44, croton oil

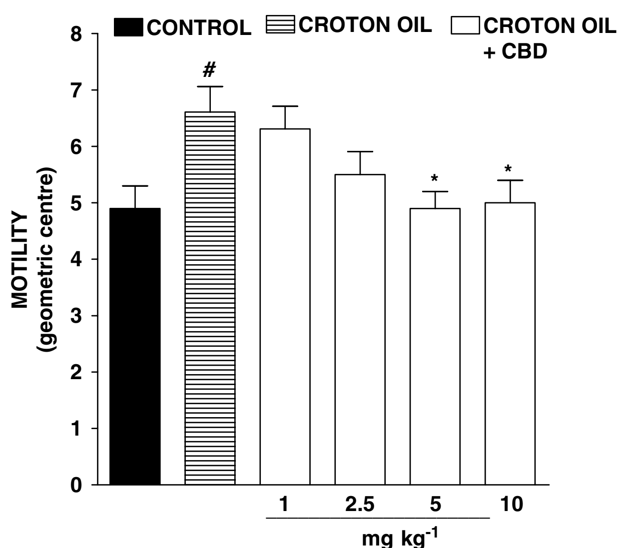

Figure 1 Inhibitory effect of cannabidiol (CBD, 1–10 mg kg^{-1}, i.p.) on intestinal transit in croton oil-treated mice *in vivo*. Transit was expressed as the geometric centre (GC) of the distribution of a fluorescent marker along the small intestine. GC ranged from 1 (minimal motility) to 10 (maximal motility) (see Methods section). Bars represent the mean ± s.e.mean of 8–10 animals for each experimental group. $^{\#}P<0.05$ vs control and $^{*}P<0.05$ vs croton oil.

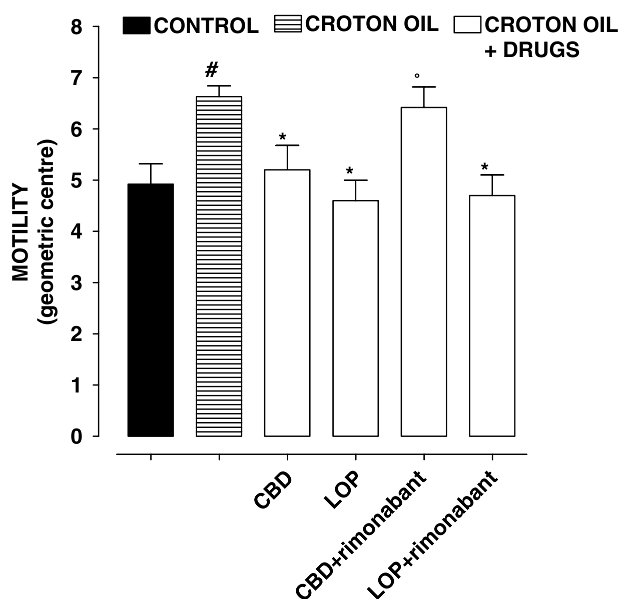

Figure 2 Croton oil-treated mice: effect of i.p.-injected cannabidiol (CBD, $5\,mg\,kg^{-1}$) and loperamide (LOP, $0.075\,mg\,kg^{-1}$) (alone or in the presence of the cannabinoid CB_1 receptor antagonist rimonabant ($0.1\,mg\,kg^{-1}$, i.p.)) on intestinal transit *in vivo*. Transit was expressed as the geometric centre (GC) of the distribution of a fluorescent marker along the small intestine. GC ranged from 1 (minimal motility) to 10 (maximal motility) (see Methods section). Bars represent the mean ± s.e.mean of 8–10 animals. $^{\#}P<0.05$ vs control, $^{*}P<0.05$ vs croton oil and $^{\circ}P<0.05$ vs CBD.

