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Short communication

Inhibition of small intestinal secretion by cannabinoids is CB_1 receptor-mediated in rats

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Abstract

We tested the hypothesis that cannabinoids, acting via a neuronal mechanism of action decrease small intestinal secretion. In vitro electrical stimulation induced ileal secretion in rats, that was attenuated by a cannabinoid receptor agonist, WIN 55212-2, (mesylate(*R*)-(+)-[2,3-dihydro-5-methyl-3-[4-morpholino)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl)methanone) but not its optical isomer WIN 55212-3. The inhibition of secretion induced by WIN 55212-2 was reversed by SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride), a cannabinoid CB_1 receptor antagonist. An ileal secretory response stimulated by acetylcholine was unaffected by WIN 55212-2. These findings show that cannabinoids inhibit neurally mediated secretion via cannabinoid CB_1 receptors. Thus, cannabinoids may have therapeutic potential for diarrhea unresponsive to available therapies.

Keywords: Cannabinoid receptor agonist; WIN 55212-2; Cannabinoid receptor antagonist; SR14716A; Ileum; Electrical field stimulation; Short-circuit current, (Rat)

1. Introduction

Marijuana is a well known recreational drug that has for centuries been prescribed therapeutically in herbal medicine for the treatment of a wide array of health concerns (Yousif and Oriowo, 1999), including many gastrointestinal disorders. Furthermore, constituents of marijuana (cannabinoids) have been implicated as a valid treatment in the US for both chemotherapy-induced and intractable cancer-related nausea and vomiting when other antiemetic medications fail (Gonzalez-Rosales and Walsh, 1997). Recent studies have shown that cannabinoids produce their effects through specific cannabinoid receptors, of which, two types have been identified (CB_1 and CB_2). These receptors are coupled to G proteins and have both been cloned (Yousif and Oriowo, 1999). Cannabinoid CB_1 receptors are present in the central nervous system (CNS) and in some peripheral tissues, including the gastrointestinal tract (Pertwee, 1993), while cannabinoid CB_2 receptors are present almost exclusively in peripheral tissues (Munro et al., 1993). Several potent cannabinoid receptor agonists have been synthesized including HU-210 (1′1,-dimethylheptyl homologue of (−)-11-hydroxy-Δ^8-THC), CP-55940 ((−)-3-[2-hydroxy-4-(1,1-dimethyl heptyl)phenyl]-4-(3-hydroxy propyl) cyclohexan-1-ol) and WIN 55212-2 (mesylate (*R*)-(+)-[2,3-dihydro-5-methyl-3-[4-morpholino)methyl] pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl) methanone) (Pertwee, 1997). These cannabinoid receptor ligands have been used in studies designed to investigate cannabinoid effects on gastrointestinal function including motility (Anderson et al., 1975; Shook and Burks, 1989; Colombo et al., 1998; Izzo et al., 1999), smooth muscle contractility (Pertwee et al., 1992; Pertwee et al., 1996; Coutts and Pertwee, 1997; Lopez-Redondo et al., 1997; Yousif and Oriowo, 1999; Heinemann et al., 1999) gastrointestinal transit and intestinal fluid accumulation (Sofia et al., 1978; Shook and Burks, 1989; Colombo et al., 1998; Izzo et al., 1999). In the gut,

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cannabinoids decrease gastric motility, slow transit and have an inhibitory effect on neurally mediated smooth muscle contractility. Cannabinoids also reduce intestinal fluid accumulation in vivo (Izzo et al., 1999). However, it is not known whether the cannabinoid effects on fluid accumulation: involved a direct inhibitory action on mucosal transport, were the result of changes in intestinal motility, or were due to changes in intestinal blood flow. All of these could produce significant inhibition of intestinal fluid accumulation.

The objective of the current study was, therefore, to further investigate the effect of cannabinoids on mucosal transport of fluids and electrolytes across the gastrointestinal tract. Specifically, we examined the direct effect of the cannabinoids on intestinal secretion using isolated mucosal/sub-mucosal plexus sheets of ileum mounted in modified Ussing chambers. Using this in vitro preparation, we tested our hypothesis that cannabinoids, acting via a neuronal mechanism, inhibit epithelial secretion. We also investigated the alternative hypothesis that cannabinoids exert direct (non-neuronal) effects to modulate intestinal fluid and electrolyte transport.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats weighing 230–260 g (Charles River, Wilmington, MA) were housed two per cage in standardized conditions (12/12 h light/dark cycles and 23°C). Rats were acclimatized to the animal facility with free access to food and water for a minimum of 1 week prior to tissue harvesting. Following humane euthanasia, via sedation with isoflo (5%) and decapitation, a midline incision exposed the small intestine. The ileum was then quickly removed, flushed of intestinal contents and placed into ice-cold oxygenated Krebs buffer. All studies were approved by the Animal Care and Use Committee at the Oklahoma City, V.A. Medical Center which is an American Association for Accreditation for Laboratory Animals Care approved facility.

