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(+)-Cannabidiol analogues which bind cannabinoid receptors but exert peripheral activity only

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Abstract

Δ^9-Tetrahydrocannabinol (Δ^9-THC) and (−)-cannabidiol are major constituents of the *Cannabis sativa* plant with different pharmacological profiles: (−)-Δ^9-tetrahydrocannabinol, but not (−)-cannabidiol, activates cannabinoid CB_1 and CB_2 receptors and induces psychoactive and peripheral effects. We have tested a series of (+)-cannabidiol derivatives, namely, (+)-cannabidiol-DMH (DMH—1,1-dimethylheptyl-), (+)-7-OH-cannabidiol-DMH, (+)-7-OH- cannabidiol, (+)-7-COOH- cannabidiol and (+)-7-COOH-cannabidiol-DMH, for central and peripheral (intestinal, antiinflammatory and peripheral pain) effects in mice. Although all (+)-cannabidiols bind to cannabinoid CB_1 and CB_2 receptors, only (+)-7-OH-cannabidiol-DMH was centrally active, while all (+)-cannabidiol analogues completely arrested defecation. The effects of (+)-cannabidiol-DMH and (+)-7-OH-cannabidiol-DMH were partially antagonized by the cannabinoid CB_1 receptor antagonist *N*-(piperidiny-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (SR141716), but not by the cannabinoid CB_2 receptor antagonist *N*-[-(1S)-endo-1,3,3-trimethil bicyclo [2.2.1] heptan-2-yl-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528), and had no effect on $CB_1^{-/-}$ receptor knockout mice. (+)-Cannabidiol-DMH inhibited the peripheral pain response and arachidonic-acid-induced inflammation of the ear. We conclude that centrally inactive (+)-cannabidiol analogues should be further developed as antidiarrheal, antiinflammatory and analgesic drugs for gastrointestinal and other peripheral conditions.

Keywords: Cannabidiol; Cannabinoid; Cannabinoid receptor; SR141716; SR144528; Intestinal motility

1. Introduction

Δ^9-Tetrahydrocannabinol (Δ^9-THC) and (−)-cannabidiol are the two major constituents of the *Cannabis sativa* (marihuana) plant. Δ^9-THC is psychoactive and binds to cannabinoid CB_1 receptors located in brain and the periphery (Herkenham, 1995; Pertwee, 1997), as well as to cannabinoid CB_2 receptors which are located predominantly on nonneural tissue, such as immune cells and glial cells in the brain (Walter et al., 2003). (−)-Cannabidiol binds neither receptor and is not psychoactive. Δ^9-THC is considered to be responsible for virtually all central effects observed with the cannabis plant and for many of its peripheral effects (Fride, 2002; Mechoulam et al., 1998; Pertwee, 1997). Peripheral effects include inhibition of gastrointestinal activity (Pinto et al., 2002) and antiinflammatory effects (Mechoulam et al., 1998).

In view of the abundance of cannabinoid CB_1 and CB_2 receptors on immune cells (Galiegue et al., 1995; Pertwee, 1997), it is not surprising that cannabinoids are effective regulators of the inflammatory process and

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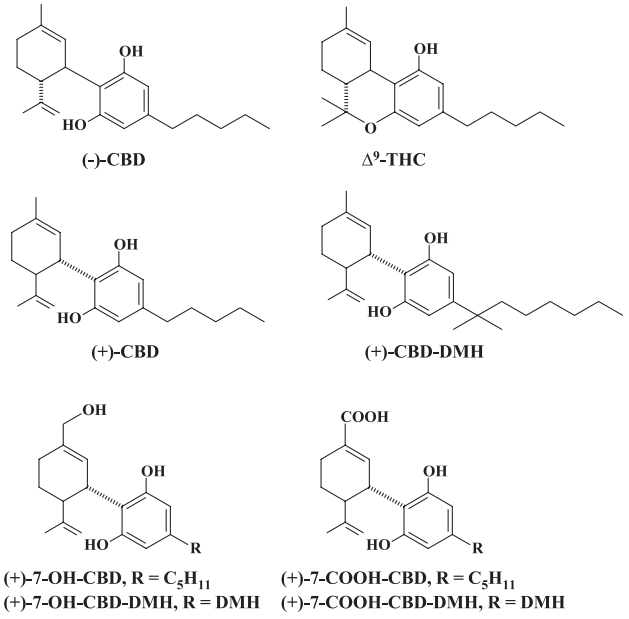

Fig. 1. Structures of the natural Δ^9-tetrahydrocannabinol (THC) and (−)-cannabidiol, the synthetic (+)-cannabidiol and its derivatives; DMH—dimethyl-heptyl.

peripheral pain (Hanus et al., 1999; Malfait et al., 2000; Mechoulam et al., 1998).

There is ample evidence in vitro and in vivo for an inhibitory action of exogenous cannabinoids and endocannabinoids (anandamide, 2-arachidonoyl glycerol and noladine ether, see Fride, 2002) on intestinal motility in various species, such as mice, rats and guinea pigs (Pertwee, 2001). Early work includes in vivo evidence for an inhibitory effect of Δ^9-THC on intestinal motility in mice (Chesher et al., 1973). Endocannabinoid-induced inhibition of intestinal motility was first demonstrated for anandamide as a near cessation of defecation in mice (Fride, 1995; Fride and Mechoulam, 1993).

Most evidence suggests that the cannabinoid-induced gastrointestinal inhibition is mediated by cannabinoid CB_1 receptors (Calignano et al., 1997; Colombo et al., 1998; Pertwee, 2001; Pinto et al., 2002). This is in agreement with a presence of cannabinoid CB_1 receptors and CB_1 receptor mRNA (Casu et al., 2003; Griffin et al., 1997), but not of cannabinoid CB_2 receptor mRNA in the mysenteric plexus of the gut. It has also been determined that gastrointestinal transit is regulated locally in the periphery rather than by centrally located cannabinoid CB_1 receptors (Izzo et al., 2000; Landi et al., 2002).

We have shown previously that the selective cannabinoid CB_2 receptor agonist, HU-308 [(+)-(1-α-H, -β-H, 5-a-H)-4-[2,6-dimethoxy-4-(1,1-dimethylheptyl)phenyl]-6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-carbinol], inhibited defecation which was antagonized by the selective 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], but not by the cannabinoid CB_1 receptor antagonist SR141716 [*N*-(piperidiny-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; Hanus et al., 1999].

Taken together, these findings suggest that cannabinoids may be developed as therapeutic agents in conditions, such as inflammatory pain and inflammatory bowel diseases. The significant drawback for the use of cannabis or Δ^9-THC is the unwanted psychoactive side effects, such as anxiety, confusion and memory impairment, which may be observed with higher doses (Robson, 2001).

