British Journal of Pharmacology (2000) 129, 1627–1632

www.nature.com/bjp

Central and peripheral cannabinoid modulation of gastrointestinal transit in physiological states or during the diarrhoea induced by croton oil

[1]Angelo A. Izzo, [1]Luisa Pinto, [1]Francesca Borrelli, [2]Raffaele Capasso, [2]Nicola Mascolo & *,[1]Francesco Capasso

[1]Department of Experimental Pharmacology, University of Naples 'Federico II', via D. Montesano 49, 80131 Naples, Italy; [2]Department of Pharmaceutical Sciences, University of Salerno, Via Ponte Don Melillo 84084 Fisciano (SA), Italy

1 We have evaluated the effect of cannabinoid drugs, administered intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.) on upper gastrointestinal transit in control and in croton oil-treated mice.

2 The cannabinoid agonists, WIN 55,212-2 (2–239 nmol mouse^{-1}) and cannabinol (24–4027 nmol mouse^{-1}), decreased while the CB_1 antagonist SR141716A (2–539 nmol mouse^{-1}) increased transit in control mice. WIN 55,212-2, cannabinol and SR141716A had lower ED_{50} values when administered i.c.v., than when administered i.p. The CB_2 antagonist SR144528 (52 nmol mouse^{-1}, i.p.) was without effect.

3 During croton oil (0.01 ml mouse^{-1}, p.o.)-induced diarrhoea, the ED_{50} values of i.p.-injected WIN 55,212-2 and cannabinol (but not SR141716A) were significantly decreased (compared to control mice). However, the ED_{50} values of WIN 55,212-2 were similar after i.p. or i.c.v. administration.

4 The inhibitory effects of WIN 55,212-2 and cannabinol were counteracted by SR141716A (16 nmol mouse^{-1}, i.p.) but not by SR144528 (52 nmol mouse^{-1}, i.p.) both in control and croton-oil treated mice.

5 Ganglionic blockade with hexamethonium (69 nmol mouse^{-1}, i.p.) did not modify the inhibitory effect of i.p.-injected cannabinoid agonists either in control or in croton-oil treated mice.

6 The lower ED_{50} values of cannabinoid drugs after i.c.v. administration suggest a central (CB_1) site of action. However, a peripheral site of action is suggested by the lack of effect of hexamethonium. In addition, croton oil-induced diarrhoea enhances the effect of cannabinoid agonists by a peripheral mechanism.

British Journal of Pharmacology (2000) **129,** 1627–1632

Keywords: Intestinal motility; cannabinoid receptors; inflammatory bowel disease; antidiarrhoeal drugs; small intestine

Abbreviations: CO, croton oil; Δ^9-THC, Δ^9-tetrahydrocannabinol

Introduction

Preparations of *Cannabis sativa* have been used medicinally for over 4000 years for the treatment of a variety of disorders, including migraine, muscle spasm, seizures, glaucoma, pain, nausea and diarrhoea (Felder & Glass, 1998). In 1964 Δ^9-tetrahydrocannabinol (Δ^9-THC) was isolated, which was later shown to be responsible for many of the pharmacological actions of *Cannabis* preparations (Mechoulam *et al*., 1998). With regard to the gastrointestinal tract, Dewey *et al*. (1972) were the first to report that Δ^9-THC reduced the rate of passage of a charcoal meal in the mouse small intestine and these findings were confirmed by others (Chesher *et al*., 1973; Jackson *et al*., 1976; Shook & Burks, 1989).

Understanding of the mechanism by which Δ^9-THC exerts its pharmacological actions has seen considerable progress in the last ten years following the discovery of two distinct cannabinoid receptors, named CB_1, (expressed mainly by central and peripheral neurons) and CB_2 (that occur mainly in immune cells) (Matsuda *et al*., 1990; Munro *et al*., 1993; Pertwee, 1998). The discovery of these receptors has led to the demonstration that there are endogenous agonists for these receptors, namely anandamide and 2-arachidonylglycerol (Devane *et al*., 1992; Stella *et al*., 1997), the latter found in the intestine of the dog (Mechoullam *et al*., 1995).

The myenteric plexus of the guinea-pig intestine contains CB_1-, but not CB_2-like cannabinoid receptor mRNA (Griffin *et al*., 1997). Activation of prejunctional CB_1 receptors produces inhibition of excitatory transmission (Pertwee *et al*., 1996; Izzo *et al*., 1998) in the isolated guinea-pig ileum and these inhibitory effects are associated with a decrease in acetylcholine release from enteric nerves (Coutts & Pertwee, 1997). However, a preliminary report indicates that cannabinoid agonists potentiate electrically-induced contractions in the porcine ileum and this effect is mediated by CB_2 receptors (Albasan *et al*., 1999).

