British Journal of Pharmacology (2000) 129, 984–990
www.nature.com/bjp

Modulation of peristalsis by cannabinoid CB_1 ligands in the isolated guinea-pig ileum

*,[1]Angelo A. Izzo, [2]Nicola Mascolo, [3]Marcello Tonini & [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 and [3]Department of Internal Medicine and Therapeutics, Division of Pharmacology and Toxicology, University of Pavia, Piazza Botta 10, 27100 Pavia, Italy

1 The effect of cannabinoid drugs on peristalsis in the guinea-pig ileum was studied. Peristalsis was induced by delivering fluid into the oral end of an isolated intestinal segment. Longitudinal muscle reflex contraction, threshold pressure and threshold volume to trigger peristalsis, compliance of the intestinal wall during the preparatory phase (a reflection of the resistance of the wall to distension) and maximal ejection pressure during the emptying phase of peristalsis were measured.

2 The cannabinoid agonists WIN 55,212-2 (0.3–300 nM) and CP55,940 (0.3–300 nM) significantly decreased longitudinal muscle reflex contraction, compliance and maximal ejection pressure, while increased threshold pressure and volume to elicit peristalsis. These effects were not modified by the opioid antagonist naloxone (1 μM) and by the α-adrenoceptor antagonist phentolamine (1 μM).

3 The inhibitory effect of both WIN 55,212-2 and CP55,940 on intestinal peristalsis was antagonized by the cannabinoid CB_1 receptor antagonist SR141716A (0.1 μM), but not by the cannabinoid CB_2 receptor antagonist SR144528 (0.1 μM).

4 In absence of other drugs, the CB_1 receptor antagonists SR141716A (0.01–1 μM) and AM281 (0.01–1 μM) slightly (approximatively 20%) but significantly increased maximal ejection pressure during the empty phase of peristalsis without modifying longitudinal muscle reflex contraction, threshold pressure, threshold volume to trigger peristalsis and compliance.

5 It is concluded that activation of CB_1 receptors reduces peristalsis efficiency in the isolated guinea-pig, and that the emptying phase of peristalsis could be tonically inhibited by the endogenous cannabinoid system.

British Journal of Pharmacology (2000) **129,** 984–990

Keywords: Cannabinoid; peristalsis; myenteric plexus; intestine; intestinal motility

Abbreviations: DMSO, dimethyl sulphoxide

Introduction

Cannabinoids are natural compounds found in the aerial parts of *Cannabis sativa* L. (Cannabinaceae). Medicinal properties of cannabis were recognized some 5000 years ago and potential therapeutic applications that are of either historical or contemporary interest include analgesia, attenuation of nausea and vomiting of cancer chemotherapy, antirheumatic and antipyretic actions, decreased bronchial constriction and decreased intestinal motility (Howlett, 1995). The synthetic cannabinoid drug nabilone and Δ^9-tetrahydrocannabinol are already used clinically to suppress nausea and vomiting provoked by anticancer drugs or to boost the appetite of AIDS patients (Pertwee, 1998).

Many of the pharmacological effects previously reported for natural cannabinoids are mediated by specific receptors (Howlett, 1995). These are CB_1, that are expressed mainly by central and peripheral neurons, and CB_2 receptors, that occur mainly in immune cells (Pertwee, 1998). The discovery of these receptors has led to the demonstration that there are endogenous ligands (agonists) for these receptors in several mammalian tissues. The most important of the endogenous cannabinoids discovered to date are anandamide (Devane *et al*., 1992) and 2-arachidonylglycerol, the latter found in the intestine of the dog (Mechoullam *et al*., 1995).

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

The guinea-pig small intestine contains cannabinoid binding sites that closely resemble CB_1 binding sites of guinea-pig brain (Ross *et al*., 1998). Prejunctionally located CB_1 receptors regulate in a negative fashion the release of acetylcholine from myenteric neurons (Coutts & Pertwee, 1997). The activation of such receptors inhibits excitatory transmission in the guinea-pig (Coutts & Pertwee, 1997; Izzo *et al*., 1998) and human ileum (Croci *et al*., 1998), while SR141716A, a specific CB_1 receptor antagonist, potentiates excitatory transmission in the guinea-pig (Coutts & Pertwee, 1997; Izzo *et al*., 1998) but not in the human ileum.

On the basis of these studies, which utilized the electrically-evoked contractions of the longitudinal and circular muscle or the release of tritiated acetylcholine from myenteric neurons, it is not clear whether cannabinoid receptors are really involved in the control of peristalsis as it occurs under physiological conditions. Indeed, there is evidence that some molecules (i.e. histamine H_3 agonists) may suppress electrical twitch responses without affecting the distension-evoked peristaltic motility (Poli & Pozzoli, 1997).

In this work we have therefore studied the role of cannabinoid receptors on intestinal peristalsis *in vitro*. For this purpose, we have used the cannabinoid non-selective receptor agonists WIN 55,212-2 and CP55,940 (Compton *et al*., 1992; Gatley *et al*., 1997), the CB_1 receptor antagonists

SR141716A and AM840 (Rinaldi-Carmona *et al*., 1995; Gifford *et al*., 1997) and the CB_2 receptor antagonist SR144528 (Rinaldi-Carmona *et al*., 1998).