Table 1
Summary of cannabinoid CB_1 and CB_2 receptor binding and in vivo-induced effects of Δ^9-THC and (+)-cannabidiol derivatives

Compound	CB_1/CB_2 receptor binding (K_i, nM)	Locomotion (%MPE)[a]	Rearing (%MPE)[a]	Catalepsy (%MPE)[a]	Analgesia hot plate (%MPE)[a]	Hypothermia (Δbody temperature, °C)	Inhibition intestinal motility (%MPE)[a]
Δ^9-THC	66.5±5.8/36.4±10.0[b]	44[c]	12[c]	44[c]	45[c]	−2.1[c]	100[c]
(+)-Cannabidiol	842±36/203±16[d]	108	103	05	13	0.5	−13
(+)-Cannabidiol-DMH	17.4±1.8/211±23[d]	82	61	16	10	−0.3	100[b]
(+)-7-OH-Cannabidiol	5.3±0.5/101.0±5.1	107	81	0	77	0.3	89[b]
(+)-7-OH-Cannabidiol-DMH	2.5±0.03/44.0±3.12	01[c]	0[c]	95[c]	100[c]	−5.2[c]	100[c]
(+)-COOH-Cannabidiol	13.2±0.4/321.8±15.8	80	71	06	-	−0.6	100[b]
(+)-COOH-Cannabidiol-DMH	5.8±0.7/155.5±5.3	102	100	0	-	0.2	100[c]

Female Sabra mice (8–12 weeks old) were injected at a time interval, which had been shown previously to yield maximal effects (30 or 60 min) before testing in a series of six consecutive assessments: motor activity (locomotion and rearing) and defecation (intestinal motility) in an open field (for 8 min); catalepsy on an elevated ring (for 4 min); response to a painful stimulus (hot plate kept at 55 °C, mouse was allowed to remain on the plate for maximally 45 s) and rectal temperature (hypothermia). All groups consisted of five mice. All drugs were injected at 20 mg/kg in a mixture of ethanol/cremophor/saline=1:1:18 ("Vehicle"). Each compound was tested at least twice, with almost identical results; several compounds, such as (+)-cannabidiol-DMH and (+)-7-OH-cannabidiol-DMH, were tested more than five times.

[a] %MPE—"Maximum Possible Effect". The formula's are described in the Materials and methods.

[b] Data from Showalter et al., 1996.

[c] Significantly different from vehicle controls (at least $P<0.05$).

[d] Data from Bisogno et al., 2001.

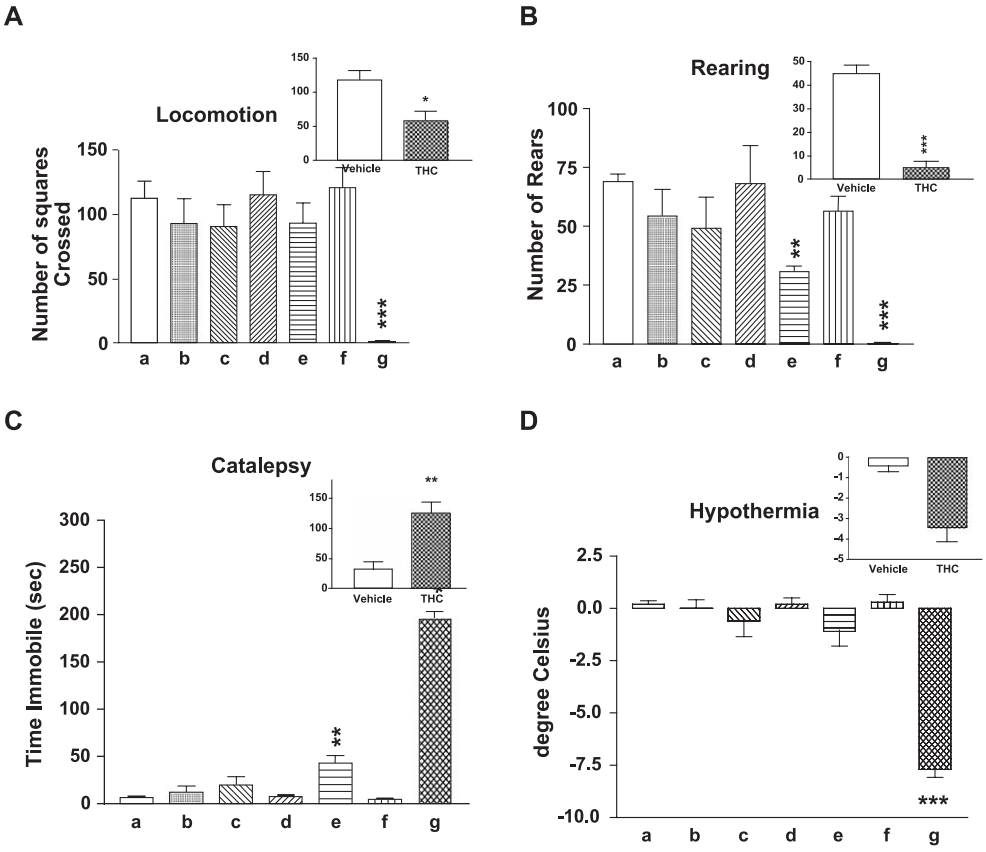

Fig. 2. Central cannabinoid effects of (+)-7-OH-cannabidiol, (+)-7OH-cannabidiol-DMH, (+)-COOH-cannabidiol, (+)-COOH-cannabidiol-DMH and (+)-cannabidiol-DMH. Compounds (20 mg/kg) were injected i.p. into female Sabra mice. Mice were tested 60 min later for centrally mediated effects [locomotion and rearing in an open field, catalepsy (immobility) on an elevated ring, analgesia on a hot plate[1] and hypothermia]. In the insets, the effect of Δ^9-THC (20 mg/kg) is given for comparison. (+)-7-OH- cannabidiol-DMH was very potent, whereas none of the other (+)-cannabidiol derivatives had any effect. Data are presented as means±S.E.M.'s. Comparisons between two groups (in the insets) were analyzed with Student's *t*-tests. Comparisons between vehicle and the (+)-cannabidiols were analyzed using one-way analysis-of-variances with Student Newman–Keul post hoc tests. ***P<0.001, cf., vehicle-injected mice.

Therefore, current efforts are aimed at developing cannabinoids with medical benefits but which are devoid of psychoactive side effects.

Despite the dichotomy between Δ^9-THC and cannabidiol, canabidiol displays a number of pharmacological activities which are similar to those of Δ^9-THC. These include antiemetic (Parker et al., 2002) and antiinflammatory effects (Malfait et al., 2000). Being devoid of psychoactive effects, cannabidiol is a good candidate for future development of peripherally acting cannabinoid-like drugs.

In a previous report, Bisogno et al. (2001) have described biochemical/pharmacological properties of a number of derivatives of the natural (−)-cannabidiol, as well as the synthetic (+)-cannabidiol, (+)-cannabidiol-DMH (cannabidiol-1,1-dimethylheptyl) and (+)-7-OH-cannabidiol-DMH. Only the latter two (+)-analogues were found to bind cannabinoid CB_1 and/or CB_2 receptors. Vanilloid VR_1 receptors or increased levels of the endocannabinoid anandamide may mediate effects of some, but not all, analogues. Based on such findings, candidates for antiinflammatory or other therapeutic activity may be developed.

