

The role of cannabinoid receptors in intestinal motility, defaecation and diarrhoea in rats

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Abstract

We have studied the effects of the cannabinoid receptor agonists (*R*)-(+)[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN 55,212-2, 0.3–5 mg/kg, i.p.) and (–)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol (CP 55,940, 0.03–1 mg/kg, i.p.), the cannabinoid CB₁ receptor antagonist (*N*-piperidin-1-yl)-5-(4-chlorophenyl)-1-2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (SR141716A, 0.3–5 mg/kg, i.p.) and the cannabinoid CB₂ receptor antagonist *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 (SR144528, 1 mg/kg, i.p.) on intestinal motility, defaecation and castor-oil (1 ml/100 g rat, orally)-induced diarrhoea in the rat. SR141716A, but not SR144528, increased defaecation and upper gastrointestinal transit, while WIN 55,212-2 and CP 55,940 decreased upper gastrointestinal transit but not defaecation. WIN 55,212-3 (5 mg/kg), the less active enantiomer of WIN 55,212-2, was without effect. A per se non-effective dose of SR141716A (0.3 mg/kg), but not of SR144528 (1 mg/kg) or the opioid receptor antagonist, naloxone (2 mg/kg i.p.), counteracted the inhibitory effect of both WIN 55,212-2 (1 mg/kg) and CP 55,940 (0.1 mg/kg) on gastrointestinal motility. WIN 55,212-2 did not modify castor-oil-induced diarrhoea, while CP 55,940 produced a transient delay in castor-oil-induced diarrhoea at the highest dose tested (1 mg/kg), an effect counteracted by SR141715A (5 mg/kg). These results suggest that (i) intestinal motility and defaecation could be tonically inhibited by the endogenous cannabinoid system, (ii) exogenous activation of cannabinoid CB₁ receptors produces a reduction in intestinal motility in the upper gastrointestinal tract but not in defaecation, (iii) endogenous or exogenous activation of cannabinoid CB₂ receptors does not affect defaecation or intestinal motility and (iv) the cannabinoid receptor agonist, CP 55,940, possesses a weak and transient antidiarrhoeal effect while the cannabinoid receptor agonist, WIN 55,212-2, does not possess antidiarrhoeal activity. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cannabinoid receptors are the molecular targets of hashish and marijuana, the drugs of abuse obtained from *Cannabis sativa* L. (Cannabaceae). Two classes of cannabinoid receptors, named CB₁ and CB₂, have been identified (Pertwee, 1997). The cannabinoid CB₁ receptor occurs in the brain (Matsuda et al., 1990), where it is responsible for psychoactive effects of cannabis and in certain peripheral tissues (Gérard et al., 1991; Kaminski et al., 1992; Schlicker et al., 1996), whereas the cannabinoid

CB₂ receptor is present outside the nervous system (Munro et al., 1993), mostly in cells of the immune system, presumably mediating cannabinoid-induced immunosuppression and possibly also antiinflammatory effects (Pertwee, 1997). The guinea-pig myenteric plexus-longitudinal muscle preparation contains cannabinoid binding sites that closely resemble cannabinoid CB₁ receptors in the guinea-pig brain (Ross et al., 1998).

Results from functional experiments have led to the conclusion that activation of cannabinoid CB₁ receptors can mediate the inhibition of electrically evoked contractions in the guinea-pig (Pertwee et al., 1996; Izzo et al., 1998b) and human ileum (Crocì et al., 1998), while the selective cannabinoid CB₁ receptor antagonist, (*N*-piperidin-1-yl)-5-(4-chlorophenyl)-1-2,4-dichlorophenyl)-4-me-

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thyl-1*H*-pyrazole-3-carboxamide (SR141716A), potentiates excitatory transmission in the guinea-pig ileum (Pertwee et al., 1996; Izzo et al., 1998b) but not in the human ileum (Crocì et al., 1998).