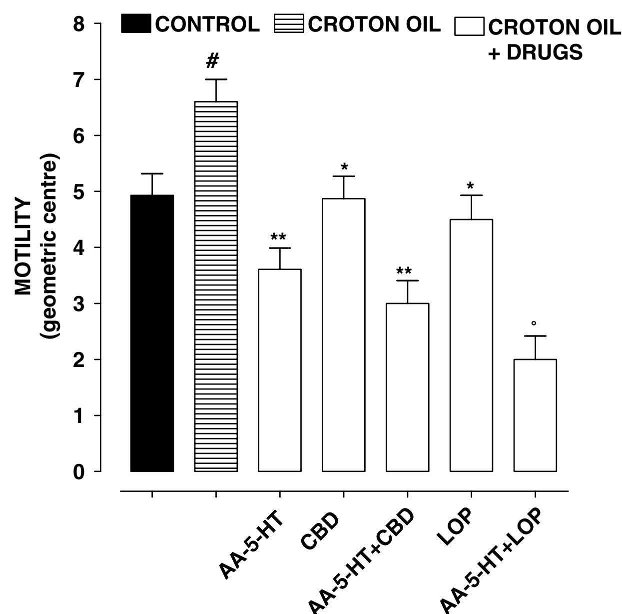

Figure 3 Croton oil-treated mice: effect of i.p.-injected cannabidiol (CBD, $5\,mg\,kg^{-1}$) and the fatty acid amide hydrolase inhibitor *N*-arachidonoyl-5-hydroxytryptamine (AA-5-HT, $7.5\,mg\,kg^{-1}$) (alone or in combination) on intestinal transit *in vivo*. Transit was expressed as the geometric centre (GC) of the distribution of a fluorescent marker along the small intestine. GC ranged from 1 (minimal motility) to 10 (maximal motility) (see Methods section). Bars represent the mean ± s.e.mean of 8–10 animals. $^{\#}P<0.05$ vs control, $^{*}P<0.05$ and $^{**}P<0.05$ vs croton oil and $^{\circ}P<0.05$ vs LOP.

Table 1 Croton oil (CO)-treated mice: effect of i.p.-injected cannabidiol (CBD, $5\,mg\,kg^{-1}$) alone or in the presence of the CB_2 antagonist SR144528 ($1\,mg\,kg^{-1}$), the opioid antagonist naloxone ($2\,mg\,kg^{-1}$) or the α_2-adrenoceptor antagonist yohimbine ($1\,mg\,kg^{-1}$) on intestinal transit *in vivo*

Treatment	*Motility (geometric centre)*
Control (no croton oil)	4.91 ± 0.43
Croton oil (CO)	6.65 ± 0.41#
CO + CBD	5.01 ± 0.36*
CO + CBD + SR144528	4.99 ± 0.38*
CO + CBD + naloxone	4.98 ± 0.44*
CO + CBD + yohimbine	4.97 ± 0.43*

$^{\#}P<0.05$ vs control.
$^{*}P<0.05$ vs croton oil.
$N=8$–10 animals for each experimental group. Transit was expressed as the geometric centre (GC) of the distribution of a fluorescent marker along the small intestine. GC ranged from 1 (minimal motility) to 10 (maximal motility) (see Methods section).

6.65 ± 0.45; croton oil + loperamide 4.55 ± 0.36, croton oil + loperamide + naloxone 6.60 ± 0.44) and yohimbine ($1\,mg\,kg^{-1}$) counteracted the inhibitory effect of clonidine $0.075\,mg\,kg^{-1}$ on motility (control 4.98 ± 0.42, croton oil 6.67 ± 0.36, croton oil + clonidine 4.50 ± 0.37, croton oil + clonidine + yohimbine 6.58 ± 0.38). In the absence of any agonist, SR144528, naloxone or yohimbine did not modify significantly motility in croton oil-treated animals (croton oil 6.70 ± 0.52; croton oil + SR144528 6.49 ± 0.62; croton oil + naloxone 6.65 ± 0.49; croton oil + yohimbine 6.79 ± 0.55, $n=7$–8, $P>0.2$).