In this study, we examined the aforementioned (+)-cannabidiol-DMH and (+)-7-OH-cannabidiol-DMH, and several novel (+)-cannabidiol analogues [(+)-7-OH- cannabidiol, (+)-COOH-cannabidiol and (+)-COOH-cannabidiol-

[1] Due to a technical failure of the apparatus, the hot plate data were not valid and are not presented here, see however Fig. 5 for hot plate data in a different experiment.

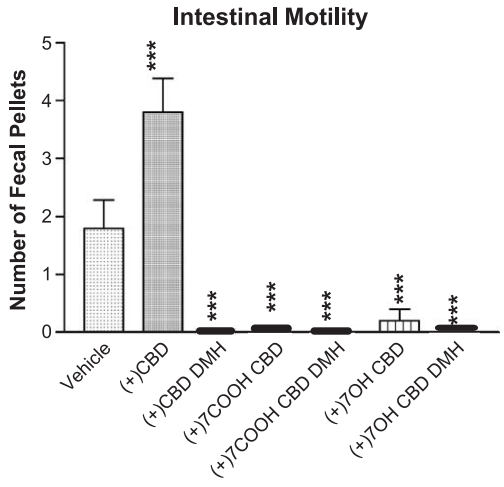

Fig. 3. Inhibition of intestinal motility by (+)-cannabidiol analogues. Sixty minutes after i.p. injections of vehicle, (+)-7-COOH-cannabidiol, (+)-7-COOH- cannabidiol-DMH, (+)-cannabidiol-DMH, (+)-7-OH-cannabidiol or (+)-7-OH- cannabidiol-DMH, defecation (intestinal motility in an open field) was almost, or completely, blocked when recorded for 8 min. Data are presented as means±S.E.M.'s. Data were analyzed using one-way analysis-of-variances with Student Newman–Keul post hoc tests. *Different from vehicle control ($P<0.001$).

DMH] for central as well as peripheral activity in mice. Central cannabinoid receptor-mediated activity was assessed in the "tetrad", a series of in vivo assays which profile central cannabinoid activity (Fride and Mechoulam, 1993; Martin et al., 1991). Peripheral activity was measured as intestinal motility (measured as defecation rates), which has high baseline levels, even over a short period, in the mouse strain (Sabra) used in this study. Antiinflammatory potential was assessed using arachidonic-acid-induced swelling of the external ear (Hanus et al., 1999; Young et al., 1984). Peripheral pain was measured by the response to an intraplantar injection of a 4% formalin solution.

2. Materials and methods

2.1. Mice

Female Sabra mice (2–3 months) of age were used, purchased from Harlan (Israel). Cannabinoid $CB_1^{-/-}$ receptor knockout mice were generously supplied by Dr. Zimmer (University of Bonn, Germany). C57BL/6 mice, the background strain for the cannabinoid $CB_1^{-/-}$ receptor knockouts, were purchased from Harlan (Israel). Between five and eight mice were used for each treatment group. Procedures were approved by the Ethics committee of the College of Judea and Samaria.

2.2. Drugs

All cannabidiol-derived compounds were prepared in our laboratory (Department of Medicinal Chemistry and Natural Products, Hebrew University of Jerusalem). The cannabinoid CB_1 and CB_2 receptor antagonists, SR141716 and SR144528, respectively, were kindly supplied by NIDA (Research Triangle). Capsazepin was purchased from Tocris, (England). All compounds were prepared in a mixture of ethanol/cremophor (Sigma)/saline=1:1:18 (see, for example, Fride and Mechoulam, 1993).

2.3. Central activity

Mice were injected with antagonist 90 min before testing and/or with agonist 60 min before testing in a series of four assays which reflect central cannabinoid activity (Martin et al., 1991, modified by Fride and Mechoulam, 1993). This "tetrad" consists of, consecutively, ambulation and rearing in an open field (8 min), immobility on an elevated ring of 5-cm diameter (4 min), rectal temperature (Yellow Springs Instruments, Yellow Springs, OH, USA) and hot plate or tail

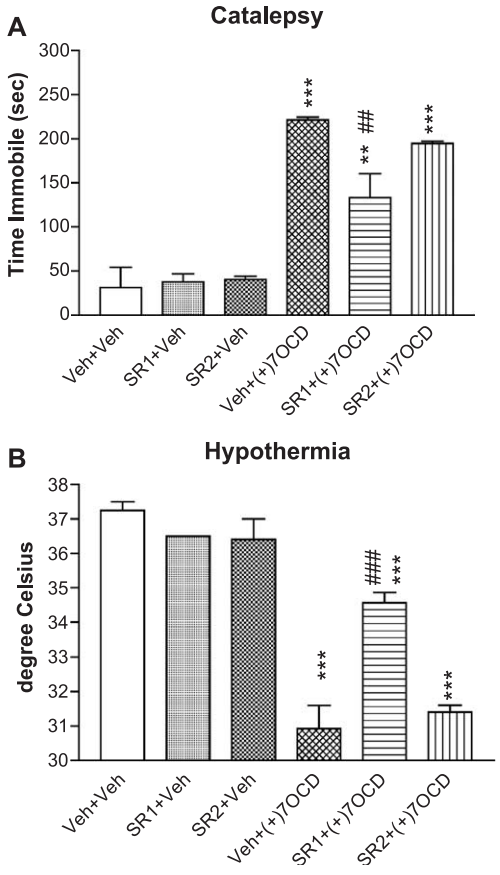

Fig. 4. Reversal of the central effects of (+)-7-OH-cannabidiol-DMH by SR141716. SR141716 (SR1, 5 mg/kg), SR144528 (SR2, 3 mg/kg) or vehicle were injected (i.p.) 30 min before vehicle or (+)-7-OH-cannabidiol-DMH [(+)7OCD, 20 mg/kg]. (A) Catalepsy (immobility on a ring) and (B) hypothermia (rectal temperature) were measured 60 min after the second injection. SR1, but not SR2, significantly antagonized the effect of (+)7OH-cannabidiol-DMH. **,***Different from Veh+Veh, SR1+Veh or SR2+Veh, $P<0.01$, $P<0.001$, respectively. ##,###Different from the Veh + (+)-7-OH-cannabidiol-DMH group, $P<0.01$, $P<0.001$, respectively.

flick analgesia (we use the hot plate, which is described below, see Pain, central vs. peripheral).

2.4. Peripheral activity

2.4.1. Intestinal motility (defecation: the number of fecal pellets voided in the open field)

In the first series of experiments (Fig. 2), the number of fecal pellets was assessed during exposure to the open field (i.e., 8 min). In later experiments (see Figs. 5–7), defecation was assessed for a prolonged period (3 h).