Cannabinoids also modulate intestinal motility in vivo. Activation of cannabinoid CB₁ receptors inhibits while blockade of cannabinoid CB₁ receptors increases defaecation (Izzo et al., 1999) and upper gastrointestinal transit in mice (Calignano et al., 1997; Colombo et al., 1998; Izzo et al., 1999). From the above, it appears that the role of cannabinoid receptors in intestinal motility in vivo has been studied exclusively in the mouse. The present study was performed to investigate the role of cannabinoid receptors in intestinal motility and defaecation in the rat. In addition, since marijuana is a traditional remedy to treat diarrhoea (Grinspoon and Bakalar, 1993), the potential antidiarrhoeal activity of cannabinoids was also evaluated. For these purposes, the cannabinoid receptor agonists, (*R*)-(+)[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenyl-methanone (WIN 55,212-2) and (–)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol (CP 55,940) (Compton et al., 1992; Pertwee, 1997), the cannabinoid CB₁ receptor antagonist, SR141716A (Rinaldi-Carmona et al., 1995), and the cannabinoid CB₂ receptor antagonist, *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 (SR144528) (Rinaldi-Carmona et al., 1998), were used. Antidiarrhoeal activity was studied using the castor-oil test. This test has been used extensively in several laboratories as a basic pharmacological test to screen and evaluate antidiarrhoeal drugs.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Fondazione “Mario Negri” Sud, Imbaro, Chieti), weighing 170–200 g were used after 1 week of adaptation to the housing conditions (23 ± 2°C; 60% humidity). The animals were deprived of food 3 h before the experiments on faecal excretion and 12 h before the experiments on intestinal motility and diarrhoea. All animal experiments complied with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC).

2.2. Upper gastrointestinal transit

Upper gastrointestinal transit was assessed according to the method described by Mascolo et al. (1993). WIN 55,212-2 (0.3–5 mg/kg), CP 55,940 (0.03–1 mg/kg), SR141716A (0.3–5 mg/kg), SR144528 (1 mg/kg) or

loperamide (1 mg/kg) was administered [in some experiments the cannabinoid receptor agonists (or loperamide) was given immediately after administration of SR 141716A (0.3 mg/kg) or SR141716A (1 mg/kg) or naloxone (2 mg/kg)] i.p. 30 min before the oral administration of the marker (10% charcoal suspension in 5% gum arabic, 1 ml/100 g). After 20 min the rats were killed by asphyxiation with CO₂ and the gastrointestinal tract was removed. The distance travelled by the marker was measured and expressed as a percentage of the total length of the small intestine from pylorus to caecum.

2.3. Faecal excretion

Faecal excretion was studied as previously described by Crocì and Bianchetti (1992). On the day of the experiment, the animals were placed individually in grid-floor cages, with food and water ad libitum, and were randomly allocated to different treatments. Food was withdrawn at 0900 h and 3 h later the animals were given (i.p.) graded doses of WIN 55,212-2 (1 and 5 mg/kg), CP 55,940 (1 mg/kg) or SR 141716A (0.3–5 mg/kg), SR144528 (1 mg/kg) or loperamide (5 mg/kg). The pellets discharged 1, 3, 5 and 7 h after drugs administration were collected and weighed immediately (wet weight) and after drying (24 h at 50°C). Effects on secretion or reabsorption of fluids were assessed from the ratio of wet to dry faecal weights 7 h after drug administration.

2.4. Castor-oil-induced diarrhoea

WIN 55,212-2 (1 and 5 mg/kg), CP 55,940 (0.1–1 mg/kg) SR141716A (5 mg/kg), SR144528 (1 mg/kg) or loperamide (5 mg/kg) was administered (i.p.) 30 min before castor oil (1 ml/100 g, orally). The pellets discharged 1, 3, 5 and 7 h after castor oil were collected and weighed immediately (wet weight) and after drying (24 h at 50°C). The incidence of diarrhoea was assessed (by an observer unaware of the particular treatment) as the number of animals producing the characteristic wet diarrhoeal droppings. In some experiments CP 55,940 (1 mg/kg) was given immediately after SR 141716A (1 and 5 mg/kg) or SR144528 (1 mg/kg).

2.5. Drugs

Drugs used were: castor oil, dimethyl sulfoxide (DMSO), loperamide hydrochloride, naloxone hydrochloride (Sigma, Milan, Italy), WIN 55,212-2 mesylate, WIN 55,212-3 mesylate (RBI, Milan, Italy), CP 55,940 (Tocris, Bristol, UK), SR141716A and SR144528 were a gift from Dr. Madeleine Mossè and Dr. Francis Barth (SANOFI-Recherche, Montpellier, France). WIN 55,212-2, CP 55,940, SR141716A and SR144528 were dissolved in DMSO, loperamide and naloxone in saline. DMSO (0.05 ml/rat) had no effect on the responses under study.