The involvement of CB_1 receptors in intestinal motility has been confirmed also *in vivo*. Indeed, the endogenous cannabinoid agonist anandamide (Calignano *et al*., 1997) and the synthetic cannabinoid agonist WIN 55,212-2 (Colombo *et al*., 1998; Izzo *et al*., 1999a) inhibited, whilst the CB_1 receptor antagonist, SR141716A increased gastrointestinal transit in mice. However, in these studies, cannabinoid drugs were administered intraperitoneally or subcutaneously and therefore it was not clear if cannabinoids were acting at central or

*Author for correspondence; E-mail: aaizzo@unina.it

peripheral cannabinoid receptors. In addition, there are no data in the literature concerning the effects of cannabinoid drugs in the control of upper gastrointestinal motility during pathophysiological states.

The present study, therefore, has two objectives: (i) to compare the effect of cannabinoid drugs on intestinal motility after intracerebroventricular and intraperitoneal administration and (ii) to evaluate the effect of cannabinoid agonists on intestinal motility during experimental diarrhoea. In order to achieve this experimental condition, we have used croton oil, a well-known cathartic agent (Pol *et al*., 1996). The cannabinoid drugs used were: the natural agonist cannabinol (Petitet *et al*., 1998) and the synthetic agonist WIN 55,212-2 (Compton *et al*., 1992), the CB_1 receptor antagonist SR141716A (Rinaldi-Carmona *et al*., 1995) and the CB_2 receptor antagonist SR144528 (Rinaldi-Carmona *et al*., 1998).

Methods

Animals

Male ICR mice (Harlan Italy, Corezzana, MI) (24–26 g) were used after 1 week of acclimation (temperature $23 \pm 2^{\circ}C$; humidity 60%). Food was withheld 3 h before experiments but there was free access to drinking water.

Upper gastrointestinal transit

Gastrointestinal transit was measured in control mice or 3 h after treatment with croton oil (0.01 ml mouse^{-1}). At this time, 0.1 ml of a black marker (10% charcoal suspension in 5% gum arabic) was administered orally to assess upper gastrointestinal transit as previously described (Pol *et al*., 1996; Izzo *et al*., 1999a). After 20 min the mice were killed by asphyxiation with CO_2 and the gastrointestinal tract 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 (Izzo *et al*., 1999a).

The cannabinoid agonists WIN 55,212-2 (2–239 nmol mouse^{-1}), cannabinol (24–4027 nmol mouse^{-1}), the CB_1 receptor antagonist SR141716A (2–539 nmol mouse^{-1}), the CB_2 receptor antagonist SR144528 (52 nmol mouse^{-1}) or vehicle (DMSO, 4–8 μl mouse^{-1}) were given intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.) 20 min before charcoal administration. In some experiments SR141716A (16 nmol mouse^{-1} = 0.3 mg kg^{-1}), SR144528 (52 nmol mouse^{-1} = 1 mg kg^{-1}) or hexamethonium (69 nmol mouse^{-1} = 1 mg kg^{-1}) were given (i.p.) 10 min before the cannabinoid agonists. The doses of hexamethonium and SR144528 were selected on the basis of previous published work (Schirgi-Degen & Beubler, 1995; Rinaldi-Carmona *et al*., 1998)

Intracerebroventricular injections

Intracerebroventricular injections were performed as described by Haley & McCormick (1957)). Mice were briefly anaesthetized with enflurane and the drugs were delivered in a volume of 4 μl, using a Hamilton microlitre syringe fitted with 26-gauge needle.

Drugs

Drugs used were: WIN 55,212-2 mesylate (Tocris Cookson, Bristol, U.K.), hexamethonium bromide and cannabinol (SIGMA, Milan, Italy). SR141716A [(N-piperidin-l-yl)-5-(4-chlorophenyl)-1-2,4-dichlorophenyl) - 4 -methyl-lH-pyrazole-3-carboxamide hydrochloride and SR144528 (N-[-1S-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl) - pyrazole - 3 - carboxamide-3-carboxamide) were a gift from Dr Madaleine Mossé and Dr Francis Barth (SANOFI-Recherche, Montpellier, France). Cannabinoid drugs were dissolved in DMSO, while hexamethonium was dissolved in saline.

Statistics

Data are mean ± s.e.mean. To determine statistical significance, Student's *t*-test for unpaired data or one-way analysis of variance followed by Tukey–Kramer multiple comparisons test was used. A *P*-value less than 0.05 was considered significant. ED_{50} (dose which produced a 50% variation of gastrointestinal transit) and E_{max} (maximal effect) values were calculated using the computer program of Tallarida & Murray (1986).

Results

Effect of cannabinoid drugs on upper gastrointestinal transit in control mice

The effect of i.p.- or i.c.v.- injected WIN 55,212-2 (2–239 nmol mouse^{-1}) and cannabinol (24–4027 nmol mouse^{-1}) on percentage inhibition of upper gastrointestinal transit are presented in Figure 1. Both WIN 55,212-2 and cannabinol produce a dose-dependent inhibition of gastrointestinal transit. However, the ED_{50} values after i.p. or i.c.v. administration were statistically different. The ED_{50} and E_{max} values of cannabinoid drugs are shown in Table 1.