2.2. Tissue preparation

Using a previously described methodology (Greebwood-Van Meerveld et al., 1999), the external, longitudinal muscle layer and attached myenteric plexus were gently peeled off and mucosal sheets were mounted in a modified Ussing chamber system (0.6 cm^2 exposed surface area and 10 ml bathing reservoirs) (WPI, FL, USA). The tissues were bathed in 20 ml of Kreb's buffer containing 11.2 mM glucose (serosal buffer) and 11.2 mM mannitol (mucosal buffer). The buffer was maintained at 37°C and continuously aerated (95% oxygen, 5% carbon dioxide).

2.3. Measurement of electrogenic ion transport in Ussing chambers.

Each mucosal sheet was allowed to equilibrate for 20 min and the transmural PD was recorded by a pair of agar salt bridge electrodes connected to an EVC-4000 multichannel voltage/current clamp apparatus designed for the study of electrical properties of epithelial tissues (World Precision Instruments, FL, USA). Preparations showing low values of basal transmural PD (less than 0.5 mV) were discarded. Following the equilibration period, the voltage was clamped to zero potential difference and the short circuit current (Isc) was continuously recorded via a Power Macintosh equipped with a MacLab Data Acquisition System (AD Instruments, Castle Hill, Australia).

2.4. Electrical field stimulation

Electrical field stimulation at 5 Hz, 100 V, 0.5 ms in trains for a total of 5 s across the tissue were accomplished using a Grass S88 Stimulator, a SIU5 Stimulation Isolation Unit (Astro-Med, RI, USA) and foil ribbon electrodes (Cooke et al., 1983). Electrical field stimulation was initially performed under basal conditions and then repeated following addition of test substances.

2.5. Drugs and chemicals

The cannabinoid receptor ligands WIN 55212-2, (mesylate (*R*)-(+)-[2,3-dihydro-5-methyl-3-[4-morpholino)-methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-naphthyl)methanone) its inactive optical isomer WIN 55212-3 were obtained from RBI (Natick, MA, USA), SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydro-chloride) was kindly supplied by Sanofi Researche (Montpellier, France). All cannabinoids were dissolved in dimethylsulphoxide (Sigma MO, USA) and then diluted in Kreb's buffer containing 0.05% bovine serum albumin. The final bath concentration of dimethylsulphoxide was $< 1\%$ at all drug concentrations. Acetylcholine chloride and compounds used in the Kreb's buffer were acquired from Sigma. The Kreb's buffer was made up as follows (in mM): NaCl (120), $NaHCO_3$ (14.4), glucose/mannitol (11.2), KCl (6), $CaCl_2$ (2.5), $MgCl_2$ (1.2) and NaH_2PO_4 (1.2).

2.6. Experimental

Following the 20-min equilibration period, basal Isc, was recorded for 10–15 min to attain steady-state Isc. To examine the effect of either WIN 55212-2, WIN 55212-3, SR141716A or the dimethylsulphoxide vehicle on basal Isc, the compounds were added to the serosal bathing solution and in separate experiments, basal Isc was monitored for an additional 90 min.

In experiments to study the effects of WIN 55212-2 and WIN 55212-3 on the electrical field stimulation-induced ΔIsc, we allowed the tissue to equilibrate for 20 min and recorded basal Isc for 10–15 min. Two control electrical stimulations were then performed and the maximal increase in Isc was measured. As soon as the Isc had returned to basal levels (3–5 min), the tissue was pretreated with a single concentration of WIN 55212-2 or WIN 55212-3. Dimethylsulphoxide-treated tissues served as controls to ensure that the increase in Isc was reproducible over time. After, 60-min electrical field stimulation was repeated and the maximal increase in Isc was measured. This time point was selected based upon pilot data demonstrating that WIN 55212-2 produced its maximal inhibitory effect between 50–70 min following addition into the serosal bathing medium, and support previous observations of Pertwee et al. (1992). In the next series of experiments to examine the effect of SR141716A on the WIN 55212-2 induced change in Isc, induced by electrical field stimulation, we pretreated the tissue with SR141716A 15 min prior to the addition of WIN 55212-2. After, 60-min electrical field stimulation was repeated and the maximal increase in Isc was measured.