Methods

Male guinea-pigs (300–450 g, Harlan Italy, Corezzana, Milano) were killed by asphyxiation with CO_2. Portions of the ileum lying 5–15 cm proximal to the ileocaecal junction were excised, flushed of luminal contents and placed, for up to 4 h, in Krebs solution kept at room temperature and oxygenated with a mixture of 95% O_2 and 5% CO_2. The composition of the Krebs solution was (mM): NaCl 119, KCl 4.75, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.5, $CaCl_2$ 2.5 and glucose 11). After dissection, ileal segments were mounted in organ baths that contained oxygenated Krebs solution maintained at 37°C.

Peristalsis

Peristalsis was studied with a constant intraluminal perfusion system that has been described in detail previously (Waterman *et al*., 1992). Briefly, ileal segments (approximately 5–7 cm in length) were secured horizontally in an organ bath containing 80 ml of Krebs solution. Peristalsis was elicited by delivering Krebs solution into the oral end of the intestinal lumen; the infusion rate was 0.85 ml min^{-1}. Longitudinal muscle contraction was recorded with an isotonic transducer (load 1 g). Two transducers measured aboral pressure; one recorded at high sensitivity and the other at low sensitivity. Each peristaltic wave caused the expulsion of fluid from the aboral end of the intestine *via* a one-way valve. The volume expelled at the end of each wave of contraction was measured with a measuring cylinder. Fluid expelled activated a photocell system that switched off the infusion pump. The arrest of the inflow, controlled by a timer, was maintained for 30 s in order not to force the contracted organ during propulsion and to allow a period of rest between two peristalses.

Physiological parameter of peristalsis

Two phases of peristalsis can be identified: the preparatory phase, when the intestine is being filled with fluid, causing the longitudinal muscle to contract, and the emptying phase, when the circular muscle contracts and forces fluid from the aboral end (Kosterlitz & Lees, 1964). The following parameters were measured: (i) longitudinal muscle contraction (calculated as the percentage shortening of the intestine at the threshold, relative to the resting length), (ii) threshold pressure value required for a peristaltic response, (iii) threshold volume to trigger peristalsis, (iv) compliance of the intestinal wall at the end of the preparatory phase, and (iv) maximal ejection pressure during the emptying phase. A schematic drawing of a peristaltic wave showing variables measured has been previously reported (Waterman *et al*., 1992; Holzer & Maggi, 1994). The compliance is defined as the change in intraluminal pressure in response to a given change in intraluminal volume and reflects the resistance of the intestinal wall to infused fluid (Waterman *et al*., 1992).

Experimental design

After stable control peristaltic activity had been recorded (at least 5 min), the response was observed in the presence of increasing cumulative concentration of WIN 55,212-2 (0.3–300 nM), CP55,940 (0.3–300 nM), SR141716A (0.01–1 μM), AM281 (0.01–1 μM) or single concentration of SR144528 (0.1 μM), atropine (1 μM), tetrodotoxin (0.3 μM), phentolamine (1 μM) and naloxone (1 μM). These concentrations were selected on the basis of previous work (Croci *et al*., 1998; Izzo *et al*., 1998).

The contact time for each concentration was 4 min for WIN 55,212-2, 6 min for CP55,940, 8 min for SR141716A and AM281, 10 min for tetrodotoxin and 20 min for the other drugs. Preliminary experiments showed that these contact times were sufficient to achieve maximal effect. In some experiments SR141716A (10, 30 and 100 nM), AM281 (0.1 μM) SR144528 (0.1 μM), phentolamine (1 μM) or naloxone (1 μM) were included in the Krebs.

Drugs

Drugs used were: WIN 55,212-2 mesylate, CP55,590, AM840 (Tocris Cookson, Bristol, U.K.), tetrodotoxin, atropine sulphate, naloxone hydrochloride, phentolamine hydrochloride (Sigma, Milan, Italy), SR141716A [(N-piperidin-1-yl)-5-(4-chlorophenyl)-1-2,4-dichlorophenyl)-4-methyl-1H-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 Mossè and Dr Francis Barth (SANOFI-Recherche, Montpellier, France). The drugs were dissolved in dimethyl sulphoxide (DMSO), with the exception of atropine, phentolamine, naloxone and tetrodotoxin which were dissolved in saline. All drugs were added in volumes less than 0.01% of the bath volume. DMSO (6 μl/80 ml) neither affected peristalsis efficiency nor modified the concentration-response to cannabinoid agonists.

Statistical analysis

Data are presented as the means ± s.e.mean of experiment using n guinea-pigs. Each measurement was made with a separate segment of intestine. Comparisons between two sets of data were made by Student's t-test for paired data. When multiple comparisons against a single control were made, analysis of variance was used. Probability less than 0.05 was regarded as significant.

Results

Continuous intraluminal infusion of Krebs solution resulted in peristaltic activity which was reproducible and stable during the experimental period of 20–30 min ($n=5$). In the absence of any drug the physiological parameters measured had the following values: threshold pressure: 118 ± 5 Pa, threshold volume 381 ± 31 μl, longitudinal muscle contraction: 16 ± 2% inhibition, maximal ejection pressure 832 ± 34 Pa, compliance 3.56 ± 0.09 μl Pa^{-1}. The application of tetrodotoxin (0.3 μM) or atropine (1 μM) completely prevented the appearance of peristaltic waves, while phentolamine (1 μM) or naloxone (1 μM) were ineffective ($n=4$ for each drug).