2.6. Statistics

The chi-square test was used to determine the significance of differences between groups with or without diarrhoea. Defaecation and upper gastrointestinal transit were expressed as means $\pm$ S.E.M. and compared using Student's *t*-test or analysis of variance followed by the Tukey–Kramer multiple comparisons test. A *P* value less than 0.05 was considered significant.

3. Results

3.1. Upper gastrointestinal transit

Administration of the cannabinoid CB₁ receptor antagonist, SR141716A (0.3–5 mg/kg), dose-dependently enhanced upper gastrointestinal transit (Fig. 1). This effect was significant (*P* < 0.05) for the 1–5 mg/kg doses. In contrast, WIN 55,212-2 and CP 55,940 significantly inhibited intestinal motility from 1 mg/kg and 0.1 mg/kg onward respectively and this effect was dose-related (Fig. 1). WIN 55,212-3, the less active isomer of WIN 55,212-2, did not significantly affect intestinal motility (% transit: control 65 ± 5 , WIN 55,212-3 63 ± 4 , *n* = 8, *P* > 0.2). Pretreatment of rats with SR141716A (0.3 mg/kg) per se did not significantly alter intestinal motility, but counteracted the inhibitory effect of WIN 55,212-2 (1 mg/kg) and CP 55,940 (0.1 mg/kg) (Fig. 2). The cannabinoid CB₂ receptor antagonist, SR144528 (1 mg/kg), itself neither significantly affected intestinal motility (% transit: control 68 ± 4 , SR144528 64 ± 3 , *n* = 12, *P* > 0.2) nor counteracted the inhibitory effect of WIN 55,212-2 and CP 55,940 on intestinal motility (Fig. 2). Loperamide (1 mg/kg) also significantly (*P* < 0.05) delayed upper gastrointestinal transit ($50 \pm 6\%$ inhibition, *n* = 6) and this effect was unchanged after pretreatment with SR141716A

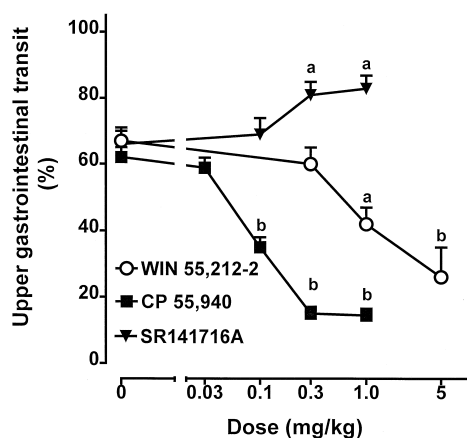


Fig. 1. Effect of WIN 55,212-2 (0.3–5 mg/kg, i.p.), CP 55,940 (0.03–1 mg/kg, i.p.) and SR141716A (0.3–5 mg/kg, i.p.) on upper gastrointestinal transit in rats. Results are means $\pm$ S.E.M. for 12 animals for each experimental group. ^a*P* < 0.05 and ^b*P* < 0.01 vs. control.

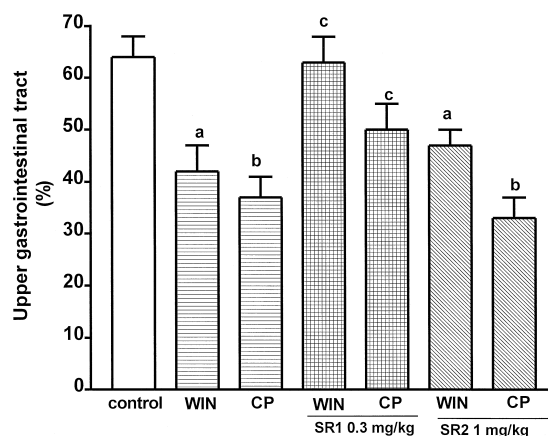


Fig. 2. Effect of WIN 55,212-2 (WIN, 1 mg/kg, i.p.) and CP 55,940 (CP, 0.1 mg/kg) alone or in combination with SR141716A (SR1, 0.3 mg/kg, i.p.) or SR144528 (SR2, 1 mg/kg, i.p.) on upper gastrointestinal transit in rats. Results are means $\pm$ S.E.M. for six animals for each experimental group. ^a*P* < 0.05 vs. control and ^b*P* < 0.01 vs. control; ^c*P* < 0.05 vs. WIN 1 mg/kg (or CP 0.3 mg/kg).