The CB_1 receptor antagonist SR141716A (16 nmol mouse^{-1}, i.p.), but not the CB_2 receptor antagonist SR144528 (52 nmol mouse^{-1}, i.p.) counteracted the inhibitory effect of WIN 55,212-2 (5 nmol mouse^{-1}, i.c.v. or 50 nmol mouse^{-1}, i.p.) and cannabinol (201 nmol mouse^{-1},

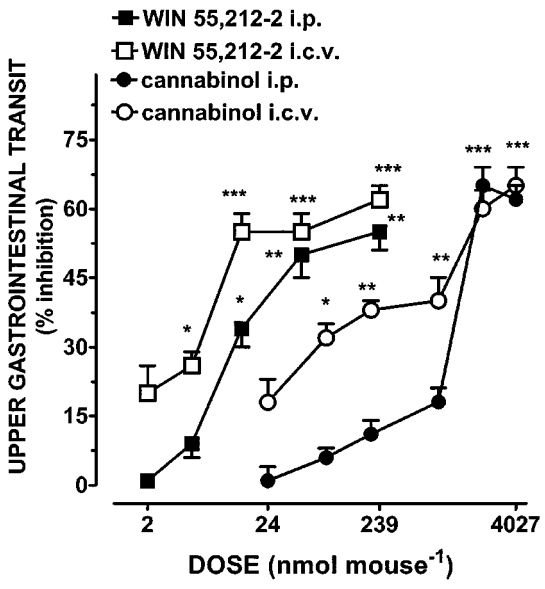

Figure 1 Dose related inhibition of upper gastrointestinal transit by WIN 55,212-2 and cannabinol after i.p. or i.c.v. administration in control mice. Each point represents the mean ± s.e.mean of 10–13 animals for each experimental group. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ *vs* corresponding control.

i.c.v. or 2010 nmol mouse^{-1}, i.p.) after both i.c.v. (Figure 2) and i.p. (Figure 3) routes of administration. Hexamethonium (69 nmol mouse^{-1}, i.p.) abolished the effect of both WIN 55,212-2 and cannabinol after i.c.v. (Figure 2) but not after i.p. (Figure 3) administration.

SR 14176A (i.p. or i.c.v.), *per se*, dose-dependently increased upper gastrointestinal transit (Figure 4a). However, the ED_{50} value after i.c.v. administration was significantly ($P<0.01$) lower than the ED_{50} after i.p. administration (Table 1). At a dose of 16 nmol mouse^{-1}, SR141716A (i.c.v.) significantly ($P<0.05$) increased intestinal motility (Figure 4a) and this effect was significantly ($P<0.05$) counteracted by hexamethonium (69 nmol mouse^{-1} i.p.) (per cent increase of SR141716A: 44±3; per cent increase of SR141716A in the presence of hexamethonium; 1±3, $n=10$).

The CB_2 receptor antagonist SR144528 (52 nmol mouse^{-1}, i.p.), given alone, did not significantly modify gastrointestinal transit (control 47±4%; SR144528 48±2%, $n=10$, $P>0.2$). Hexamethonium (69 nmol mouse^{-1} i.p.) did not significantly modify gastrointestinal transit (17±8% increase, $n=12$). DMSO (4 μl mouse^{-1} i.c.v. or 4–8 μl mouse^{-1} i.p.) had no effect on the response under study (data not shown).

Effect of cannabinoid drugs on upper gastrointestinal transit during croton oil-induced diarrhoea

Oral administration of croton oil produced diarrhoea which was associated with a significant increase in gastrointestinal transit (per cent transit: control 46±2; croton oil, 56±2, $P<0.01$, $n=24$). Both WIN 55,212-2 (2–239 nmol mouse^{-1}, i.p.) and cannabinol (24–4027 nmol mouse^{-1}, i.p.) produced a dose-related inhibition of transit (Figure 5) and both agonists had a lower ED_{50} value compared to the corresponding i.p. treatment in control mice (Table 1). In croton oil-treated animals, WIN 55,212-2 (i.p.) and cannabinol (i.p.) had a significant inhibitory effect with threshold doses of 5 nmol mouse^{-1} and 80 nmol mouse^{-1} doses respectively whilst in control mice, significant inhibitory effects were achieved at doses of 14 nmol mouse^{-1} (WIN 55,212-2) and 2010 nmol mouse^{-1} (cannabinol) respectively (Figure 5).

Table 1 ED_{50}±s.e.mean and E_{max}±s.e.mean of cannabinoid drugs after i.p. or i.c.v. administration in control mice and in mice receiving croton oil (0.01 ml mouse^{-1}, orally)

	ED_{50} (nmol mouse^{-1})		E_{max} (variation in motility)	
Drug	*i.p.*	*i.c.v.*	*i.p.*	*i.c.v.*
Control mice				
WIN 55,212-2	169±8	104±8*	61±2	67±1
Cannabinol	2760±144	1829±98#	72±3	69±5
SR141716A	375±31	117±8†	59±3	93±2
Croton oil-treated mice				
WIN 55,212-2	68±5**	74±10	77±2	78±3
Cannabinol	1681±99#	n.d.	79±3	n.d.
SR141716A	418±32	n.d.	59±2	n.d.