The cannabinoid agonists WIN 55,212-2 and CP55,940 affected all the measured parameter of peristalsis. WIN 55,212-2 (0.3–300 nM) and CP55,940 (0.3–300 nM) increased threshold pressure and volume to elicit peristalsis (Figure 1a,b), reduced in a concentration dependent fashion longitudinal muscle contraction (Figure 1c), and decreased maximal ejection pressure (Figure 1d). The compliance of the intestine

during the preparatory phase was reduced by WIN 55,212-2 300 nM and CP55,940 300 nM by approximately 30% (Figure 2). WIN 55,212-2-induced changes in peristalsis were not modified by phentolamine (1 μM $n=5$) or naloxone (1 μM, $n=5$) (data not shown).

The emptying phase of peristalsis was abolished by 300 nM WIN 55,212-2 in two out of eight experiments. WIN 1 μM and CP55,940 1 μM also completely abolished peristalsis in five out of eight experiments and two out of five experiments, respectively. A significant rise in the triggering pressure, followed by a complete paralysis of the peristaltic motility was observed. The fluid moved straight through the intestine as if it were a passive tube. Figure 3 shows a recording of the effect of WIN 55,212-2 1 μM on peristalsis. In the experiments in which the emptying phase of peristalsis was not abolished, a strong inhibition of physiological parameter of peristalsis was observed (data not shown).

The effect of WIN 55,212-2 and CP55,940 on peristalsis was completely counteracted by SR141716A (0.1 μM) (Figures 1 and 2) and by AM281 (0.1 μM) (data not shown), two cannabinoid CB_1 receptor antagonists, but not by the cannabinoid CB_2 receptor antagonist SR144528

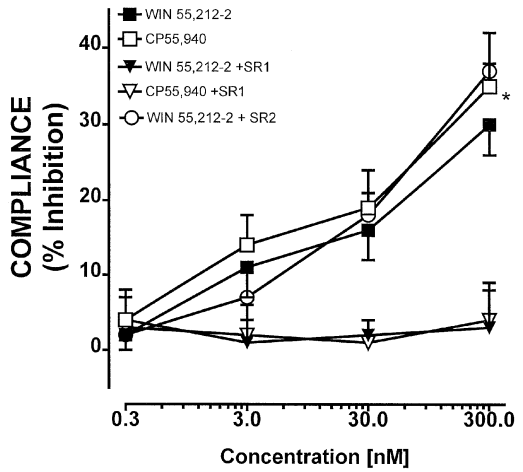

Figure 2 Effect of WIN 55,212-2 (0.3–300 nM) and CP55,940 (0.3–300 nM) alone or in combination with SR141716A (SR1 0.1 μM) or SR144528 (SR2 0.1 μM) on intestinal compliance. The ordinates show the percentage of inhibition compared to control response. Each point represents the mean of 6–8 experiments; vertical lines show s.e.mean $^*P<0.05$ compared to control response.