($48 \pm 5\%$ inhibition, *n* = 6). Naloxone (2 mg/kg), per se, did not modify intestinal motility, but counteracted the inhibitory effect of loperamide (data not shown). However naloxone (2 mg/kg) was not able to modify the inhibitory effect of WIN 55,212-2 1 mg/kg (% transit: control 66 ± 5 , WIN 55,212-2 38 ± 5 , WIN 55,212-2 + naloxone 37 ± 4 , *n* = 8).

3.2. Faecal excretion

Table 1 shows the effect of the i.p.-injected cannabinoid drugs and loperamide on faecal excretion for a period of 7 h. The cannabinoid CB₁ receptor antagonist, SR141716A (0.3–1 mg/kg), increased significantly (*P* < 0.05–0.01) defaecation, starting from the third hour (Table 1). However, neither the cannabinoid CB₂ receptor antagonist, SR144528 (1 mg/kg), nor the cannabinoid receptor agonists, WIN 55,212-2 (1 and 5 mg/kg) and CP 55,940, modified defaecation 1–7 h after their administration (Table 1). In contrast, pretreatment of rats with loperamide (5 mg/kg) completely prevented defaecation (Fig. 3). Faecal water content (indicated by the ratio of wet to dry weight) was also significantly (*P* < 0.05) increased 7 h after SR141716A administration, but was unchanged by WIN 55,212-2 (1 and 5 mg/kg), CP 55,940 (1 mg/kg) or by SR144528 (1 mg/kg).

3.3. Castor-oil-induced diarrhoea

Castor oil (1 ml/100 g) produced diarrhoea that lasted at least 7 h in 83% of the rats (Fig. 3a). One hour after castor-oil administration diarrhoea was evident in 83% animals and 3 h after castor-oil administration, all rats produced copious diarrhoea (Fig. 3a). The cannabinoid receptor agonist WIN 55,212-2 did not alter significantly

Table 1

Effect of SR141716A (0.3–5 mg/kg, i.p.), SR144528 (1 mg/kg, i.p.), WIN 55,212-2 (1 and 5 mg/kg, i.p.), CP 55,940 (1 mg/kg, i.p.) or loperamide (5 mg/kg, i.p.) on faecal excretion in rats. Results are means $\pm$ SEM for 10–12 rats for each experimental group

Treatment	Faecal wet weight (g) at various times (h) after drug administration				Wet/dry weight ^a of faeces
	1	3	5	7	
Control	0.06 $\pm$ 0.06	0.16 $\pm$ 0.14	0.57 $\pm$ 0.23	1.30 $\pm$ 0.25	1.62 $\pm$ 0.11
SR141716A (mg/kg)					
0.3	0.39 $\pm$ 0.24	0.55 $\pm$ 0.78	1.07 $\pm$ 0.52	1.43 $\pm$ 0.50	1.694 $\pm$ 0.12
1	0.22 $\pm$ 0.22	1.13 $\pm$ 0.30*	1.71 $\pm$ 0.38*	2.30 $\pm$ 0.24**	2.21 $\pm$ 0.09**
5	0.75 $\pm$ 0.66	1.24 $\pm$ 0.26*	1.70 $\pm$ 0.40**	2.12 $\pm$ 0.18**	2.40 $\pm$ 0.10**
SR1445.28 (1 mg/kg)	0.00 $\pm$ 0.00	0.06 $\pm$ 0.06	0.16 $\pm$ 0.16	0.94 $\pm$ 0.22	1.55 $\pm$ 0.36
WIN 55,212-2 (mg/kg)					
1	0.10 $\pm$ 0.10	0.10 $\pm$ 0.10	0.42 $\pm$ 0.12	1.00 $\pm$ 0.36	1.50 $\pm$ 0.35
5	0.14 $\pm$ 0.05	0.20 $\pm$ 0.06	0.53 $\pm$ 0.11	1.12 $\pm$ 0.20	1.63 $\pm$ 0.35
CP 55,940 (1 mg/kg)	0.09 $\pm$ 0.04	0.12 $\pm$ 0.08	0.32 $\pm$ 0.15	0.81 $\pm$ 0.28	1.55 $\pm$ 0.27
Loperamide (5 mg/kg)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00**	0.00 $\pm$ 0.00*	–

^a 7 h after drug administration.