Data are means±s.e.mean of 10–13 animals; *$P<0.05$ and **$P<0.001$ *vs* WIN 55,212-2 i.p. in control mice; #$P<0.05$ *vs* cannabinol i.p. in control mice; †$P<0.01$ *vs* SR141716A i.p. in control mice; n.d. = not determined

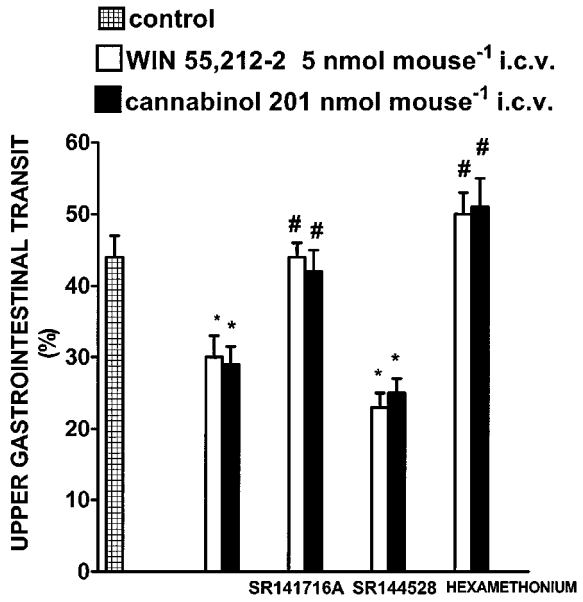

Figure 2 Effect of WIN 55,212-2 (5 nmol mouse^{-1} i.c.v) and cannabinol (201 nmol mouse, i.c.v.) on upper gastrointestinal transit alone or in mice treated with SR141716A (16 nmol mouse^{-1}, i.p.) or SR144528 (52 nmol mouse^{-1}, i.p.) or hexamethonium (69 nmol mouse^{-1}, i.p.). Results are mean±s.e.mean of 8–11 animals for each experimental group. *$P<0.01$ *vs* control and #$P<0.05$ *vs* WIN 55,212-2 (or cannabinol).

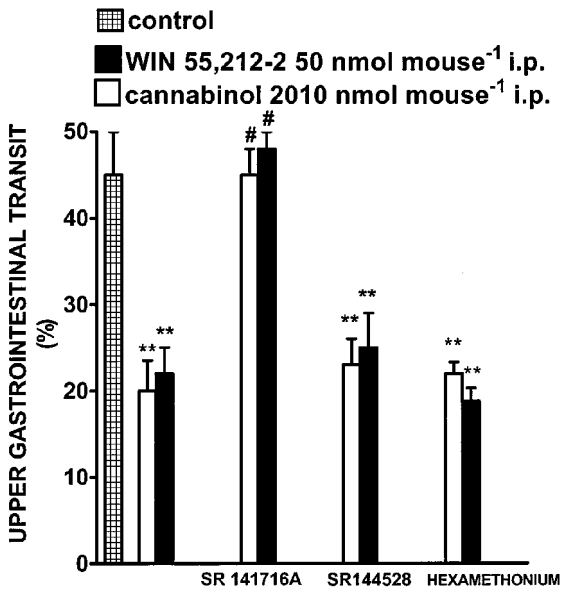

Figure 3 Effect of WIN 55,212-2 (50 nmol mouse^{-1}, i.p.) and cannabinol (2010 nmol mouse^{-1}, i.p.) on upper gastrointestinal transit alone or in mice treated with SR141716A (16 nmol mouse^{-1}, i.p.) or SR144528 (52 nmol mouse^{-1}, i.p.) or hexamethonium (69 nmol mouse^{-1}, i.p.). Results are mean±s.e.mean of 8–11 animals for each experimental group. **$P<0.01$ *vs* control and #$P<0.01$ *vs* WIN 55,212-2 (or cannabinol).