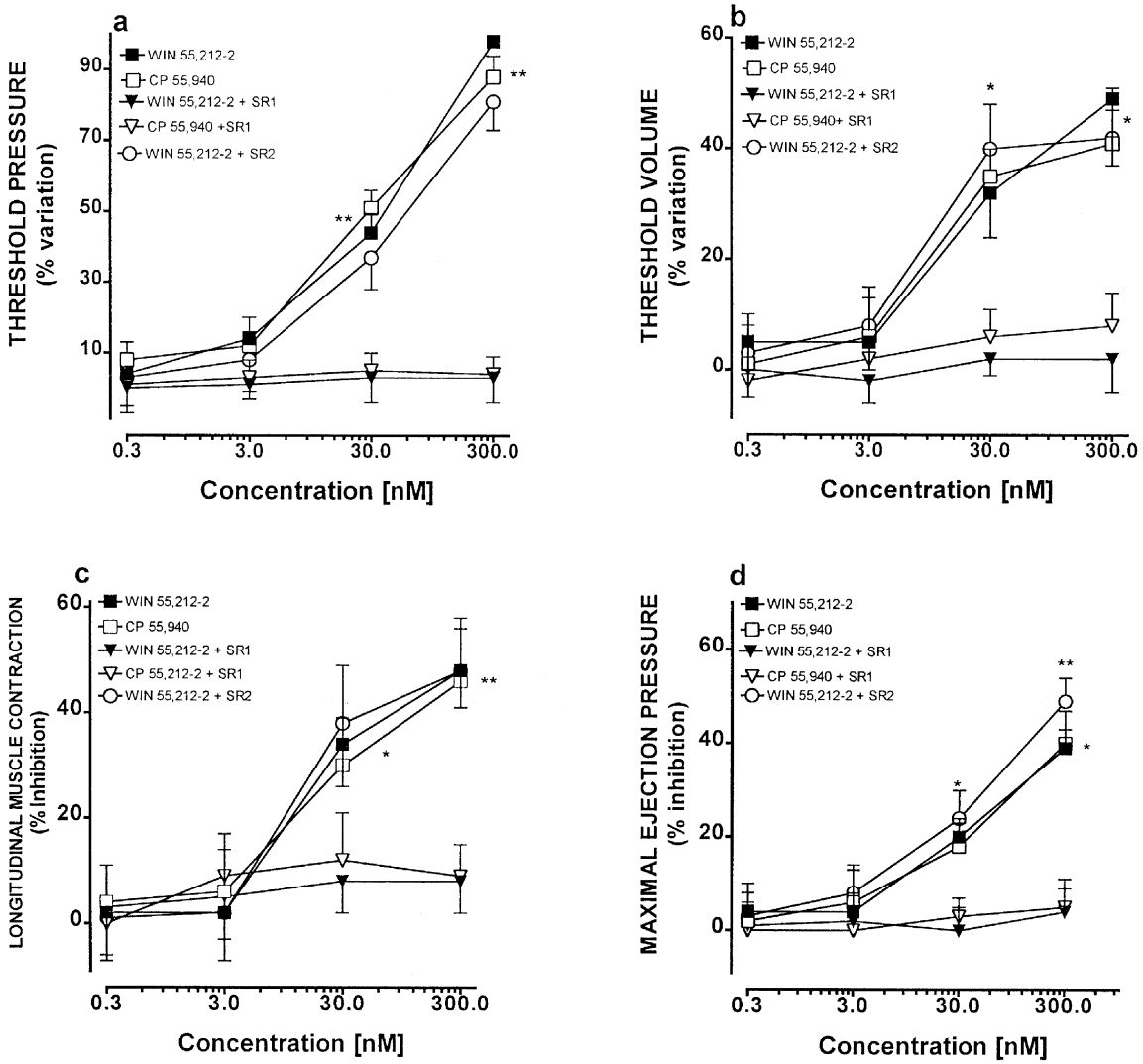

Figure 1 Effect of WIN 55,212-2 (0.3–300 nM) and CP55,940 (0.3–300 nM) alone or in combination with SR141716A (SR1 0.1 μM) or SR144528 (SR2 0.1 μM) on threshold pressure (Figure 1a), threshold volume (Figure 1b), longitudinal muscle contraction (Figure 1c) and maximal ejection pressure (Figure 1d). The ordinates show the percentage of variation (Figure 1a,b) or the percentage of inhibition (Figure 1c,d) compared to control response. Each point represents the mean of 6–8 experiments; vertical lines show s.e.mean $^*P<0.05$ and $^{**}P<0.01$ vs corresponding control.

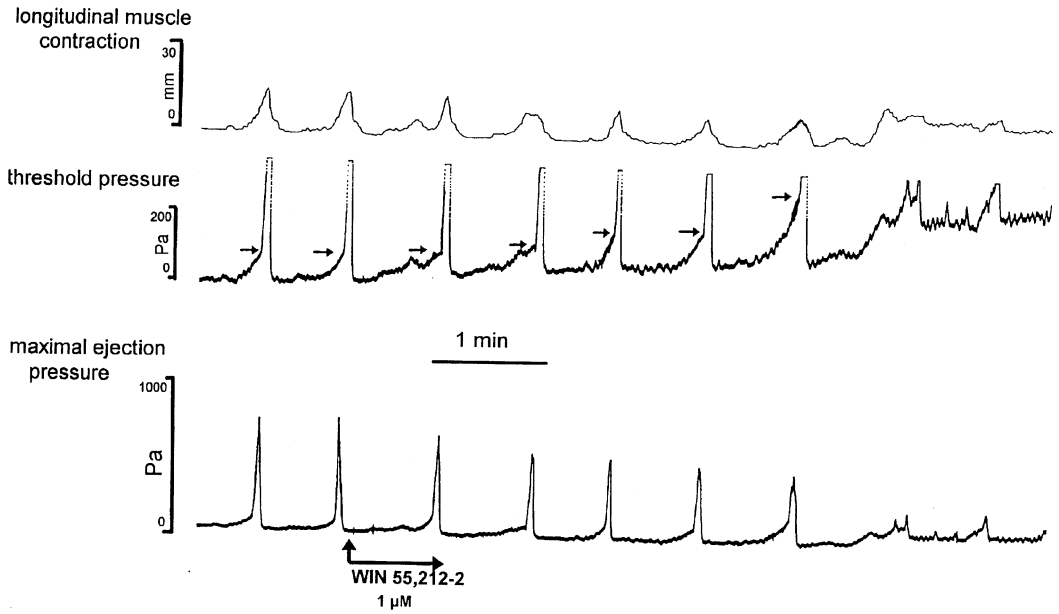

Figure 3 Trace showing the effect of WIN 55,212-2 (1 μM) on intestinal peristalsis (longitudinal muscle contraction, threshold pressure during the preparatory phase and maximal ejection pressure during the emptying phase) in the guinea-pig ileum. Longitudinal muscle contraction is measured with an isotonic transducer, threshold pressure (indicated by the arrow) with a pressure transducer at the aboral end recorded at high sensitivity and maximal ejection pressure with a pressure transducer at the aboral end recorded at low sensitivity.

(0.1 μM) (Figures 1 and 2). Figure 4 shows the effect of SR141716A (10, 30 and 100 nM) on WIN 55,212-2 (300 nM)- and CP55,940 (300 nM)-induced changes threshold pressure (Figure 4a), threshold volume (Figure 4b), longitudinal muscle contraction (Figure 4c) and maximal ejection pressure (Figure 4d). SR141716A (10, 30 and 100 nM) also counteracted the effect of WIN (300 nM) and CP55,940 (300 nM) on compliance in a concentration-dependent manner (data not shown).