To investigate the effect of WIN 55212-2 on the increase in Isc induced by exogenous addition of acetylcholine, a single concentration of acetylcholine (1 μM), known to increase Isc from previous studies (Isaacs et al., 1976) was added to the serosal bathing solution and the maximal increase in Isc was recorded. After the acetylcholine-induced increase in Isc returned to basal values (usually within 5 min), a single concentration of WIN 55212-2 was added to the serosal bathing solution and 60 min later, the effect of acetylcholine (1 μM) on Isc was investigated. Dimethylsulphoxide-treated tissues served as controls to ensure that the increase in Isc produced by acetylcholine was reproducible.

2.7. Expression of results

The effect of the agonist and antagonist on Isc were measured as peak changes from baseline values and expressed as $\mu A/cm^2$. These values were transferred to an Excel spreadsheet and all results were expressed as mean ± S.E.M. Graphs were drawn using Cricket graph software. Statistical significance was determined by one-way analysis of variance with Dunnett's post test or by one-way analysis of variance with Tukey–Kramer Multiple Comparisons post test, where appropriate. Statistical significance was set at $P < 0.05$. All statistical analysis was performed using GraphPad InStat version 3.1 for Windows 95/NT (GraphPad Software, CA, USA).

3. Results

In the current series of experiments, none of the compounds tested including WIN 55212-2, WIN 55212-3, SR141716A or the dimethylsulphoxide vehicle had significant effect on basal Isc when added to the serosal bathing medium during the 90-min recording period. However, electrical field stimulation produced a significant increase in basal Isc, from 41.2 ± 2.8 to 82.6 ± 4.6 $\mu A/cm^2$ ($P < 0.001$). In vehicle-control experiments, we found that the increase in Isc induced by electrical field stimulation was consistent over time (46.5 ± 4.6 $\mu A/cm^2$ at the start of the experiments and 48.5 ± 4.5 $\mu A/cm^2$ after 90 min). Following the addition of WIN 55212-2 into the serosal

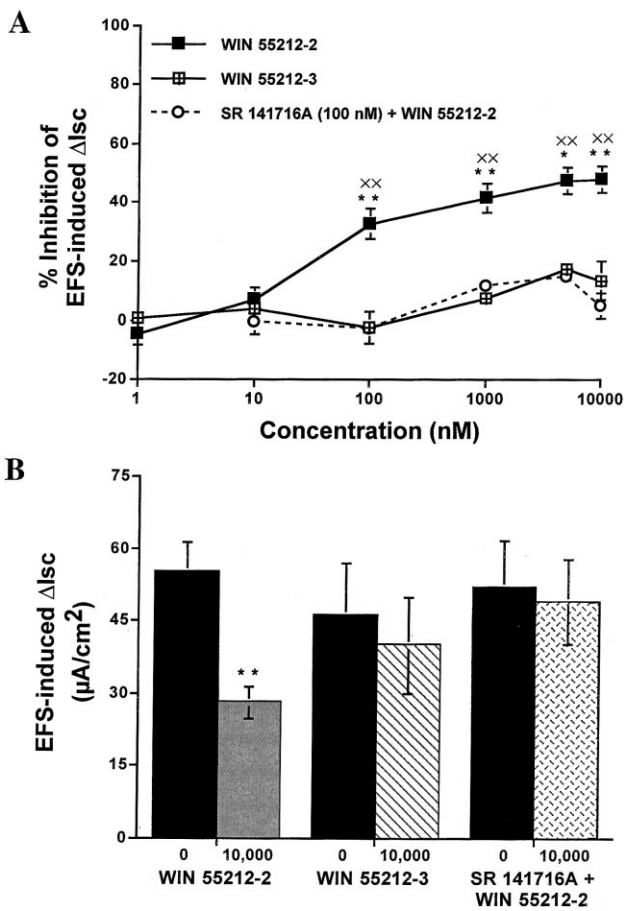

Fig. 1. (A) Direct comparison of WIN 55212-2 ($n = 6$ sheets/concentration), WIN 55212-3 ($n = 4$ sheets/concentration) and WIN 55212-2 in the presence of 100 nM SR141716A ($n = 6$ sheets/concentration) on the electrical field stimulation-induced increase in Isc. WIN 55212-2 produced a significant inhibition of the electrical field stimulation-induced increase in Isc at concentrations of 100, 1000, 5000 and 10,000 nM. Addition of WIN 55212-3 had no significant inhibitory effect on the electrical field stimulation-induced increase in Isc. Pre-treatment of sheets with 100 nM SR141716A before additions of WIN 55212-2 resulted in reversal of the inhibitory effect (* denotes $P < 0.01$, ** denotes $P < 0.001$ compared to WIN 55212-3 and xx denotes $P < 0.001$ compared to WIN 55212-3 in the presence of SR141716A). (B) Representation of the effect of 10,000 nM concentrations of WIN 55212-2, WIN 55212-3 and WIN 55212-2 in the presence of 100 nM SR141716A on the electrical field stimulation-induced increase in Isc. Control electrical field stimulation, performed before the addition of cannabinoids and represented by the solid bars, are compared to electrical field stimulation performed following cannabinoid additions.