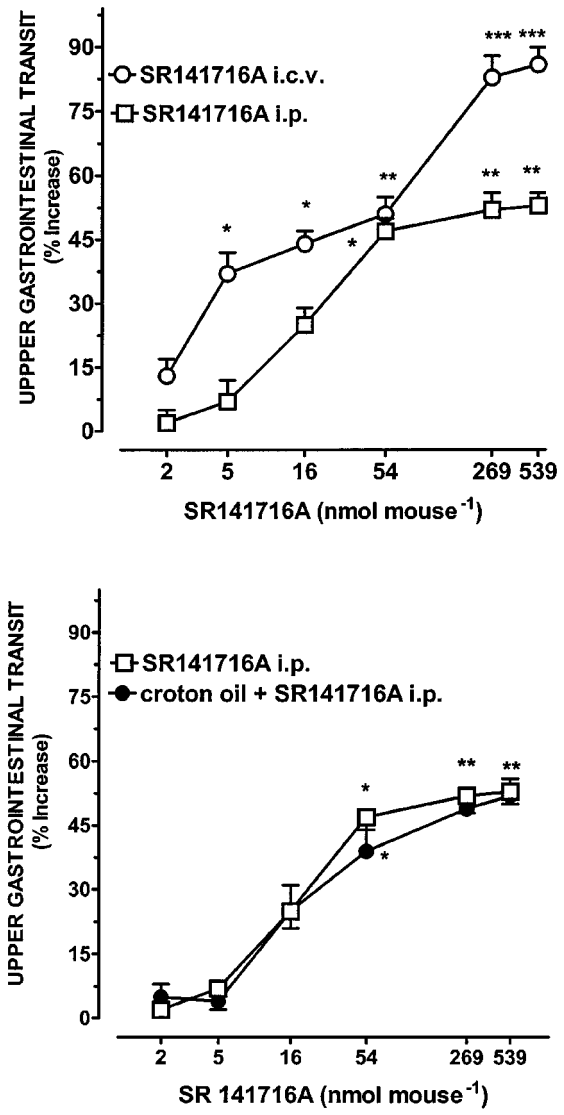

Figure 4 Dose-related increase of upper gastrointestinal transit by SR141716A in control mice (a) or mice treated with croton oil (0.01 ml mouse^{-1}, orally) (b). Results are mean$\pm$s.e.mean of 10–12 animals for each experimental group. *$P<0.05$ and **$P<0.01$ *vs* corresponding control.

Administered i.c.v. WIN 55,212-2 (2–239 nmol mouse^{-1}) also decreased intestinal motility, but the ED_{50} value (74$\pm$10 nmol mouse^{-1}) was not statistically different from the ED_{50} value (68$\pm$5 nmol mouse^{-1}) after i.p. administration (Table 1).

The inhibitory effect of i.p.-injected WIN 55,212-2 (14 nmol mouse^{-1}) or cannabinol (805 nmol mouse^{-1}) was reduced by the CB_1 receptor antagonist SR141716A (16 nmol mouse^{-1}, i.p.) but not by the CB_2 receptor antagonist SR144528 (52 nmol mouse^{-1}, i.p.) or by the ganglion blocker hexamethonium (69 nmol mouse^{-1}, i.p.) (Figure 6).

Figure 4b shows the potentiating effect of SR141716A (2–539 nmol mouse, i.p.) in mice treated with croton oil. The ED_{50} value (418$\pm$32 nmol mouse^{-1}) was not statistically different from the corresponding ED_{50} value in control animals (375$\pm$31 nmol mouse^{-1}). By contrast, SR144528 (52 nmol mouse^{-1}, i.p.) or hexamethonium (69 nmol mouse^{-1}, i.p.) did not modify gastrointestinal transit (per cent transit: croton oil: 58$\pm$6, croton oil+SR144528 61$\pm$5, croton oil+hexamethonium 68$\pm$4, $n=6$, $P>0.2$).

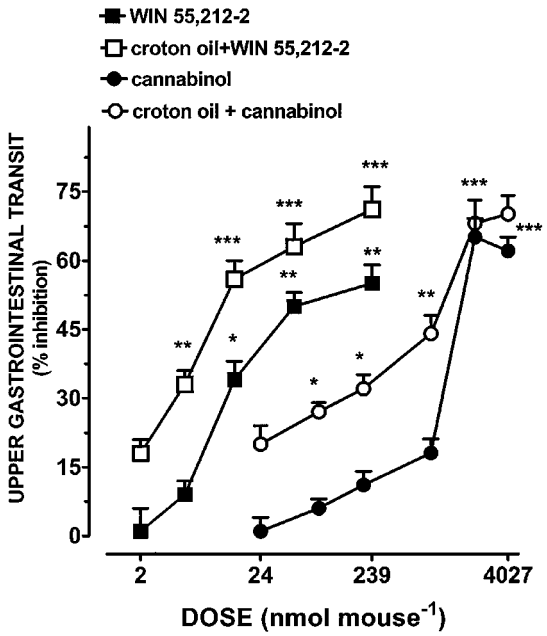

Figure 5 Dose-related inhibition of upper gastrointestinal transit by WIN 55,212-2 (i.p.) and cannabinol (i.p.) in control mice or in mice receiving croton oil (0.01 ml mouse^{-1}, orally). Results are mean$\pm$ s.e.mean of 10–12 animals for each experimental group. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ *vs* corresponding control.