In absence of any drug, SR141716A and AM281 did not modify threshold pressure, threshold volume, longitudinal muscle contraction (Figure 5a–c) and compliance (data not shown), but significantly ($P<0.05$) increased maximal ejection pressure to elicit peristalsis. By contrast the CB_2 receptor antagonist SR144528 (0.1 μM), when administered alone, did not modify the physiological parameter of peristalsis measured (per cent variation: threshold volume 1 ± 4, threshold pressure -2 ± 5, longitudinal muscle contraction $0\pm4\%$, maximal ejection pressure 2 ± 5, compliance $5\pm4\%$, $n=5$).

Discussion

Peristalsis is a coordinated pattern of motor behaviour which occurs in the gastrointestinal tract and allows the contents to be propelled in an anal direction. Two phases of peristalsis have been described in response to the slow infusion of liquid which radially stretches the intestinal wall: a preparatory phase, in which the intestine gradually distends until a threshold distension and an emptying phase, in which the circular muscle at the oral end of the intestine contracts, an effect followed by a wave of contraction that propagates aborally along the intestine. The pathways mediating peristalsis involve intrinsic primary enteric sensory neurons and interneurons, as well as excitatory and inhibitory motor neurons (Furness *et al*., 1998). Acetylcholine acting through both muscarinic and nicotinic receptors and tachykinins are excitatory neurotransmitters participating in the peristaltic activity (Tonini *et al*., 1981; Holzer & Maggi, 1994; Holzer *et al*., 1998), whereas vasoactive intestinal polypeptide, nitric oxide and an apamine-sensitive inhibitory transmitter act as inhibitory mediators (Grider, 1993; Waterman & Costa, 1994).

Prejunctional or presynaptic receptor systems, such as opioids, α_2-adrenoceptors or adenosine A_1 receptors are involved in the control of peristaltic movements (Kromer *et al*., 1980; Candura *et al*., 1992; Waterman *et al*., 1992; Poli & Pozzoli, 1997) while histamine H_3 agonists are not able to modulate the reflex-evoked peristaltic response (Poli & Pozzoli, 1997). In the present study we have observed multiple actions of cannabinoid drugs on different parameters of peristalsis.

Longitudinal muscle contraction

The longitudinal shortening of the intestine during the preparatory phase is a reflex response elicited by radial distension; it is mediated largely by excitatory cholinergic motoneurons to the longitudinal muscle (Kosterlitz & Robinson, 1959). The present study indicates that this contraction is reduced in amplitude by activation of CB_1 receptors. Consistent with this, a CB_1-mediated inhibition of the longitudinal muscle in strips of the guinea-pig small intestine, associated with a reduction of acetylcholine release, has previously been reported (Coutts & Pertwee, 1997; Ross *et al*., 1998). In addition, WIN 55,212-2 and CP55,940 inhibit fast and slow excitatory synaptic transmission in myenteric S-neurons through activation of CB_1 receptors (Lopez-Redondo *et al*., 1997). Therefore it is possible that cannabinoid agonists act at more than one site in the reflex pathways.

Compliance

The compliance of the intestinal wall reflects the resistance of the wall to dynamic distension. Compliance is an index of the activity of the circular muscle during the preparatory phase.