Table 1
Effect of WIN 55212-2 on the acetylcholine-induced ΔIsc

Pre-WIN 55212-2 Acetylcholine response ΔIsc ($\mu A/cm^2$)	(nM)	Post-WIN 55212-2 Acetylcholine response ΔIsc ($\mu A/cm^2$)
46.5 ± 6.9	1	46.5 ± 6.6
46.2 ± 4.7	10	46.5 ± 4.8
44.1 ± 4.6	100	44.1 ± 4.8
46.4 ± 5.6	1000	46.7 ± 5.8
51.0 ± 6.3	5000	51.3 ± 6.1
49.7 ± 6.1	10,000	50.0 ± 6.8

bathing medium, there was an inhibition of the electrical field stimulation evoked increase in Isc (Fig. 1). This inhibition was not observed in response to mucosal addition of WIN 55212-2. Although the maximum inhibition of the electrical field stimulation-induced increase in Isc by WIN 55212-2 ($47.8 \pm 4.6\%$) occurred at a concentration of 10,000 nM, this inhibitory response was not significantly different from the inhibition produced by lower concentration of WINf-2 (1000 or 5000 nM). Conversely, the inactive isomer WIN 55212-3 had no significant inhibitory effect on the electrical field stimulation-induced increase in Isc at any concentration investigated (Fig. 1).

In the next series of experiments, we investigated the effect of addition of WIN 55212-2 on the increase in Isc induced by the addition of acetylcholine (1 μM) into the serosal bathing medium. As expected, acetylcholine produced a marked increased in Isc of 48 ± 2.4 $\mu A/cm^2$, ($P < 0.001$). The acetylcholine-induced increase in Isc was unaffected by pretreatment of ileal tissue with WIN 55212-2 (1–10,000 nM) (Table 1).

In a separate series of experiments, we investigated the effect of the cannabinoid CB_1 receptor antagonist SR-171416A (100 nM) on the inhibition of the electrical field stimulation-induced Isc response produced by WIN 55212-2. In these experiments, SR141716A (100 nM) was added following the control electrical field stimulation. After a 15-min pretreatment period, a single concentration of WIN 55212-2 was added to the serosal bathing solution. After, 60-min electrical field stimulation was repeated and our results showed that SR141716A completely attenuated then WIN 55212-2 induced inhibition of Isc (Fig. 1). Additional experiments found that SR141716A alone at a concentration of 100 nM had no effect on the EFS-induced increase in Isc.

4. Discussion

In the present study, we demonstrated that the cannabinoid receptor agonist WIN 55212-2, but not its optical isomer, effectively inhibited neuronal-mediated ileal secretion, measured electrically as an increase in Isc. Furthermore, we found that the inhibition of Isc induced by WIN 55212-2 was reversed by the cannabinoid CB_1 receptor selective antagonist SR141716A. Our results suggest that the inhibition of neurally evoked ileal secretion by WIN 55212-2 occur through a reversible cannabinoid CB_1 receptor-mediated mechanism. These findings provide compelling evidence that cannabinoid CB_1 receptors are involved in the regulation of small intestinal water and electrolyte transport in the rat ileum.

Cannabinoid receptor agonists have been shown to inhibit the electrically evoked contractions of myenteric plexus-longitudinal muscle preparations through prejunctional cannabinoid CB_1 receptors. This results in inhibition of contractile activity by blocking the release of acetylcholine from neurons of the myenteric plexus (Coutts and Pertwee, 1997). The results of this present study suggest that cannabinoid receptor agonists may produce their antisecretory effects through a neuronal mechanism, which in all likelihood, involves the inhibition of acetylcholine release from neurons of the sub-mucosal plexus. This hypothesis is supported by our experiments showing that although WIN 55212-2 inhibited a neurally evoked secretory response, the compound had no inhibitory effect on the increase in Isc induced by exogenous addition of acetylcholine. In addition, because this current study was performed in vitro using isolated sheets of intestine in which the longitudinal muscle layer and myenteric plexus had been removed, the effects of cannabinoid on mucosal ion transport appear to be independent of changes in either longitudinal smooth muscle activity, myenteric neuronal activity or mucosal blood flow. Thus, we conclude that cannabinoids can play a neuromodulatory role in the small intestinal mucosal transport function and may prove to be an alternative therapeutic approach for the treatment of diarrhea, which is unresponsive to currently available therapies.

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