* $P < 0.01$ vs. control.

** $P < 0.05$ vs. control.

the percentage of rats with diarrhoea after castor oil administration (Fig. 3a). In addition, WIN 55,212-2 did not significantly affect the faecal water content (Fig. 3b) associated with castor-oil administration during the entire period considered. However the cannabinoid receptor agonist, CP 55,940 (0.1–1 mg), delayed the onset of diarrhoea

(Fig. 3a). The effect of CP 55,940 on the percentage of rats with diarrhoea (Fig. 3a) and on faecal water content (Fig. 3b) was significant at the first hour and for the highest dose tested (1 mg/kg). No significant differences were observed for the 3–7 h period. One hour after castor-oil administration, the effect of CP 55,940 1 mg/kg on the percentage of rats with diarrhoea (Fig. 4) and on faecal water content associated with castor oil administration (data not shown) was counteracted by SR141716A (1 and 5 mg/kg) but not by SR144528 (1 mg/kg).

Loperamide (5 mg/kg) completely prevented the diarrhoea caused by castor-oil administration (Fig. 3). Neither SR141716A (5 mg/kg) nor SR144528 (1 mg/kg) modified castor-oil-induced diarrhoea ($n = 6$ for each drug, data not shown).

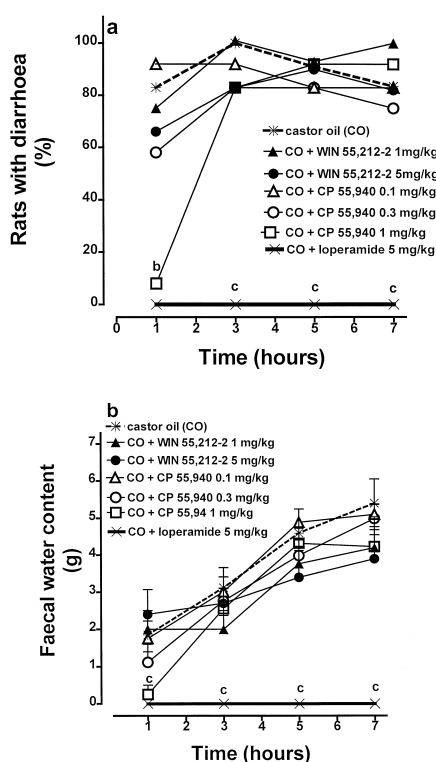


Fig. 3. Effect of WIN 55,212-2 (1 and 5 mg/kg, i.p.), CP 55,940 (0.1–1 mg/kg, i.p.) or loperamide (5 mg/kg, i.p.) on the percentage of rats with diarrhoea (a) and on faecal water content (b) at various times (hours) after castor oil administration (1 ml/100 g rat, orally). Twelve animals were used for each experimental group. ^b $P < 0.01$ and ^c $P < 0.001$ vs. castor oil.

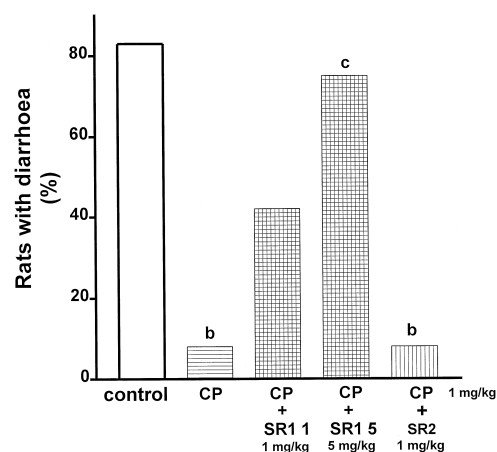


Fig. 4. Effect of CP 55,940 (CP, 1 mg/kg) alone or in combination with SR141716A (SR1, 1 and 5 mg/kg) or SR144528 (SR2, 1 mg/kg) on the percentage of rats with diarrhoea 1 h after castor oil administration. Twelve animals were used for each experimental group. ^b $P < 0.01$ vs. control and ^c $P < 0.05$ vs. CP.