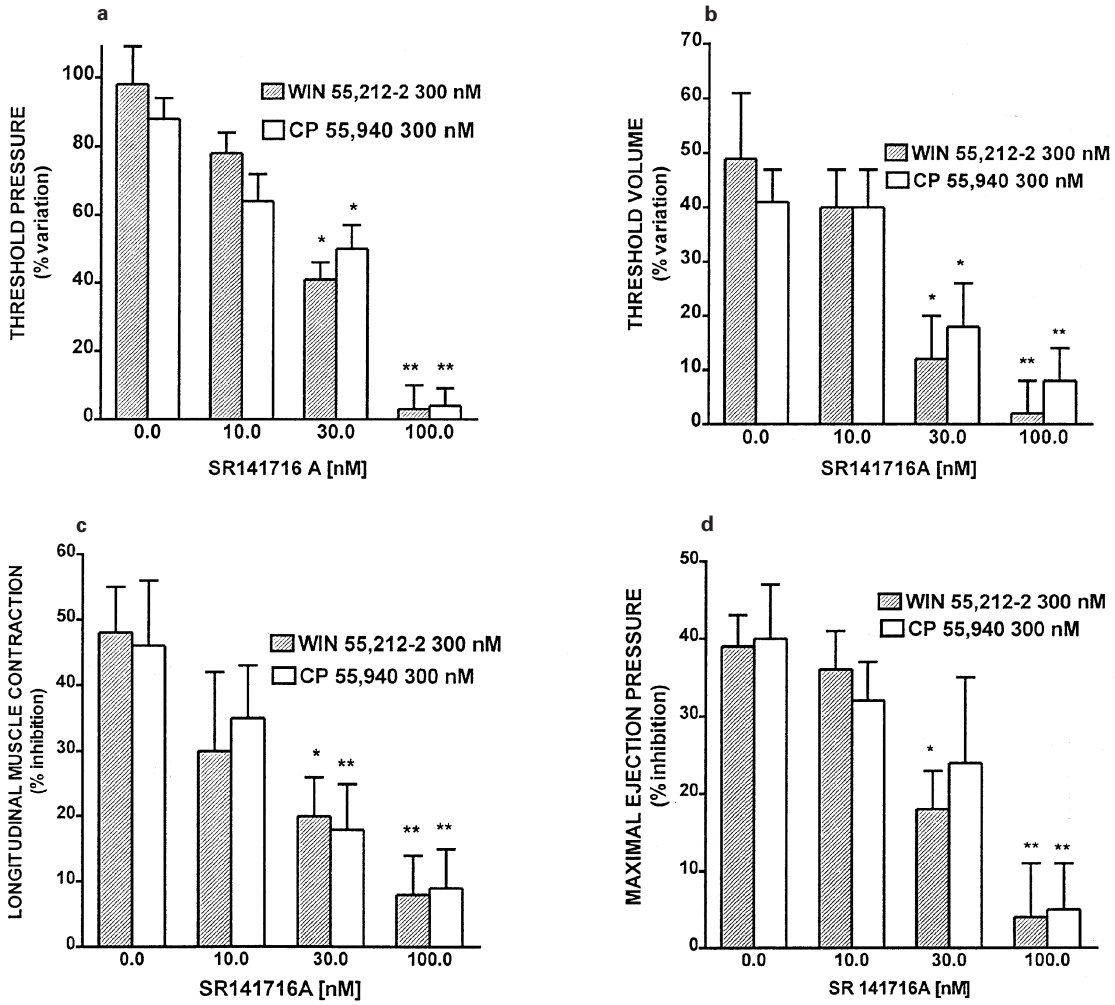

Figure 4 Effect of WIN 55,212-2 (300 nM) and CP55,940 (300 nM) alone or in the presence of various concentrations of SR141716A (10, 30 and 100 nM) on threshold pressure (Figure 4a), threshold volume (Figure 4b), longitudinal muscle contraction (Figure 4c) and maximal ejection pressure (Figure 4d). Each point represents the mean of 5–8 experiments; $*P<0.05$ and $**P<0.01$ vs WIN 55,212-2 300 nM (or CP55,940 300 nM) alone.

The greater the tone of the circular muscle, the more resistant it is to distension and consequently the less compliance. Compliance is influenced both by neural and by muscular factors. Drugs which block excitatory nerves to the circular muscle increase compliance, while drugs which inhibit enteric inhibitory nerves decrease compliance (Waterman *et al*., 1994). On the basis of these data, it was expected that cannabinoid agonists, which inhibit excitatory transmission (Izzo *et al*., 1998) could increase compliance. However, we have shown that the cannabinoid agonists WIN 55,2122-2 and CP55,940 significantly reduced compliance, an effect counteracted by the CB_1 antagonists SR141716A. The most likely explanation of these results is that cannabinoid agonists cause a decrease in the activity of enteric inhibitory nerves which mediates accommodation in the isolated guinea-pig small intestine (Waterman *et al*., 1994). There is no evidence in the literature about a modulation of enteric inhibitory nerves by cannabinoids drugs. However, it should be noted that central CB_1 receptors mediate inhibition of nitric oxide production by rat microglial cells (Waksman *et al*., 1999) and nitric oxide inhibition is associated with decreased compliance in the guinea pig ileum (Waterman *et al*., 1994). It is unlikely that cannabinoid agonists decrease compliance by acting directly on smooth muscle since it has been recently shown that they are able to inhibit electrically-evoked contractions without modifying the contractions produced by exogenous acetylcholine or substance P (Izzo *et al*., 1998).

Threshold for triggering peristalsis

During the preparatory phase, activity of circular muscle motor neurones determines the tone of the circular muscle and the resistance of the muscle to further distension. We have recently shown that activation of CB_1 receptors produces inhibition of cholinergic and tachykinergic transmission to the circular muscle of the guinea pig ileum (Izzo *et al*., 1998). In the present study we have demonstrated that the cannabinoid agonists WIN 55,212-2 and CP55,940 increased the threshold volume and pressure for triggering the emptying phase of peristalsis, an effect counteracted by the CB_1 antagonists SR141716A and AM281 but not by the CB_2 antagonist SR1445258. In some experiments, with high concentration of cannabinoid agonists (300 and 1 μM), a block of peristalsis was observed, in spite of an intraluminal pressure of 300 Pa.

Maximal ejection pressure

The extent of circular muscle contraction and its coordinated propagation determines the peak pressure generated by the intestine. This parameter was reduced, in a dose dependent