Figure 3 shows the effect of CBD ($5\,mg\,kg^{-1}$), loperamide ($0.075\,mg\,kg^{-1}$) or AA-5-HT ($7.5\,mg\,kg^{-1}$ (administered alone or in combination) in croton oil-treated mice. CBD, loperamide and AA-5-HT significantly reduced motility in croton oil-treated animals; however, the effects of CBD and AA-5-HT were not additive, while the effects of loperamide and AA-5-HT were additive (that is, loperamide (but not CBD) still inhibited motility in animals pretreated with AA-5-HT).

In vitro *results*

ACh (1 μM) evoked a contractile response that was 66 ± 5% (in control tissues) or 81 ± 3% (in the ileum from croton oil-treated mice, $P<0.\ 05$ vs control, $n=7$–9) of the contraction produced by ACh 1 mM. This concentration of ACh (1 mM) produced a maximal contractile response in the ileum (100% contraction). CBD (0.01–100 μM) had no effect on the baseline mechanical activity of the intestine, but it significantly and in a concentration-dependent manner, inhibited the contractions induced by ACh (Figure 4). The IC_{50} values of CBD were 4.39 ± 1.55 μM in control tissues and 2.66 ± 1.99 μM in inflamed tissues (no significant differences between the two IC_{50} values, $n=7$–9). The E_{max} values were 72 ± 10% in control tissues and 75 ± 14% in the inflamed gut (no significant difference between the two E_{max} values, $n=7$–9). CBD (0.01–10 μM) also reduced the contractions induced by prostaglandin $F_{2\alpha}$ (0.2 μM) (data not shown).

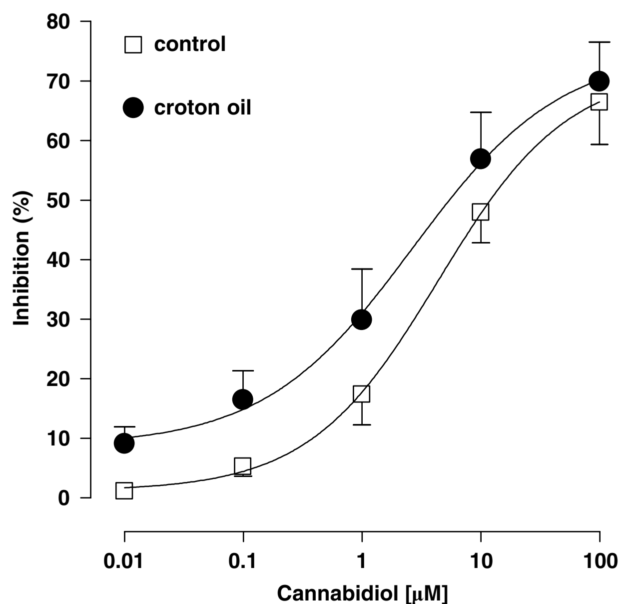

Figure 4 Inhibitory effect of cannabidiol (0.01–100 μM) on the contractions induced by ACh (1 μM) in the isolated mouse ileum of control and croton oil-treated mice. Each point represents mean ± s.e.mean of 7–8 experiments.