4. Discussion

Recent findings indicate that cannabinoid CB₁ receptors can modulate intestinal motility, based on the findings that cannabinoid receptor agonists are able to depress intestinal motility both *in vitro* and *in vivo* and that these effects are counteracted by SR141716A, a specific cannabinoid CB₁ receptor antagonist (Pertwee et al., 1996; Colombo et al., 1998; Izzo et al., 1998b, 1999). In the mouse, activation of cannabinoid CB₁ receptors decreases, while blockade of cannabinoid CB₁ receptors increases, upper gastrointestinal transit and defaecation (Izzo et al., 1999). We now report evidence that cannabinoids also modulate upper gastrointestinal transit and defaecation in the rat, but some differences in the control of defaecation can be observed between rats and mice. In addition, the supposed antidiarrhoeal activity of cannabinoids was only partly confirmed in the present study.

The present results indicate that activation of cannabinoid CB₁ receptors inhibits intestinal motility in the upper gastrointestinal tract. Indeed (i) the cannabinoid receptor agonists, WIN 55,212-2 and CP 55,940, but not WIN 55,212-3, the less active enantiomer of WIN 55,212-2, delayed upper gastrointestinal transit in a dose-dependent fashion and (ii) the selective cannabinoid CB₁ receptor antagonist SR141716A, at a dose inactive per se, counteracted the effect of the two cannabinoid agonists. In addition, the effect of WIN 55,212-2 was not modified by naloxone, indicating that the inhibitory effect of WIN 55,212-2 was not due to an action on opioid receptors. Similar results have been observed in the isolated guinea-pig ileum (Pertwee et al., 1996; Izzo et al., 1998b).

SR141716A increased marker transit in the rat upper gastrointestinal tract. This indicates that either SR141716A is antagonizing an endogenous cannabinoid agonist which acts on cannabinoid CB₁ receptors or that it is an inverse agonist. Indeed, although two endogenous cannabinoid ligands (anandamide and 2-arachidonylglycerol) have been isolated (Devane et al., 1992; Mechoulam et al., 1995), it has been shown that SR141716A behaves as an inverse agonist at human recombinant cannabinoid CB₁ and cannabinoid CB₂ receptors (Landsman et al., 1997; MacLennan et al., 1998), and this property might explain why the drug causes effects opposite to those of cannabinoid agonists.

SR141716A also increased faecal output in rats, suggesting a modulatory role of cannabinoid CB₁ receptors in defaecation in rats. The induction of faecal excretion was accompanied by an increase in faecal water content (faecal wet/dry weight significantly higher in SR141716A-treated rats). These observations suggest that SR141716A may induce faecal excretion by either or both of the following mechanisms: increased intestinal motility and altered exchange of fluid from gut and lumen. Consistent with this, SR141716A increased intestinal secretion (Izzo et al., 1999) and motility (present results) in rats. The ability of

SR141716A to increase defaecation in the mouse has been recently reported (Izzo et al., 1999). However, in contrast to effects in the mouse, in the present study we have shown that the cannabinoid receptor agonists, WIN 55,212-2 and CP 55,940, did not inhibit defaecation in the rat. The reason for this discrepancy is, at present, not clear. A possible explanation is that, in physiological states, defaecation is maximally inhibited by the endogenous cannabinoid system and therefore further addition of exogenous cannabinoids does not produce further inhibition.

Although cannabinoid CB₂ receptors are not present in the guinea-pig ileal myenteric neurons, they could mediate cannabinoid-induced changes in intestinal motility as CB₂-like cannabinoid receptor mRNA, possibly derived from resident macrophages and/or other immune cells (Munro et al., 1993), was detected in the guinea-pig whole gut (Griffin et al., 1997). In addition, cannabinoid CB₂ receptors modulate intestinal motility in the isolated porcine ileum (Albasan et al. 1999). However it is unlikely that cannabinoid CB₂ receptors play a significant role in regulating intestinal motility as the selective cannabinoid CB₂ receptor antagonist, SR144528, at doses previously shown to bind the cannabinoid CB₂ receptor in the spleen (Rinaldi-Carmona et al., 1998), neither modified the inhibitory effect of WIN 55,212-2 or CP 55,940 on upper gastrointestinal transit nor affected, per se, intestinal motility and defaecation.