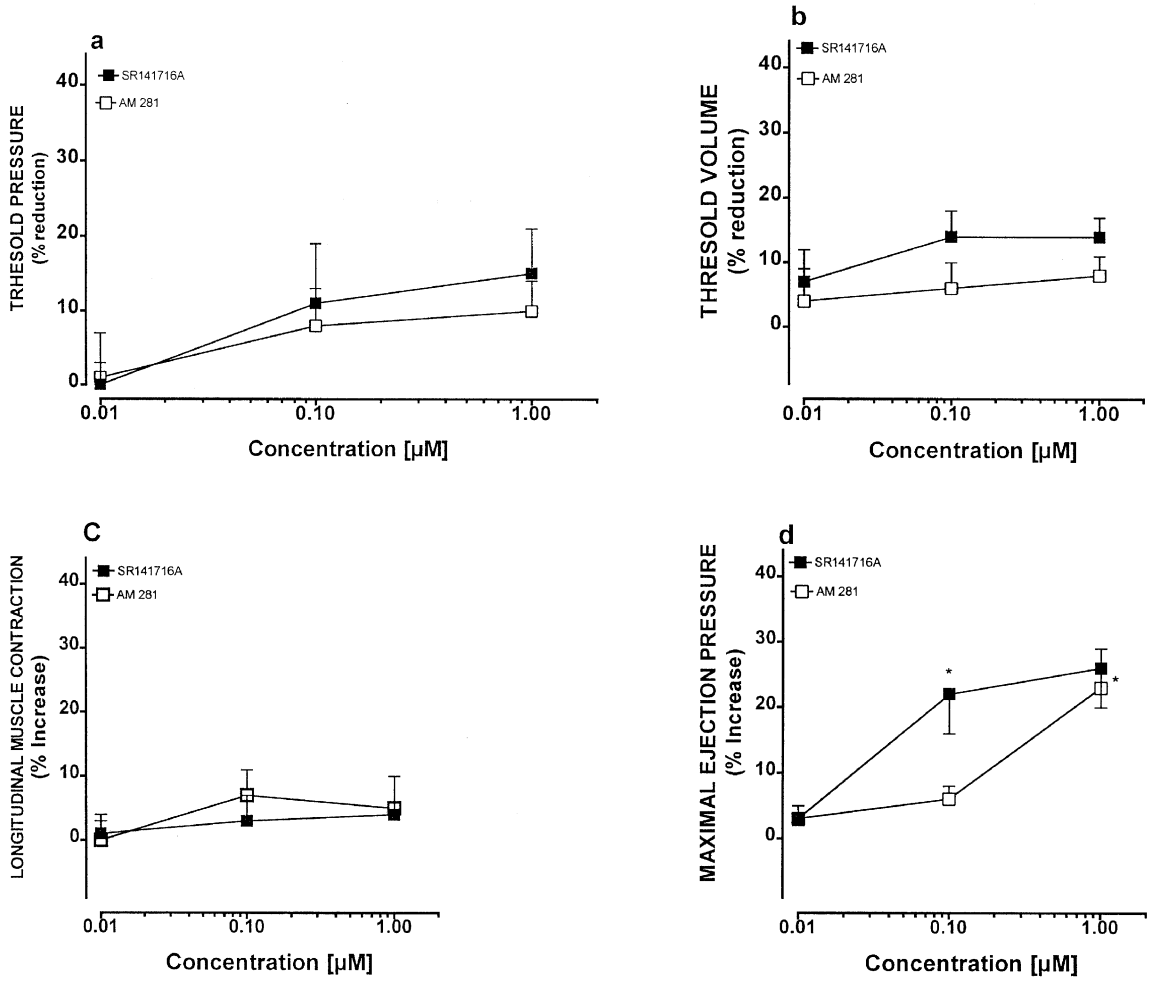

Figure 5 Effect of SR141716A (0.01 – 1 μM and AM281 (0.01 – 1 μM) on threshold pressure (Figure 5a), threshold volume (Figure 5b), longitudinal muscle contraction (Figure 5c) and maximal ejection pressure (Figure 5d). The ordinates show the percentage of reduction (Figure 5a,b) or the percentage of increase (Figure 5c,d) compared to control response. Each point represents the mean of 8 – 10 experiments; vertical lines show s.e.mean $*P<0.05$ vs corresponding control.

fashion, by selective activation of CB_1 but not CB_2 receptors. These effects can be explained by the inhibitory action of the cannabinoid agonists on excitatory (both cholinergic and tackykinergic) motor neurones to the circular muscle recently reported (Izzo *et al*., 1998).

The role of the endogenous cannabinoid system

There is evidence in the literature that intestinal motility can be tonically inhibited by the endogenous cannabinoid system. Indeed the presence of the endocannabinoid agonist 2-arachidonylglicerol has been demonstrated in the canine gut (Mechoullam *et al*., 1995), while the rat small intestine contains high amounts of anandamide hydrolase, the enzyme responsible of anandamide inactivation (Katayama *et al*., 1997). Functional studies indicate that excitatory transmission to the circular and longitudinal muscle of the guinea-pig ileum is potentiated by SR141716A, a CB_1 receptor antagonist (Izzo *et al*., 1998; Ross *et al*., 1998). Consistent with these *in vitro* results, SR141716A was found to increase upper gastrointestinal transit and defecation in mice (Colombo *et al*., 1998; Izzo *et al*., 1999a). The constitutive activity of CB_1 receptors in these systems, however, could not be attributed unequivocally to displacement of endocannabinoids as SR141716A behaves as inverse agonist at the human CB_1 receptors (MacLennan *et al*., 1998). In the present study we have observed a tendency of SR141716A and AM281 to decrease threshold pressure and volume to elicit peristalsis, but this effect was not significant. Therefore an activation of the endogenous cannabinoid system seems unlikely during the preparatory phase of the peristalsis. However, SR141716A and AM281, two selective CB_1 receptor antagonists increased maximal ejection pressure during the empty phase of peristalsis, thus indicating the possible activation of the endogenous cannabinoid system during the emptying phase of peristalsis. In other studies, it has been shown that cholinergic transmission to the longitudinal muscle of the human ileum *in vitro* (Croci *et al*., 1998) and gastric emptying in rats *in vivo* were unaffected by SR141716A (Izzo *et al*., 1999b).