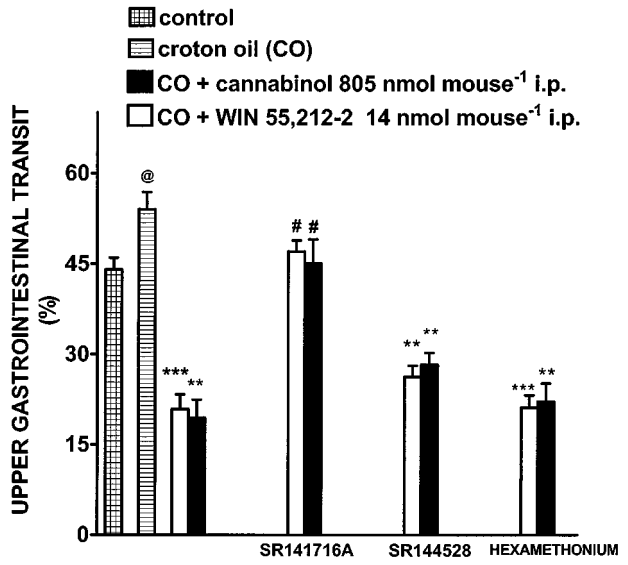

Figure 6 Upper gastrointestinal transit in mice with diarrhoea induced by croton oil (0.01 ml mouse^{-1}, orally): effect of WIN 55,212-2 (14 nmol mouse^{-1}, i.p.) and cannabinol (805 nmol mouse^{-1}, i.p.) alone or in mice treated with SR141716A (16 nmol mouse^{-1}, i.p.) or SR144528 (52 nmol mouse^{-1}, i.p.) or hexamethonium (69 nmol mouse^{-1}, i.p.). Results are mean$\pm$s.e.mean of 8–11 animals for each experimental group. @$P<0.05$ *vs* control, **$P<0.01$ and ***$P<0.001$ *vs* croton oil and #$P<0.01$ *vs* croton oil+WIN 55,212-2 (or croton oil+cannabinol).

Discussion

The role of cannabinoid receptors in control mice

It is now well known that cannabinoid agonists can reduce intestinal motility through activation of CB_1

receptors. Indeed activation of CB_1 receptors can mediate, (i) inhibition of electrically-evoked contractions in the isolated guinea-pig (Pertwee *et al*., 1996; Izzo *et al*., 1998) and human ileum (Croci *et al*., 1998), (ii) inhibition of fast and slow synaptic transmission in guinea-pig myenteric nerves (Lopez-Redondo *et al*., 1997), (iii) inhibition of electrically-evoked acetylcholine release from myenteric nerves (Coutts & Pertwee, 1997) and (iv) reduction of peristalsis efficiency in the isolated guinea-pig ileum (Heinemann *et al*., 1999; Izzo *et al*., 2000). These findings are in keeping with the presence of CB_1, but not CB_2-like receptor messenger RNA in the myenteric plexus of the guinea-pig small intestine (Griffin *et al*., 1997). Consistent with these *in vitro* findings, it has been shown that cannabinoid agonists reduced intestinal motility in mice (Calignano *et al*., 1997; Colombo *et al*., 1998; Izzo *et al*., 1999a) and rats (Izzo *et al*., 1999c) and this effect was counteracted by SR141716A, a specific CB_1 antagonist. However, whether the effect of cannabinoid drugs *in vivo* is mediated *via* a central or a peripheral site of action was not demonstrated in these studies. Indeed the CB_1 receptor is located within both the central nervous system (Matsuda *et al*., 1990) and within the enteric nervous system (Griffin *et al*., 1997).

In the present study we have shown that the synthetic cannabinoid agonist WIN 55,212-2 and the natural cannabinoid agonist cannabinol produced a dose-related inhibition of upper gastrointestinal transit when administered i.p. or i.c.v. The inhibitory effect of cannabinoid agonists was abolished by SR141716A, a specific CB_1 antagonist, but not by SR144528, a CB_2 receptor antagonist, indicating an involvement of CB_1 but not CB_2 receptors.

The ED_{50} values of WIN 55,212-2 and cannabinol after i.c.v. administration were significantly lower than the corresponding ED_{50} values after i.p. administration. The low doses that were needed to inhibit transit after i.c.v. injection implies that cannabinoid agonists may inhibit intestinal motility through activation of central CB_1 receptors. However, the effect of i.p.-injected cannabinoid agonists was not modified by the ganglion blocker hexamethonium. These results probably indicate that the effect of i.p.-injected cannabinoid agonists is mediated by peripheral CB_1 cannabinoid receptors.

Although some reports indicate that the CB_1 receptor antagonist SR141716A does not affect intestinal motility in the isolated human ileum (Croci *et al*., 1998) and gastric emptying in the rat (Izzo *et al*., 1999b), other studies indicate that intestinal motility could be tonically inhibited by the endogenous cannabinoid system. Indeed SR141716A increased electrically-induced contractions in the isolated guinea-pig ileum (Pertwee *et al*., 1996; Izzo *et al*., 1998) and intestinal motility and defaecation in the mouse (Colombo *et al*., 1998; Izzo *et al*., 1999a). The observation that SR141716A, *per se*, increased intestinal motility does not necessary imply that endogenous cannabinoids are involved in the control of intestinal motility in view of the inverse agonist properties of SR141716A at human recombinant CB_1 (Landsman *et al*., 1997) and both CB_1 and CB_2 receptors (MacLennan *et al*., 1998).

In the present study, we have shown that SR141716A (i.c.v. or i.p.) produced a dose-dependent increase in upper gastrointestinal transit. The ED_{50} value after i.c.v. administration was significantly lower than the ED_{50} value after i.p. administration, suggesting a central site of action of SR141716A. The most likely explanation of these results is that the endogenous cannabinoid system, within the central nervous system, can inhibit intestinal motility through activation of CB_1 receptors. In a recent study, we have shown that SR141716A (i.p.)-induced changes in intestinal motility are not modified by the ganglionic blocker hexamethonium (Izzo *et al*., 1999a), suggesting a peripheral site of action of i.p.-injected SR141716A.