Several anecdotal reports suggest the efficacy of marijuana in alleviating dysentery (Grinspoon and Bakalar, 1993). Howlett (1995) mentioned the treatment of diarrhoea among potential therapeutic applications of cannabinoids. WIN 55,212-2 is a cannabinoid receptor agonist with an aminoalkylindole structure able to inhibit intestinal secretion (Izzo et al., 1999) and motility (present results) in the rat, and antidiarrhoeal agents have antisecretory and/or antimotility effects. In the light of these observations, it was to be expected that the cannabinoid receptor agonist, WIN 55,212-2, could possess antidiarrhoeal activity. This assumption, testing of which was one of the main purposes of the present work, was not confirmed in the present study, as WIN 55,212-2 was not able to prevent the diarrhoea and faecal water content associated with castor oil administration. In contrast, CP 55,940, which is an analogue of Δ^9 -tetrahydrocannabinol that lacks a pyran ring (Pertwee, 1997), had an antidiarrhoeal effect that lasted 1 h, while the opioid loperamide, used as a positive control, completely prevented the diarrhoea for the entire period considered. However it should be noted that the dose of CP 55,940 (1 mg/kg) able to delay significantly castor-oil-induced diarrhoea was tenfold higher than the minimal dose of CP 55,940 (0.1 mg/kg) able to reduce significantly intestinal motility. It is unlikely that the transient effect produced by CP 55,940 was due to a loss of activity (i.e., metabolic degradation) as CP 55,940 (0.1 mg/kg) produced pharmacological effects for up to 24 h following its administration (McGregor et al., 1996).

Consistent with the results on intestinal motility, the antidiarrhoeal effect of CP 55,940 was mediated by the cannabinoid CB₁, but not the cannabinoid CB₂ receptor, as it was counteracted by SR141716A but not by SR144528. Previous studies have shown that the diarrhoea induced by castor oil is reduced or prevented by several drugs affecting intestinal motility and/or secretion including α_2 -adrenoceptor agonists, cyclooxygenase inhibitors, platelet activating factor receptor antagonists, tachykinin NK₁ and NK₂ receptor antagonists and nitric oxide synthase inhibitors (Izzo et al., 1998a).

In conclusion, we suggest that endogenous activation of cannabinoid CB₁, but not cannabinoid CB₂ receptors, has a negative effect on intestinal motility and defaecation in the rat. Exogenous activation of cannabinoid CB₁ receptors inhibits intestinal motility but not defaecation. In addition using the castor oil test, we have shown that the cannabinoid receptor agonist, WIN 55,212-2, does not possess antidiarrhoeal activity, while the cannabinoid receptor agonist, CP 55,940, produced a weak and transient antidiarrhoeal effect.