2.4.2. Arachidonic-acid-induced inflammation of the external ear

Inflammation of the external ear was assessed by measuring tissue swelling after topical application of arachidonic acid (Hanus et al., 1999). Thus, arachidonic acid was applied to the inner surface of the external ear (4.5 mg dissolved in 5 μl ethanol). The opposite ear served as control (5 μl of ethanol). Ear thickness was determined (in 0.01-mm units) by using a dial thickness gauge (Mitutoyo, Japan), every 15 min for 90 min, starting immediately after arachidonic acid application. Test drugs were injected i.p. 60 min before arachidonic acid.

2.5. Pain, central vs. peripheral

Pain perception on a hot plate is thought to be mediated by a central mechanism, whereas the second, late phase of the response to an intraplantar injection of formalin reflects inflammatory pain mechanisms (Tjolsen and Hole, 1997).

Therefore, central pain perception was assessed by the analgesic response on a hot plate (55 °C, Columbus Instruments, OH, USA). Peripheral pain was measured as the response to intraplantar injection of formalin (4%), 45 min after injection of the drug, by recording the number of licks of the formalin-injected hind paw, in 5-min intervals, for 1 h.

2.6. Cannabinoid CB_1 receptor binding

Cannabinoid CB_1 receptor concentration and affinity were measured by a competition binding assay as described

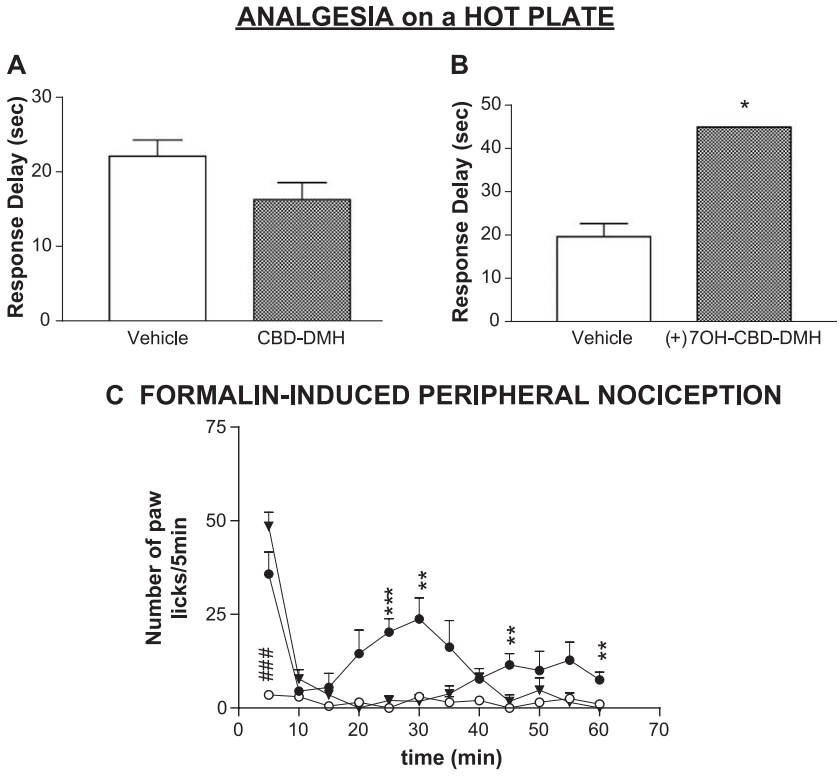

Fig. 5. Centrally and peripherally mediated nociception of (+)-cannabidiol-DMH and (+)-7-OH-cannabidiol-DMH. Female Sabra mice were injected with (A) cannabidiol-DMH or (B) (+)-7-OH- cannabidiol-DMH (20 mg/kg) 60 min before assaying their response latency on a hot plate. (+)-7-OH-cannabidiol-DMH, but not (+)-cannabidiol-DMH, induced hypoalgesia. (C) Formalin (4%) was injected in the left hind footpad, and the number of licks of the injected foot was recorded for each 5-min interval for the 60 min starting immediately after formalin application. (+)-Cannabidiol-DMH almost completely prevented the second phase of nociception. ○: i.p. and *intraplantar* vehicle; ●: i.p. vehicle and *intraplantar* formalin; ▼: i.p. (+)-cannabidiol-DMH and *intraplantar* formalin. Data are presented as means±S.E.M.'s. Comparisons between two groups (A and B) were analyzed with Student's *t*-tests. Peripheral nociception was analyzed using two-way analysis-of-variance for repeated measures (time), followed by one-way analysis-of-variance's with Student Newman–Keul post hoc tests. (B): $^{*}P<0.001$ vs. control (vehicle). (C): $^{**}P<0.01$, different from (+)-cannabidiol-DMH-treated mice. $^{***}P<0.001$, different from (+)-cannabidiol-DMH-treated mice. $^{\#\#\#}P<0.001$, different from the other two groups.

in detail previously (Ben-Shabat et al., 1998; Rhee et al., 1997). Thus, the high-affinity receptor probe, [^{3}H]HU-243 (Tocris Cookson, United Kingdom), with a dissociation constant of 45±7 pM for the cannabinoid CB_1 receptor was incubated with synaptosomal membranes (3 to 4 μg) for cannabinoid CB_1 receptor assays and/or transfected cells for cannabinoid CB_2 receptor assays for 90 min at 30 °C with different concentrations of the assayed cannabidiol derivatives or with the vehicle alone (fatty-acid-free bovine serum albumin at a final concentration of 0.5 mg/ml). Bound and free radioligand were separated by centrifugation. The data were normalized to 100% of specific binding, which was determined with 50 nM unlabeled HU-243. The results presented are the average of triplicate determinations from three independent experiments. The K_i value was determined using GraphPad Prism (Version 3.02) software, which follows the Cheng–Prusoff equation. A sigmoid dose–response (variable slope) built-in equation in this Prism program was used to fit the curves.

2.7. Data presentation and statistical analyses

To compare between the pharmacological effects of the various compounds and their binding constants, the data from the tetrad and intestinal motility assays were normalized using various formula's for percent maximum possible effect (%MPE; Fride, 1995).

Horizontal and vertical movements in the open field:

$$\%\mathrm{MPE} = \left(1 - \frac{\mathrm{Vehicle} - \mathrm{Experiment}}{\mathrm{Vehicle}}\right) \times 100$$

Immobility on a ring (catalepsy):

$$\%\mathrm{MPE} = \frac{\mathrm{Experiment} - \mathrm{Vehicle}}{240 - \mathrm{Vehicle}} \times 100$$

Analgesia on a hot plate:

$$\%\mathrm{MPE} = \frac{\mathrm{Experiment} - \mathrm{Vehicle}}{45 - \mathrm{Vehicle}} \times 100$$

Inhibition of intestinal motility:

$$\%\mathrm{MPE} = \frac{\mathrm{Vehicle} - \mathrm{Experiment}}{\mathrm{Vehicle}} \times 100$$

Hypothermia was calculated as the difference between rectal temperature measured before injection and 60 min after injection.

Comparisons between the two groups were analyzed with Student's *t*-tests. Comparisons between three or more treatment groups were analyzed using one-way analyses-of-variance with Student Newman–Keul post hoc tests. When repeatedly tested over time, two-way analysis-of-variances for repeated measures was used, followed by one-way analysis-of-variances at individual time points, followed by Student Newman–Keul post hoc tests.