Effect of cannabinoid drugs during croton oil-induced diarrhoea

Croton oil is a well known irritant that has been widely used to produce experimental inflammation in different tissues, especially skin and mucosa, and induces diarrhoea associated with intestinal inflammation in the mouse small intestine (Pol *et al*., 1996). According to Pol *et al*., (1996), we have shown that croton oil increases upper gastrointestinal transit 3 h after oral administration. The cannabinoid agonists WIN 55,212-2 and cannabinol blocked the increase in intestinal motility induced by croton oil; in addition, the ED_{50} values of i.p.-injected WIN 55,212-2 and cannabinol were significantly decreased (compared to control mice). However, during croton oil-induced diarrhoea the ED_{50} value of WIN 55,212-2 was similar after i.p. or i.c.v. treatment and ganglionic blockade with hexamethonium did not alter the inhibitory effect of i.p.-injected cannabinoids.

Taken together, these results indicate that the enhanced effect of cannabinoid agonists are mediated by peripheral receptors. By contrast, using the castor oil test, we have recently shown that cannabinoid agonists possess either weak or no antidiarrhoeal activity in the rat (Izzo *et al*., 1999c). The use of a different cathartic (castor oil *vs* croton oil), different species (rat *vs* mouse) and different region of the gut (whole gut *vs* upper gastrointestinal tract) could explain this discrepancy. Consistent with this hypothesis, Shook & Burks (1989) showed that Δ^9-THC produced a greater inhibition of small intestinal transit than large bowel transit.

In line with the result obtained in control mice and those reported in the isolated guinea-pig ileum (Pertwee *et al*., 1996; Izzo *et al*., 1998), the antitransit response of cannabinoid agonists involves CB_1, but not CB_2 receptors, as the inhibitory effect of both WIN 55,212-2 and cannabinol were reduced by SR141716A, but not SR144528. Administration of SR141716A (i.p.), *per se*, increased intestinal motility in control mice and those given croton oil with a similar ED_{50} value, thus indicating that during the experimental diarrhoea the endogenous cannabinoid system is activated as in control animals. By contrast, SR144524, a specific CB_2 receptor antagonist, at doses previously shown to bind the CB_2 receptor in the rat spleen (Rinaldi-Carmona *et al*., 1998), failed to modify the inhibitory effect of both WIN 55,212-2 and cannabinol and did not modify, *per se*, intestinal motility during the diarrhoea induced by croton oil. Thus, a role for CB_2 receptors in modulating intestinal motility during experimental diarrhoea seems unlikely.

Conclusions

Our results suggest that both central and peripheral CB_1 receptors can modulate upper gastrointestinal motility. However, the effect of systemic (i.p.) cannabinoid drugs is probably mediated by peripheral receptors. Diarrhoea induced by the irritant croton oil enhances the inhibitory effect of cannabinoid agonists by a peripheral mechanism, while CB_2 receptors are not involved in the control of

intestinal motility, either in physiological or in pathophysiological states. Thus, selective non-psychotropic CB_1 agonists could represent novel drugs to treat motility disorders associated with inflammatory diarrhoea.

This work was supported by *Cofinanziamento Murst 1999* and Enrico and Enrica Sovena Foundation (Roma). The Authors are grateful to Drs Antonio Calignano and Carla Cicala for their help. SR141716A and SR144528 were a kind gift from SANOFI (Montpellier, France).

References

ALBASAN, H., SOLDANI, G. & BROWN, D.R. (1999). Synthetic cannabinoids potentiate electrically-induced contractions in porcine ileum *in vitro*. *Pharm. Res.,* **39** (Supplement), 5.

CALIGNANO, A., LA RANA, G., MAKRIAYANNIS, A., LIN, S.Y., BELTRAMO, M. & PIOMELLI, D. (1997). Inhibition of intestinal motility by anandamide, an endogenous cannabinoid. *Eur. J. Pharmacol.,* **340,** R7–R8.

CHESHER, G.B., DAHL, C.J., EVERNGHAM, 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.

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.

COUTTS, A.A. & PERTWEE, R.G. (1997). Inhibition by cannabinoid receptor agonists of acetylcholine release from the guinea-pig myenteric plexus. *Br. J. 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 CB_1 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,* **277,** 119–131.

DEWEY, W.L., HARRIS, L.S. & KENNEDY, J.S. (1972). Some pharmacological and toxicological effects of 1-trans-8 and 1-trans-9tetrahydrocannabinol in laboratory rodents. *Arch. Int. Pharmacod. Ther.,* **196,** 133–145.

FELDER, C.C. & GLASS, M. (1998). Cannabinoid receptors and their endogenous agonists. *Annu. Rev. Pharmacol. Toxicol.,* **38,** 179–200.

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 CB_2-like cannabinoid receptors on peripheral nerve terminals. *Eur. J. Pharmacol.,* **339,** 53–61.

HALEY, T.J. & MCCROMICK, W.G. (1957). Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Br. J. Pharmacol.,* **12,** 12–15.

HEINEMANN, A., SHAHBAZIAN, A. & HOLZER, P. (1999). Cannabinoid inhibition of guinea-pig intestinal peristalsis via inhibition of excitatory and activation of inhibitory neural pathways. *Neuropharmacology,* **38,** 1289–1297.