Discussion

The presence of motility changes in inflammatory diseases of small or large intestine is a well-recognized and clinically accepted phenomenon (Ohama *et al*., 2007). The croton oil model of intestinal hypermotility has been extensively used to evaluated drugs with clinical or potential clinical use. Intestinal inflammation induced by croton oil is characterized by disruption of the mucosa and an infiltration of lymphocytes into the submucosa associated with an increase of intestinal transit (Pol and Puig, 1997). Motility in the croton oil model of ileitis may be attenuated by a number of drugs, including cannabinoid CB_1 (Izzo *et al*., 2001b), α_2-adrenoceptor (Pol *et al*., 1996) and opioid (Puig and Pol, 1998; Capasso *et al*., 2008) receptor agonists. In the present study, we have shown that CBD, a non-psychotropic component of the marijuana plant *C. sativa*, reduced motility in this experimental model of intestinal ileitis. Two points should be considered here: first, our method to evaluate motility does not distinguish between an effect on gastric emptying and transit through the small intestine and, second, we used a liquid non-nutrient meal and thus there is the possibility that our results will not translate to the transit of solid and/or caloric meals. Interestingly, CBD did not affect transit (present results) and defecation (Fride *et al*., 2005) in control mice, suggesting that this compound is pharmacologically active only when intestinal homoeostasis is perturbed by an inflammatory stimulus. Although we cannot exclude the possibility that *in vivo* CBD attenuates the systemic inflammatory response to croton oil rather than having direct effects on intestinal transit (see also below) and although there is evidence that rodent data on cannabinoids might not translate to humans (Sanger, 2007), the present results make CBD an attractive compound for possible therapeutic use to reduce motility during inflammation.

To investigate the mechanism of action of CBD-induced delay in motility, we considered the possible involvement of FAAH, that is, the enzyme involved in endocannabinoid degradation, for several reasons. Thus, FAAH mRNA has been detected in the mouse small intestine and its inhibition resulted in increased intestinal anandamide and 2-arachidonoylglycerol levels and reduction of transit along the small intestine in mice (Capasso *et al*., 2005). Intestinal FAAH activity is increased in the croton oil model of ileitis (Izzo *et al*., 2001a, b) and, more importantly, CBD has been shown to inhibit anandamide hydrolysis (Watanabe *et al*., 1998; Bisogno *et al*., 2001). In the present study, we have shown that CBD, in contrast to loperamide, did not further reduce transit in animals treated with the FAAH inhibitor, AA-5-HT. The fact that the effects of CBD and AA-5-HT were not additive suggests that the mechanism of CBD-induced delay in motility may involve FAAH. Others have shown that FAAH mediates the antitumour activity of CBD in cultured cells (Massi *et al*., 2008).

It is now well known that activation of enteric cannabinoid CB_1 receptors results in inhibition of intestinal motility in mice *in vivo* (Izzo *et al*., 2001a; Carai *et al*., 2006; Yuece *et al*., 2007). Previous studies have shown that the inhibitory effect of FAAH inhibitors on gastric and intestinal motility involves, at least in part, indirect activation of cannabinoid CB_1 receptors (via enhanced production of intestinal endocannabinoids) (Capasso *et al*., 2005; Di Marzo *et al*., 2008). Indeed, the cannabinoid CB_1 receptor antagonist rimonabant partially reduced the inhibitory effect of the FAAH inhibitor AA-5-HT on gastric (Di Marzo *et al*., 2008) and intestinal (Capasso *et al*., 2005) motility. In the present study, we have shown that a dose of rimonabant, ineffective *per se*, counteracted the inhibitory effect of CBD (but not the effect of the opioid agonist loperamide) on motility in croton oil-treated mice. On the basis of our experimental data and those previously published which showed the inhibitory effect of CBD on anandamide hydrolysis (Watanabe *et al*., 1998; Bisogno *et al*., 2001), we hypothesize that CBD may indirectly activate (via FAAH inhibition) enteric cannabinoid CB_1 receptors and thus reduce motility. A direct activation of cannabinoid CB_1 receptors seems unlikely as this *Cannabis*-derived compound has very little affinity for cannabinoid CB_1 receptors (McPartland *et al*., 2007). Interestingly, increased intestinal FAAH activity and increased cannabinoid CB_1 receptor expression have been observed in the intestine of croton oil-treated mice (Izzo *et al*., 2001b). This observation could explain why CBD reduced motility in pathophysiological states, whereas it was without effect in control mice. During the preparation of our paper, others have shown that CBD inhibited FAAH expression in the inflamed—but not in the normal—mouse gut (De Filippis *et al*., 2008), thus further supporting the involvement of this enzyme in CBD-mediated intestinal effects.