3. Results

In the "tetrad" for central cannabinoid effects, (+)-cannabidiol, which weakly binds cannabinoid CB_1 and CB_2 receptors (Fig. 1; Table 1), had no central (Fig. 2) nor peripheral (intestinal motility) effects (Fig. 3). Although all of the (+)-cannabidiol analogues showed substantial cannabinoid CB_1 receptor binding (see Table 1 and Bisogno et al., 2001), only (+)-7-OH-cannabidiol-DMH had central effects in all assays of the tetrad (Figs. 2 and 4 and Table 1); (+)-cannabidiol-DMH had a small effect on catalepsy (Fig. 2C) and on rearing in an open-field (Fig. 2B), but these effects were not consistently observed. In fact, we have repeated the experiment using (+)-cannabidiol-DMH at least five times and only observed occasional small effects in one or two assays.

On the other hand, all compounds, significantly inhibited defecation (frequently reducing fecal pellets to zero, see Figs. 3 and 7 and Table 1).

Centrally mediated pain in response to exposure to a hot plate was not affected by (+)-cannabidiol-DMH (Fig. 5A) but was significantly inhibited (to the maximal response of 45 s) by (+)-7-OH-cannabidiol-DMH (Fig. 5B). In contrast, the second, inflammatory phase of the formalin-induced pain response (Tjolsen et al., 1992), was almost completely

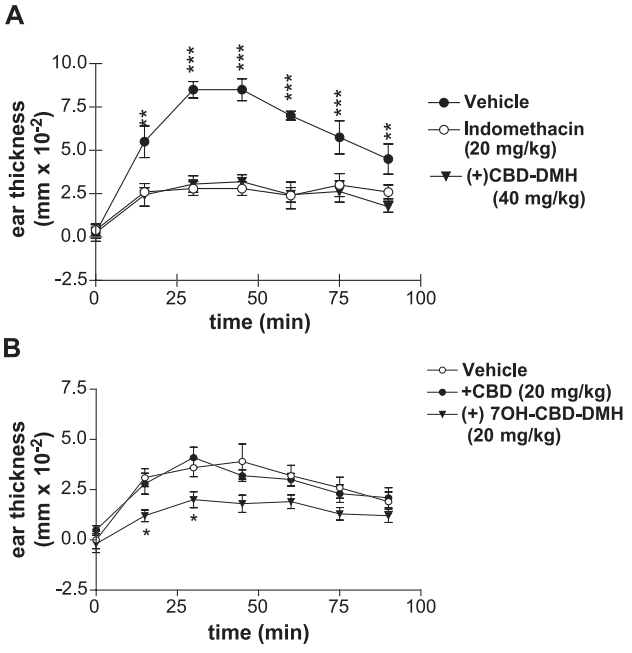

Fig. 6. Antiinflammatory effect of (A) 40 mg/kg of (+)-cannabidiol-DMH or (B) 20 mg/kg of (+)-cannabidiol or (+)-7-OH-cannabidiol-DMH, compared to indomethacin (20 mg/kg), in a model of arachidonic-acid-induced ear inflammation in the mouse: similar antiinflammatory effects of (+)-cannabidiol-DMH and indomethacin were observed (A,B). Data are presented as means±S.E.M.'s. Ear thickness was analyzed using two-way analysis-of-variance for repeated measures (time), followed by one-way analysis-of-variance's with Student Newman–Keul post hoc tests. *P<0.05 different from vehicle or (+)-cannabidiol, **P<0.01 different from indomethacin or 7-OH- cannabidiol-DMH. ***P<0.001 different from indomethacin or 7-OH-cannabidiol-DMH.

inhibited by (+)-cannabidiol-DMH (Fig. 5C; F_{group}=66, *df*=2,72, *P*<0.001).

Arachidonic-acid-induced inflammation of the external ear was almost completely inhibited by 40 mg/kg (F_{group}=88, *df*=2/56, *P*<0.001; Fig. 6A) or 10 (F_{group}=66, *df*=2/72, *P*<0.001, data not shown) of (+)-cannabidiol-DMH. The effect of (+)-cannabidiol-DMH (whether 10 or 40 mg/kg) was similar to that of 20 mg/kg of indomethacin (Fig. 6A). (+)-7-OH-cannabidiol-DMH (20 mg/kg) also inhibited ear inflammaton (F_{group}=8.5, *df*=2/56, *P*<0.001), but (+)-cannabidiol had no effect (Fig. 6B).

3.1. Receptor mechanisms: effect of antagonists

All central effects of (+)-7-OH-cannabidiol-DMH (open field activity, catalepsy, hot plate analgesia and hypothermia) were significantly reversed by the cannabinoid CB_1 receptor antagonist SR141716, but not by the cannabinoid CB_2 receptor antagonist SR144528. In the interest of space, these effects are shown for catalepsy and hypothermia only (Fig. 4). The inhibition of defecation induced by (+)-cannabidiol-DMH was almost fully reversed by SR141716 (5 mg/kg; Fig. 7A), but not at all by SR144528 (3 mg/kg; Fig. 7B).

A lower dose of SR144528 (1 mg/kg) also did not prevent intestinal immotility induced by (+)-cannabidiol-DMH (data not shown).

Δ^9-THC, (+)-cannabidiol-DMH or (+)-7-OH-cannabidiol-DMH neither affected central activity (data not shown) nor intestinal motility (peripheral) activity in $CB_1^{-/-}$ receptor-deficient mice (Fig. 7C). In the background strain (C57BL/6), intestinal motility was significantly inhibited by Δ^9-THC and (+)-cannabidiol-DMH (Fig. 7D).