Conclusions

We have shown that activation of CB_1 receptors, but not CB_2 receptors, inhibits most of physiological parameters of peristalsis. This inhibitory effect does not involve the activation of α-adrenoceptors or opioid receptors and could be attributed, at least in part, to an action of cannabinoid agonists on excitatory motor neurones innervating the circular

and longitudinal muscle as previously reported (Izzo *et al*., 1998; Ross *et al*., 1998). In addition, the emptying phase of peristalsis could be tonically inhibited by the endogenous cannabinoid system. This work emphasises the role of peripheral CB_1 receptors in the control of intestinal motility under physiological conditions and opens the possibility to investigate *in vivo* selective, non-psychotropic cannabinoid agonists or antagonists that may decrease (e.g. spasm, diarrhoea) or increase gut motility (ileus), respectively.

This work was supported by Cofinanziamento Murst 1999 and Enrico and Enrica Sovena Foundation (Roma). We wish to thank Miss Emilia Nocerino and Marianna Amato for their help. SR141716A and SR144528 were a kind gift from SANOFI (Montpellier, France).

References

CANDURA, S.M., MARRACCINI, P., COSTA, L.G., MANZO, L., ROSSI, A., COCCINI, T. & TONINI, M. (1992). Calcium entry blockade as a mechanism for chlordimeform-induced inhibition of motor activity in the isolated guinea-pig ileum. *Pharmacol. Toxicol.,* **71,** 426–433.

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.

FURNESS, J.B., KUNZE, W.A., BERTRAND, P.P., CLERC, N. & BORNSTEIN, J.C. (1998). Intrinsic primary afferent neurons of the intestine. *Prog. Neurobiol.,* **54,** 1–18.

GATLEY, S.L., LAN, R., PYATT, B., GIFFORD, A.N., VOLKOW, N.D. & MAKRIYANNIS, A. (1997). Binding of the non-classical cannabinoid CP55,940, and the diarylpyrazole AM251 to rodent brain cannabinoid receptors. *Life Sci.,* **61,** PL191–PL197.

GIFFORD, A.N., TANG, Y., GATLEY, S.J., VOLKOW, N.D., LAN, R. & MAKRIYANNIS, A. (1997). Effect of the cannabinoid receptor SPECT agent, AM 281, on hippocampal acetylcholine release from rat brain slices. *Neurosci. Lett.,* **283,** 84–86.

GRIDER, J.R. (1993). Interplay of VIP and nitric oxide in regulation of the descending relaxation phase of peristalsis. *Am. J. Physiol.,* **264,** G334–G340.

HOLZER, P., LIPPIE, I.Th., HEINEMANN, A. & BARTHO', L. (1998). Tachykinin NK_1 and NK_2 receptor-mediated control of peristaltic propulsion in the guinea-pig small intestine in vitro. *Neuropharmacology,* **37,** 131–138.

HOLZER, P. & MAGGI, C.A. (1994). Synergistic role of muscarinic acetylcholine and tachykinin NK-2 receptors in intestinal peristalsis. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **349,** 194–201.

HOWLETT, A.C. (1995). Pharmacology of cannabinoid receptors. *Annu. Rev. Pharmacol. Toxicol.,* **35,** 607–634.

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.

KATAYAMA, K., UEDA, N., KURAHASHI, Y., SUZUKU, H., YAMAMOTO, S. & KATO, I. (1997). Distribution of anandamide amidohydrolae in rat tissues with special reference to small intestine. *Biochim. Biophys. Acta.,* **1347,** 212–218.

KOSTERLITZ, H.W. & LEES, G.M. (1964). Pharmacological analysis of intrinsic intestinal reflexes. *Pharm. Rev.,* **16,** 301–339.

KOSTERLITZ, H.W. & ROBINSON, J.A. (1959). Reflex contractions of the longitudinal muscle coat of the isolated guinea-pig ileum. *J. Physiol.,* **146,** 369–379.

KROMER, W., PRETZLARR, W. & WOINOFF, R. (1980). Opioids modulate periodicity rather than efficacy of peristaltic waves in the guinea pig ileum in vitro. *Life Sci.,* **26,** 1857–1865.

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.

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.

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

POLI, E. & POZZOLI, C. (1997). Histamine H_3 receptor do not modulate reflex-evoked peristaltic motility in the isolated guinea-pig ileum. *Eur. J. Pharmacol.,* **327,** 49–56.

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.

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.

TONINI, M., FRIGO, G., LECCHINI, S., D'ANGELO, L. & CREMA, A. (1981). Hyoscine-resistant peristalsis in guinea-pig ileum. *Eur. J. Pharmacol.,* **71,** 375–381.

WAKSMAN, Y., OLSON, J.M., CARLISLE, S.J. & CABRAL, G.A. (1999). The central cannabinoid receptor (CB_1) mediates inhibition of nitric oxide production by rat microglial cells. *Br. J. Pharmacol.,* **288,** 1357–1366.

WATERMAN, S.A. & COSTA, M. (1994). The role of enteric inhibitory motoneurons in peristalsis in the isolated guinea-pig small intestine. *J. Physiol.,* **477.3,** 459–468.

WATERMAN, S.A., COSTA, M. & TONINI, M. (1992). Modulation of peristalsis in the guinea-pig isolated small intestine by exogenous and endogenous opioids. *Br. J. Pharmacol.,* **106,** 1004–1010.

WATERMAN, S.A., COSTA, M. & TONINI, M. (1994). Accommodation mediated by enteric inhibitory reflexes in the isolated guinea-pig small intestine. *J. Physiol.,* **474,** 539–546.

(Received June 2, 1999
Revised October 8, 1999
Accepted November 24, 1999)