Another possible target of the CBD action is the cannabinoid CB_2 receptor. In the gut, this receptor has been found to be expressed by inflammatory/immune cells and also identified on epithelial cells and neurons (Coutts and Izzo, 2004; Di Marzo and Izzo, 2006; Wright *et al*., 2008). Thomas

et al. (2007) have recently shown that the ability of CBD to behave as a cannabinoid CB_2 inverse agonist may contribute to its anti-inflammatory properties. Our results demonstrate that the cannabinoid CB_2 receptor is functionally active in reducing motility during ileitis, as the selective cannabinoid CB_2 receptor agonist JWH 015 (in a cannabinoid CB_2 antagonist-sensitive manner) reduced motility in mice treated with croton oil (but not in control animals). However, blockade of the cannabinoid CB_2 receptor with the selective antagonist SR144528 did not modify the inhibitory effect of CBD on motility, suggesting that CBD-mediated inhibition of transit is independent of the activation of cannabinoid CB_2 receptors. Nevertheless, the cannabinoid CB_2-mediated inhibition of intestinal motility, which has been previously documented in the model of intestinal inflammation induced by an endotoxic agent (Mathison *et al.*, 2004), is relevant in the light of the observation that cannabinoid CB_2 receptor agonists are devoid of the characteristic psychotropic effects associated with cannabis use (Izzo, 2007; Wright *et al.*, 2008).

We also investigated other mechanisms as potential contributors of the inhibitory effect of CBD on intestinal motility. Specifically, we investigated the possible involvement of α_2-adrenoceptors and opioid receptors, because such receptors are upregulated in the intestinal model of ileitis induced by croton oil (Pol *et al.*, 1996, 2001, 2003). Moreover, CBD has been recently shown to be an allosteric modulator at μ- and δ-opioid receptors (Kathmann *et al.*, 2006). However, our experimental data did not support the involvement of α_2-adrenoceptors or opioid receptors as specific antagonists of these receptors (namely naloxone and yohimbine) did not modify the inhibitory effect of CBD on motility.

Finally, to verify whether or not CBD may affect directly intestinal contractility, that is, to exert actions in the gut independently from possible systemic anti-inflammatory effects, we evaluated the effect of CBD on the contractions evoked by ACh in the isolated ileum. We found that CBD reduced, in a concentration-dependent manner, ACh-induced contractions, both in control and in croton oil-treated animals. The IC_{50} values found in our study (2.66–4.39 μM) were in the range of concentrations previously shown to reduce noradrenaline-induced contractions in the vas deferens (Thomas *et al.*, 2004) and to exert neuroprotective (Esposito *et al.*, 2006) and antitumour effects (Ligresti *et al.*, 2006; Vaccani *et al.*, 2006). In contrast to *in vivo* results, CBD inhibited ACh-induced contractions both in the healthy and in the inflamed intestine (no significant differences in potency or in efficacy were observed, although CBD showed a trend towards a greater potency in the intestine from croton oil-treated mice). Discrepancies between *in vitro* and *in vivo* actions of cannabinoids have been previously documented in the digestive tract (Coruzzi *et al.*, 2006). It is very unlikely that the antispasmodic effect of CBD observed here was due to antimuscarinic actions, as CBD also inhibited the contractions induced by prostaglandin $F_{2\alpha}$.

In conclusion, we have shown that the marijuana component CBD normalize intestinal motility in an experimental model of ileitis. *In vitro* results showed antispasmodic actions of CBD on intestinal ileal segments. The inhibitory effect of CBD involves, at least *in vivo*, cannabinoid CB_1 receptors and FAAH. In view of its safety records in humans (an average daily dose of about 700 mg/day for 6 weeks was found to be non-toxic, relative to placebo, in clinical trials; Cunha *et al.*, 1980; Consroe *et al.*, 1991) and because CBD reduced motility during inflammation and not in physiological conditions, CBD might be considered as a good candidate to be clinically evaluated for the treatment of hypermotility associated with inflammatory bowel disease.

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Conflict of interest

The authors state no conflict of interest.

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