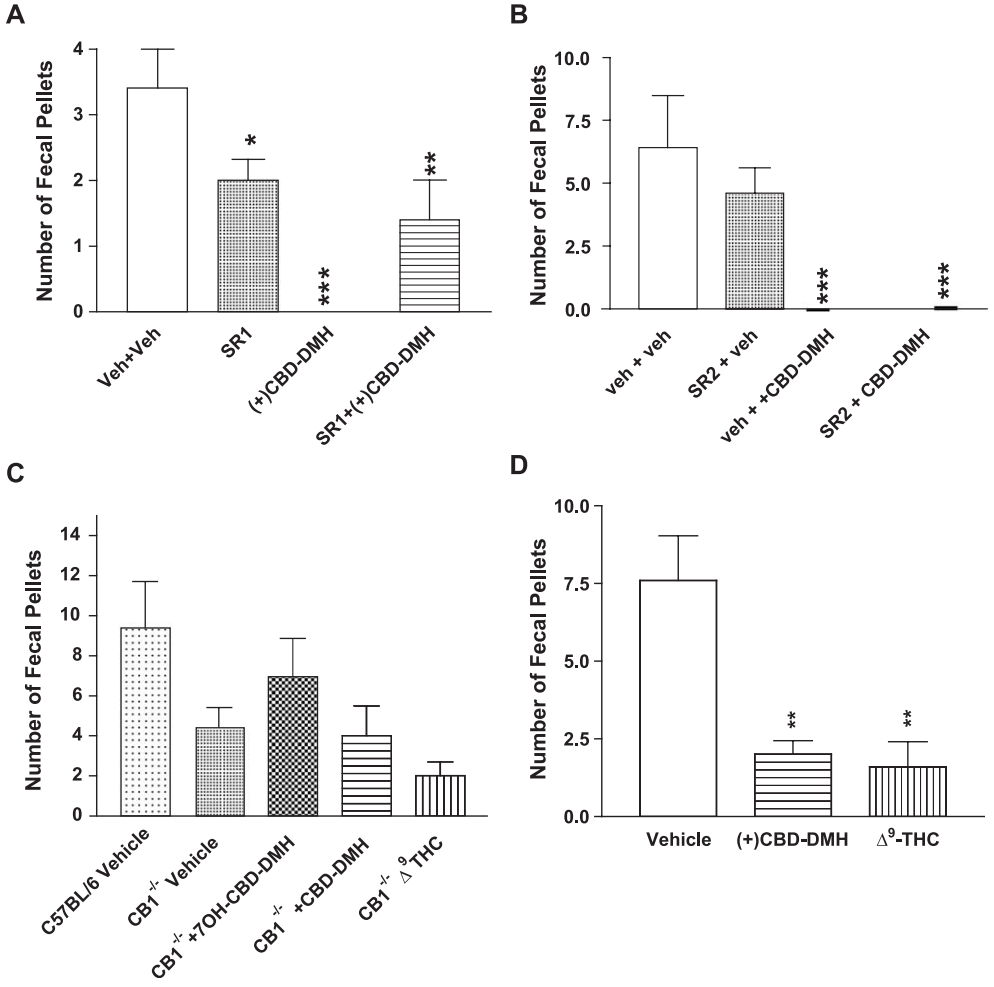

Fig. 7. Cannabinoid CB_1 receptor mediation of the effects of (+)-cannabidiol derivatives on intestinal motility. The cannabinoid CB_1 receptor antagonist SR141716, SR1, 5 mg/kg (A) or SR144528, SR2, 3 mg/kg (B) was injected (i.p.) 30 min before the agonist in female Sabra mice. Sixty minutes after agonist (20 mg/kg) injection, the number of fecal pellets was recorded for 3 h in the home cage. (C) (+)-7-OH-cannabidiol-DMH, (+)-cannabidiol-DMH, Δ^9-THC or vehicle were injected in $CB_1^{-/-}$ receptor knockout mice. Vehicle was also injected in a group of the background C57BL/6 mice. (D) (+)-cannabidiol-DMH, Δ^9-THC or vehicle were injected into CB57BL/6 mice. Results: (A) partial reversal of the effect of (+)-cannabidiol-DMH on intestinal motility by SR1; (B) No reversal effect by SR2; and (C) no significant effect of (+)-cannabidiol-DMH, (+)-7-OH-cannabidiol-DMH or Δ^9-THC on defecation in the $CB_1^{-/-}$ receptor knockout mice, compared to vehicle-treated knockouts. (D) Significant inhibition of Δ^9-THC and (+)-cannabidiol-DMH on intestinal motility. Data are presented as means±S.E.M.'s. Data were analyzed using one-way analysis-of-variance with Newman–Keuls post hoc comparisons. *Different from vehicle+vehicle (*P*<0.05). **Different from vehicle+vehicle (*P*<0.01). ***Different from vehicle+vehicle (*P*<0.001).

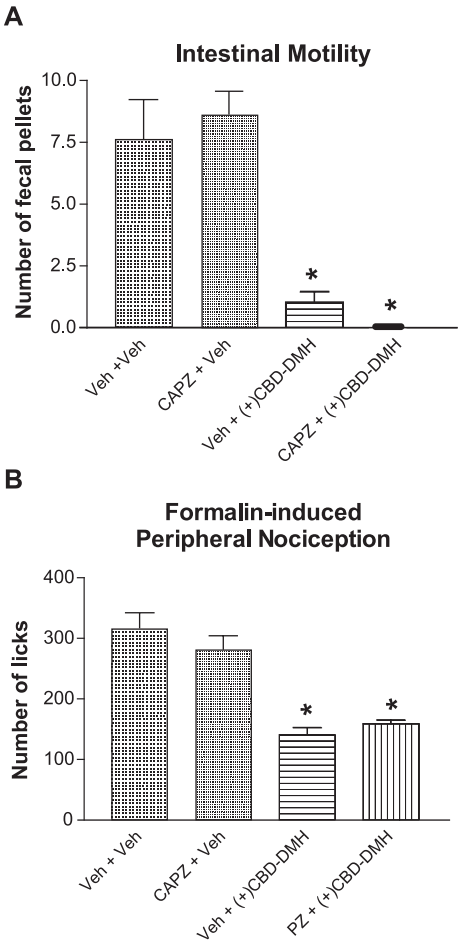

Fig. 8. Lack of reversal by capsazepine of the intestinal and nociceptive effects of (+)-cannabidiol-DMH. Female Sabra mice were injected with capsazepine (30 mg/kg), 30 min before (+)-cannabidiol-DMH (20 mg/kg). Sixty minutes later, (A) the rate of fecal boli (intestinal motility) was recorded for 3 h in the home cage, and (B) the cumulative response to a 4% formalin injection into the hind paw (number of licks) was recorded for 1 h in 5-min intervals. Veh—vehicle, CAPZ—capsazepine. Data are presented as means±S.E.M.'s. Data were analyzed using one-way analysis-of-variance with Newman–Keuls post hoc comparisons. *P<0.001 different from vehicle controls (Veh+Veh).

Although canabidiol analogues did not activate vanilloid VR_1 receptors in in vitro assays (Bisogno et al., 2001), we investigated whether the vanilloid VR_1 receptor antagonist capsazepin inhibited (+)-cannabidiol-DMH-induced effects on peripheral in vivo functions. As can be seen in Fig. 8, capsazepin did not affect (+)-cannabidiol-DMH-induced intestinal immotility (Fig. 8A) and did not antagonize (+)-cannabidiol-DMH-induced analgesia in the formalin test for peripheral pain (Fig. 8B).

4. Discussion

We have tested a series of cannabidiol analogues for central, as well as peripheral, activities in vivo. Central activity was assessed in the "tetrad", which is a series of assays commonly used to measure central cannabimimetic effects (Fride and Sanudo-Pena, 2002; Martin et al., 1991). Three parameters for peripheral activity were used: intestinal motility, archidonic acid-induced inflammation of the external ear and formalin-induced peripheral pain.