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References

- Albasan, H., Soldani, G., Brown, D.R., 1999. Synthetic cannabinoids potentiate electrically-induced contractions in porcine ileum in vitro. *Pharm. Res.* 39, 5, (Supplement).
- Calignano, A., La Rana, G., Makryannis, A., Lin, S.Y., Beltramo, M., Piomelli, D., 1997. Inhibition of intestinal motility by anandamide, an endogenous cannabinoid. *Eur. J. Pharmacol.* 340, R7–R8.
- 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.
- Compton, D.R., Gold, L.H., Ward, S.J., Balster, R.L., Martin, B.R., 1992. Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from Δ^9 -tetrahydrocannabinol. *J. Pharmacol. Exp. Ther.* 263, 1118–1126.
- Croci, T., Bianchetti, A., 1992. Stimulation of faecal excretion in rats by α -adrenergic antagonist. *J. Pharm. Pharmacol.* 121, 1557–1566.
- Croci, T., Manara, L., Aureggi, G., Guagnini, F., Rinaldi-Carmona, M., Maffrand, J.P., Le Fur, G., Mukenge, S., Ferla, G., 1998. In vitro functional evidence of neuronal cannabinoid CB₁ receptors in human ileum. *Br. J. Pharmacol.* 125, 1393–1396.
- Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258, 1946–1949.
- Gérard, C.M., Mollereau, C., Vassart, G., Parmentier, M., 1991. Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem. J.* 279, 129–134.
- Griffin, G., Fernando, S.R., Ross, R.A., McKay, N.G., Ashford, M.L.J., Shire, D., Huffman, J.W., Yu, S., Lainton, J.A.H., Pertwee, R.G., 1997. Evidence for the presence of CB₂-like cannabinoid receptors on peripheral nerve terminals. *Eur. J. Pharmacol.* 339, 53–61.
- Grinspoon, L., Bakalar, J.B., 1993. Marijuana: The Forbidden Medicine. Yale University Press, Yale, CT.
- Howlett, A.C., 1995. Pharmacology of cannabinoid receptors. *Annu. Rev. Pharmacol. Toxicol.* 35, 607–634.
- Izzo, A.A., Gaginella, T.S., Mascolo, N., Capasso, F., 1998a. Recent findings on the mode of action of laxatives: the role platelet activating factor and nitric oxide. *Trends Pharmacol. Sci.* 19, 403–405.
- Izzo, A.A., Mascolo, N., Borrelli, F., Capasso, F., 1998b. Excitatory transmission to the circular muscle of the guinea-pig ileum: evidence for the involvement of cannabinoid CB₁ receptor. *Br. J. Pharmacol.* 124, 1363–1368.
- Izzo, A.A., Mascolo, N., Borrelli, F., Capasso, F., 1999. Defaecation, intestinal fluid accumulation and motility in rodents: implications of cannabinoid CB₁ receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 359, 65–70.
- Kaminski, N.E., Abood, M., Kessler, F.K., Martin, B.R., Schatz, A.R., 1992. Identification of a functionally relevant cannabinoid receptor on mouse spleen cells that is involved in cannabinoid-mediated immune modulation. *Mol. Pharmacol.* 42, 736–742.
- Landsman, R.S., Burkey, T.H., Consroe, P., Roeske, W.R., Yamamura, H.I., 1997. SR14171A is an inverse agonist at the human cannabinoid CB₁ receptors. *Eur. J. Pharmacol.* 334, R1–R2.
- MacLennan, S.J., Reynen, P.H., Bonhaus, D.W., 1998. Evidence for inverse agonism of SR141716A at human recombinant cannabinoid CB₁ and CB₂ receptors. *Br. J. Pharmacol.* 124, 619–622.
- Mascolo, N., Izzo, A.A., Barbato, F., Capasso, F., 1993. Inhibitors of nitric oxide synthetase prevent castor-oil-induced diarrhoea in the rat. *Br. J. Pharmacol.* 108, 861–864.
- Matsuda, L.A., Lolait, S.J., Brownstein, B.J., Young, A.C., Bonner, T.L., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346, 561–564.
- McGregor, I.S., Issikidis, C.N., Prior, G., 1996. Aversive effects of the synthetic cannabinoid CP 55,940 in rats. *Pharmacol. Biochem. Behav.* 53, 657–664.
- Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffin, G., Bayewitch, M., Barg, J., Vogel, Z., 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to a cannabinoid receptor. *Biochem. Pharmacol.* 50, 83–90.
- Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365, 61–65.
- Pertwee, R.G., 1997. Pharmacology of cannabinoid CB₁ and CB₂ receptors. *Pharmacol. Ther.* 74, 129–180.
- Pertwee, R.G., Fernando, S.R., Nash, J.E., Coutts, A.A., 1996. Further evidence for the presence of cannabinoid CB₁ receptors in guinea-pig small intestine. *Br. J. Pharmacol.* 118, 2199–2205.
- Rinaldi-Carmona, M., Barth, F., Heaulme, M., Alonso, R., Shire, D., Congy, C., Soubrie, P., Breliere, J.C., Le Fur, G., 1995. Biochemical and pharmacological characterization of SR141716A, the first potent and selective brain cannabinoid receptor antagonist. *Life Sci.* 56, 1941–1947.
- Rinaldi-Carmona, M., Barth, F., Millan, J., Derocq, J.M., Casellas, P., Congy, C., Oustric, D., Sarran, M., Bouaboula, M., Calandra, B., Portier, M., Shire, D., Breliere, J.C., Le Fur, G., 1998. SR144528, the first potent and selective antagonist of the CB₂ cannabinoid receptors. *J. Pharmacol. Exp. Ther.* 284, 644–650.
- Ross, R.A., Brockie, H.C., Fernando, S.R., Saha, B., Razdan, R.K., Pertwee, R.G., 1998. Comparison of cannabinoid binding sites in guinea-pig forebrain and small intestine. *Br. J. Pharmacol.* 125, 1345–1351.
- Schlicker, E., Timm, J., Gothert, M., 1996. Cannabinoid receptor-mediated inhibition of dopamine release in the retina. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 354, 791–795.