IZZO, A.A., MASCOLO, N., BORRELLI, F. & CAPASSO, F. (1998). Excitatory transmission to the circular muscle of the guinea-pig ileum: evidence for the involvement of cannabinoid CB_1 receptor. *Br. J. Pharmacol.,* **124,** 1363–1368.

IZZO, A.A., MASCOLO, N., BORRELLI, F. & CAPASSO, F. (1999a). Defaecation, intestinal fluid accumulation and motility in rodents: implications of cannabinoid CB_1 receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **359,** 65–70.

IZZO, A.A., MASCOLO, N., CAPASSO, R., GERMANO', M.P., DE PASQUALE, R. & CAPASSO, F. (1999b). Inhibitory effect of cannabinoid agonists on gastric emptying in the rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **360,** 221–223.

IZZO, A.A., MASCOLO, N., PINTO, L., CAPASSO, R. & CAPASSO, F. (1999c). The role of cannabinoid receptors in intestinal motility, defaecation and diarrhoea in rats. *Eur. J. Pharmacol.,* **384,** 37–42.

IZZO, A.A., MASCOLO, N., TONINI, M. & CAPASSO, F. (2000). Modulation of peristalsis by cannabinoid CB_1 ligands in the isolated guinea-pig ileum. *Br. J. Pharmacol.,* **129,** 984–990.

JACKSON, D.M., MALOR, R., CHESHER, G.B., STARMER, G.A., WELBURN, P.J. & BAILEY, R. (1976). The interaction between prostaglandin E_1 and delta^9-tetrahydrocannabinol on intestinal motility and on the abdominal constriction response in the mouse. *Psychopharmacology,* **47,** 187–193.

LANDSMAN, R.S., BURKEY, T.H., CONSROE, P., ROESKE, W.R. & YAMAMURA, H.I. (1997). SR141716A is an inverse agonist at the human cannabinoid CB_1 receptors. *Eur. J. Pharmacol.,* **334,** R1–R2.

LOPEZ-REDONDO, F., LEES, G.M. & PERTWEE, R.G. (1997). Effects of cannabinoid receptor ligands on electrophysiological properties of myenteric neurones of the guinea-pig ileum. *Br. J. Pharmacol.,* **122,** 330–384.

MACLENNAN, S.L., REYNEN, P.H., KWAN, J. & BONHAUS, D.W. (1998). Evidence for inverse agonism of SR141716A at human recombinant cannabinoid CB_1 and CB_2 receptors. *Br J. Pharmacol.,* **124,** 619–622.

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.

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-monogliceride, present in canine gut, that binds to a cannabinoid receptor. *Biochem. Pharmacol.,* **50,** 83–90.

MECHOULAM, R., FRIDE, E. & DI MARZO, V. (1998). Endocannabinoids. *Eur. J. Pharmacol.,* **359,** 1–18.

MUNRO, S., THOMAS, K.L. & ABU-SHAAR, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature,* **365,** 61–65.

PERTWEE, R.G. (1998). Pharmacological, physiological and clinical implications of the discovery of cannabinoid receptors. *Biochem. Soc. Trans.,* **26,** 267–272.

PERTWEE, R.G., FERNANDO, S.R., NASH, J.E. & COUTTS, A.A. (1996). Further evidence for the presence of cannabinoid CB_1 receptors in guinea-pig small intestine. *Br. J. Pharmacol.,* **118,** 2199–2205.

PETITET, F., JEANTAUD, B., REIBAUD, M., IMPERATO, A. & DUBROEUCQ, M.C. (1998). Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of Δ^9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. *Life Sci.,* **63,** PL1–PL6.

POL, O., VALLE, L., FERRER, I. & PUIG, M. (1996). The inhibitory effects of α_2-adrenoceptor agonists on gastrointestinal transit during croton oil-induced intestinal inflammation. *Br. J. Pharmacol.,* **119,** 1649–1655.

RINALDI-CARMONA, M., BARTH, F., HEAULME, M., ALFONSO, R., SHIRE, D., CONGY, C., SOBRIE', 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 CB_2 cannabinoid receptors. *J. Pharmacol. Exp. Ther.,* **284,** 644–650.

SCHIRGI-DEGEN, A. & BEUBLER, E. (1995). Significance of nitric oxide in the stimulation of intestinal fluid absorption in the rat jejunum in vivo. *Br. J. Pharmacol.,* **114,** 13–18.

SHOOK, J.E. & BURKS, T.F. (1989). Psychoactive cannabinoids reduce gastrointestinal propulsion and motility in rodents. *J. Pharmacol. Exp. Ther.,* **249,** 444–449.

STELLA, N., SCHWEITZER, P. & PIOMELLI, D. (1997). A second endogenous cannabinoid that modulates long-term potentiation. *Nature,* **388,** 773–778.

TALLARIDA, R.J. & MURRAY, R.B. (1986). *Manual of Pharmacological Calculations with Computer Programs*. New York: Springer-Verlag.

(Received December 2, 1999
Revised January 25, 2000
Accepted January 28, 2000)