Bisogno et al. (2001) reported that the (+)-cannabidiol analogues [(+)-7-OH- cannabidiol-DMH: K_i=2.5±0.03 nM; and (+)-cannabidiol-DMH: K_i=17.4±1.8 nM] have a strong affinity for the cannabinoid CB_1 receptor and more modestly for the cannabinoid CB_2 receptor [(+)-7-OH-cannabidiol-DMH: K_i=44±3.1 nM; (+)-cannabidiol-DMH: K_i=211±23 nM]. We have shown now that additional (+)-cannabidiol analogues also bind cannabinoid CB_1 and CB_2 receptors [(+)-7-OH-cannabidiol, (+)-cannabidiol-COOH, (+)-7-OH-cannabidiol-COOH, see Table 1]. However, we observed significant and consistent central activity only with (+)-7-OH-cannabidiol-DMH. The remaining (+)-cannabidiol derivatives exerted no central effects at all, or, in the case of (+)-cannabidiol-DMH, small, sporadic central effects.

4.1. Peripheral activity

A. Since gastrointestinal transit is regulated locally in the periphery rather than by centrally located cannabinoid CB_1 receptors (Izzo et al., 2000; Landi et al., 2002), *intestinal motility* was used as one parameter of peripheral cannabinoid effects, measured as rates of defecation. All compounds potently inhibited defecation over a prolonged period (3 h) without inducing hypothermia (a measure of central activity), thus excluding a delayed psychoactive effect. We have shown in the present experiments that, at least for (+)-cannabidiol derivatives, central and peripheral effects of cannabinoids analogues can be conveniently distinguished using this paradigm.

B. In vivo inflammatory responsiveness to arachidonic-acid-induced swelling of the external ear (Young et al., 1984; Hanus et al., 1999): a dose of 40 mg/kg or 10 mg/kg of (+)-cannabidiol-DMH almost completely prevented arachidonic-acid-induced inflammation of the external ear similarly to 20 mg/kg of indomethacin. In a separate experiment, (+)-7-OH-cannabidiol-DMH but not (+)-cannabidiol prevented ear inflammation.

C. (+)-Cannabidiol-DMH also completely inhibited the second phase of formalin-induced peripheral pain, in contrast to its lack of activity in the hot plate test, a centrally mediated pain response (Tjolsen et al., 1992).

4.2. Receptor mechanisms

The central effects of (+)-7-OH-cannabidiol-DMH were antagonized by the cannabinoid CB_1 receptor antagonist SR141716. The inhibitory effect of (+)-cannabidiol-DMH on defecation was effectively antagonized by SR141716, but not at all by the cannabinoid CB_2 antagonist (SR144528), suggesting that (+)-cannabidiol-DMH partly

or fully inibited defecation via cannabinoid CB_1 receptors. Supportive of this conclusion, we further observed that, in $CB_1^{-/-}$ receptor knockout mice, none of the compounds tested [(+)-7-OH-cannabidiol-DMH, (+)-cannabidiol-DMH or the positive control Δ^9-THC] had any significant effect on the rate of defecation. SR141716 by itself reduced intestinal motility in Sabra mice. Izzo et al. (1999a,b) reported enhanced defecation rates in rats or mice injected with 1 or 5 mg/kg SR141716. We do not know the reason for this discrepency. However, since we have observed significant strain differences in defecation rates (Fride et al., submitted for publication), we suggest that sex and/or strain differences (we used female Sabra mice; Izzo et al. used male Swiss mice) may explain the inconsistency.

Why then is (+)-cannabidiol-DMH virtually devoid of central effects? Since it is unlikely that this compound does not cross the blood–brain barrier, while its 7-OH-counterpart does, we suggest that (+)-cannabidiol-DMH may have antagonist or partial agonist/antagonist properties in the central nervous system while acting as an agonist in intestinal tissue. Tissue-specific distribution of partial agonist/antagonist properties of the same compound has been thoroughly documented for benzodiazepines and muscarinic ligands (Gardner et al., 1988; Gurwitz et al., 1994; Haefely et al., 1990). We are currently investigating this hypothesis for (+)-cannabidiol-DMH.

Although (+)-7-OH-cannabidiol-DMH and (+)-cannabidiol-DMH bind to cannabinoid CB_2 receptors (Bisogno et al., 2001), the complete lack of antagonism by 1 or 3 mg/kg SR144528 of the effects of the (+)-cannabidiol analogues on defecation excludes mediation by cannabinoid CB_2 receptors. Alternative receptor mechanisms include vanilloid VR_1 receptors. In this study, we have observed however that the vanilloid VR_1 receptor antagonist capsazepine did not affect the inhibition of defecation induced by (+)-cannabidiol-DMH. This is consistent with a previous observation that capsazepine did not affect anandamide-induced intestinal immotility (Izzo et al., 2001). Moreover, (−)- or (+)-cannabidiol, but not cannabidiol analogues, activated vanilloid VR_1 receptors (Bisogno et al., 2001).

Taken together, we have presented evidence for an inhibitory effect on intestinal motility by a series of (+)-cannabidiol analogues, all of which bind cannabinoid CB_1 receptors and to a lesser extent, cannabinoid CB_2 receptors. Our observations indicate that the two analogues, (+)-cannabidiol-DMH and (+)-7-OH-cannabidiol-DMH, inhibit defecation, at least in part, via cannabinoid CB_1 receptors. Furthermore, we have shown the antiinflammatory and analgesic capacity of one of these compounds in the periphery. Except for (+)-7-OH-cannabidiol-DMH, none of the (+)-cannabidiol analogues had consistent central activity.

We suggest that (+)-cannabidiol-DMH, (+)-7-OH- cannabidiol, (+)-COOH- cannabidiol and/or (+)-COOH-cannabidiol-DMH may have partial agonist/antagonist effects in the central nervous system, as opposed to agonist properties in intestinal tissue. In addition, we suggest that the acids may not be able to cross the blood–brain barrier. If correct, this would explain their lack of central effects.

Most of the hydroxy and carboxy derivatives of (+)-cannabidiol are prepared by multistep synthetic procedures (manuscript in preparation). However (+)-cannabidiol-DMH is the result of a facile synthesis and may represent the compound of choice for further pharmaceutical development.

In conclusion, the (+)-cannabidiol analogues, which, as we have demonstrated here, do not have central cannabinoid effects but which have intestine-relaxing and antiinflammatory/peripheral nociception potential, at least in part, via cannabinoid CB_1 receptors, may be developed as cannabinoid-based medicinal drugs for peripheral conditions, such as inflammatory bowel disease, diarrhea and inflammatory pain.

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References

Ben-Shabat, S., Fride, E., Sheskin, T., Tamiri, T., Rhee, M.H., Vogel, Z., Bisogno, T., De Petrocellis, L., Di Marzo, V., Mechoulam, R., 1998. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. Eur. J. Pharmacol. 353, 23–31.

Bisogno, T., Hanus, L., De Petrocellis, L., Tchilibon, S., Ponde, D.E., Brandi, I., Moriello, A.S., Davis, J.B., Mechoulam, R., Di Marzo, V., 2001. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br. J. Pharmacol. 134, 845–852.

Calignano, A., La Rana, G., Makriyannis, A., Lin, S.Y., Beltramo, M., Piomelli, D., 1997. Inhibition of intestinal motility by anandamide, an endogenous cannabinoid. Eur. J. Pharmacol. 340, R7–R8.

Casu, M.A., Porcella, A., Ruiu, S., Saba, P., Marchese, G., Carai, M.A., Reali, R., Gessa, G.L., Pani, L., 2003. Differential distribution of functional cannabinoid CB1 receptors in the mouse gastroenteric tract. Eur. J. Pharmacol. 459, 97–105.

Chesher, G.B., Dahl, C.J., Everingham, M., Jackson, D.M., Marchant-Williams, H., Starmer, G.A., 1973. The effect of cannabinoids on intestinal motility and their antinociceptive effect in mice. Br. J. Pharmacol. 49, 588–594.

Colombo, G., Agabio, R., Lobina, C., Reali, R., Gessa, G.L., 1998. Cannabinoid modulation of intestinal propulsion in mice. Eur. J. Pharmacol. 344, 67–69.

Fride, E., 1995. Anandamides: tolerance and cross-tolerance to delta 9-tetrahydrocannabinol. Brain Res. 697, 83–90.

Fride, E., 2002. Endocannabinoids in the central nervous system—an overview. Prostaglandins Leukot. Essent. Fat. Acids 66, 221–233.

Fride, E., Mechoulam, R., 1993. Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. Eur. J. Pharmacol. 231, 313–314.

Fride, E., Sanudo-Pena, C., 2002. Cannabinoids and endocannabinoids: behavioral and developmental aspects. In: Onaivi, E. (Ed.), The Biology of Marijuana. Harwood Academic Publishers, Reading, pp. 174–204.

Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G., Casellas, P., 1995. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur. J. Biochem. 232, 54–61.

Gardner, A.L., Darroch, S.A., Choo, L.K., Mitchelson, F., 1988. The effect of some selective agonists and antagonists on peripheral muscarinic receptors. Trends Pharmacol. Sci., 40–43 (Suppl).

Griffin, G., Fernando, S.R., Ross, R.A., McKay, N.G., Ashford, M.L., Shire, D., Huffman, J.W., Yu, S., Lainton, J.A., Pertwee, R.G., 1997. Evidence for the presence of CB2-like cannabinoid receptors on peripheral nerve terminals. Eur. J. Pharmacol. 339, 53–61.

Gurwitz, D., Haring, R., Heldman, E., Fraser, C.M., Manor, D., Fisher, A., 1994. Discrete activation of transduction pathways associated with acetylcholine m1 receptor by several muscarinic ligands. Eur. J. Pharmacol. 267, 21–31.

Haefely, W., Martin, J.R., Schoch, P., 1990. Novel anxiolytics that act as partial agonists at benzodiazepine receptors. Trends Pharmacol. Sci. 11, 452–456.

Hanus, L., Breuer, A., Tchilibon, S., Shiloah, S., Goldenberg, D., Horowitz, M., Pertwee, R.G., Ross, R.A., Mechoulam, R., Fride, E., 1999. HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. Proc. Natl. Acad. Sci. U. S. A. 96, 14228–14233.

Herkenham, M., 1995. Localization of cannabinoid receptors in brain and periphery. In: Pertwee, R.G. (Ed.), Cannabinoid Receptors. Academic Press, London, pp. 145–166.

Izzo, A.A., Mascolo, N., Borrelli, F., Capasso, F., 1999a. Defaecation, intestinal fluid accumulation and motility in rodents: implications of cannabinoid CB1 receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 359, 65–70.

Izzo, A.A., Mascolo, N., Pinto, L., Capasso, R., Capasso, F., 1999b. The role of cannabinoid receptors in intestinal motility, defaecation and diarrhoea in rats. Eur. J. Pharmacol. 384, 37–42.

Izzo, A.A., Pinto, L., Borrelli, F., Capasso, R., Mascolo, N., Capasso, F., 2000. Central and peripheral cannabinoid modulation of gastrointestinal transit in physiological states or during the diarrhoea induced by croton oil. Br. J. Pharmacol. 129, 1627–1632.

Izzo, A.A., Capasso, R., Pinto, L., Di Carlo, G., Mascolo, N., Capasso, F., 2001. Effect of vanilloid drugs on gastrointestinal transit in mice. Br. J. Pharmacol. 132, 1411–1416.

Landi, M., Croci, T., Rinaldi-Carmona, M., Maffrand, J.P., Le Fur, G., Manara, L., 2002. Modulation of gastric emptying and gastrointestinal transit in rats through intestinal cannabinoid CB(1) receptors. Eur. J. Pharmacol. 450, 77–83.

Malfait, A.M., Gallily, R., Sumariwalla, P.F., Malik, A.S., Andreakos, E., Mechoulam, R., Feldmann, M., 2000. The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. Proc. Natl. Acad. Sci. U. S. A. 97, 9561–9566.

Martin, B.R., Compton, D.R., Thomas, B.F., Prescott, W.R., Little, P.J., Razdan, R.K., Johnson, M.R., Melvin, L.S., Mechoulam, R., Ward, S.J., 1991. Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. Pharmacol. Biochem. Behav. 40, 471–478.

Mechoulam, R., Hanus, L., Fride, E., 1998. Towards cannabinoid drugs—revisited. Prog. Med. Chem. 35, 199–243.

Parker, L.A., Mechoulam, R., Schlievert, C., 2002. Cannabidiol, a non-psychoactive component of cannabis and its synthetic dimethylheptyl homolog suppress nausea in an experimental model with rats. Neuro-Report 13, 567–570.

Pertwee, R.G., 1997. Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol. Ther. 74, 129–180.

Pertwee, R.G., 2001. Cannabinoids and the gastrointestinal tract. Gut 48, 859–867.

Pinto, L., Capasso, R., Di Carlo, G., Izzo, A.A., 2002. Endocannabinoids and the gut. Prostaglandins Leukot. Essent. Fat. Acids 66, 333–341.

Rhee, M.H., Vogel, Z., Barg, J., Bayewitch, M., Levy, R., Hanus, L., Breuer, A., Mechoulam, R., 1997. Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylylcyclase. J. Med. Chem. 40, 3228–3233.

Robson, P., 2001. Therapeutic aspects of cannabis and cannabinoids. Br. J. Psychiatry 178, 107–115.

Tjolsen, A., Hole, K., 1997. Animal models of analgesia. In: Dickenson, L., Bessing, J. (Eds.), The Pharmacology of Pain. Springer, Heidelberg, pp. 1–20.

Tjolsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H., Hole, K., 1992. The formalin test: an evaluation of the method. Pain 51, 5–17.

Walter, L., Franklin, A., Witting, A., Wade, C., Xie, Y., Kunos, G., Mackie, K., Stella, N., 2003. Nonpsychotropic cannabinoid receptors regulate microglial cell migration. J. Neurosci. 23, 1398–1405.

Young, J.M., Spires, D.A., Bedord, C.J., Wagner, B., Ballaron, S.J., De Young, L.M., 1984. The mouse ear inflammatory response to topical arachidonic acid. J. Invest. Dermatol. 82, 367–371.