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Cannabinoid modulation of intestinal propulsion in mice

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Abstract

The effect of cannabinoid receptor activation and blockade on the propulsive activity in the mouse small intestine was assessed in the present study by measuring the transit of an orally administered, non-absorbable marker. The cannabinoid receptor agonist WIN 55,212-2 (*R*(+)-[2,3-dihydro-5-methyl-3[(morpholinyl)methyl]pyrrolo[1,2,3-de-1,4benzoxazin-yl]-(1-naphthalenyl)methanone mesylate) inhibited, while the selective cannabinoid CB_1 receptor antagonist SR 141716A (*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide) stimulated the marker transit. Furthermore, a per se non-effective dose of SR 141716A reversed WIN 55,212-2-induced reduction of the transit. The results of the present study suggest a role for cannabinoid CB_1 receptors in the control of propulsive activity in the mouse small intestine. © 1998 Elsevier Science B.V.

Keywords: Small intestine; Intestinal propulsive activity; Cannabinoid CB_1 receptor; WIN 55,212-2; SR 141716A; (Mouse)

1. Introduction

Recent studies indicate that cannabinoid receptors, beside their distribution in the central nervous system (see Herkenham, 1995), are also located in some peripheral tissues, including the small intestine of guinea pigs (Paterson and Pertwee, 1993) and mice (Pertwee, 1993). More recent in vitro studies by Pertwee and colleagues have demonstrated that different cannabinoid receptor agonists inhibit the electrically-evoked contractions of a myenteric plexus-longitudinal muscle preparation from guinea-pig small intestine and that the selective cannabinoid CB_1 receptor antagonist, SR 141716A (*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide) (Rinaldi-Carmona et al., 1994), (a) completely abolished this effect and (b) exerted an opposite action, stimulating the amplitude of these contractions, when added alone to the preparation (Pertwee et al., 1996; Coutts and Pertwee, 1997). These effects of cannabinoid receptor agonists and SR 141716A are likely mediated by a reduction and an increase, respectively, of acetylcholine release from myenteric neurons. Collectively, these results lead to the hypothesis that the cannabinoid receptors may be involved in the regulation of small intestine propulsive activity.

The present study was designed to verify this hypothesis by assessing the effect of the cannabinoid receptor agonist, WIN 55,212-2 (*R*(+)-[2,3-dihydro-5-methyl-3[(morpholinyl)methyl]pyrrolo[1,2,3-de-1,4benzoxazin-yl]-(1-naphthalenyl)methanone mesylate), the antagonist SR 141716A and their combination on the propulsion of a non-absorbable marker chyme in mice.

The selection of the mouse, as the animal species to be used in the present study, was determined by the adoption in this laboratory of the reliable and sensitive 'upper gastrointestinal transit' ex vivo procedure, recently set up and validated by Nagakura et al. (1996) in mice.

2. Materials and methods

2.1. Animals

Male ICR mice (Harlan Nossan, Correzzana, MI, Italy), weighing 30–35 g, were used. After delivery to our animal facility, mice were left undisturbed for 7 days to adapt to the new housing conditions. Mice were housed 20 per cage in standard plastic cages (55 × 33 × 19 (h) cm) with wood

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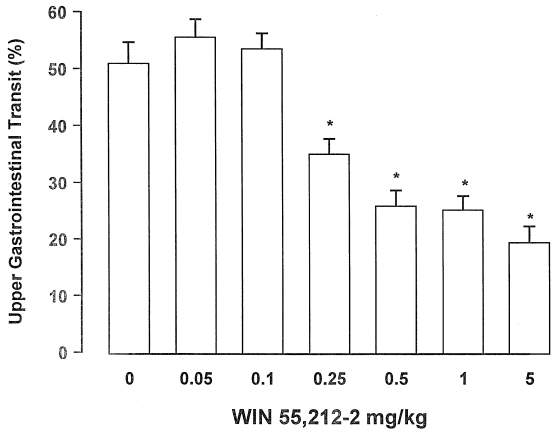

Fig. 1. Effect of the cannabinoid receptor agonist, WIN 55,212-2, on propulsive activity in the mouse small intestine. WIN 55,212-2 was administered i.p. 20 min prior to the i.g. administration of the non-absorbable marker, carmine. 20 min later, mice were killed and the distance travelled by the head of the marker, between the pylorus and the cecum, was measured and expressed as percent of total length of the small intestine. Each bar is the mean ± S.E.M. of 15 mice. $^{*}P < 0.01$ with respect to vehicle-treated mice (Newman–Keuls test).

chip bedding under a 12 h artificial light–dark cycle (lights on at 8.00 a.m.), at a constant temperature of 22 ± 2°C and relative humidity of 60%. Tap water and standard laboratory rodent chow (MIL Morini, San Polo d'Enza, RE, Italy) were provided ad libitum.

2.2. Procedure

The present study employed the procedure recently conceived and validated by Nagakura et al. (1996). Validity of the arrangement obtained in this laboratory was demonstrated by preliminary experiments where neostigmine (0.1 mg/kg, s.c.) and clonidine (0.3 mg/kg, s.c.) stimulated by 30% and inhibited by 80%, respectively, the propulsive activity in the mouse small intestine. Furthermore, as in the reference study (Nagakura et al., 1996), the marker was consistently found at approximately 50% of the small intestine, 20 min after its administration to undrugged mice.

In experiment 1, WIN 55,212-2 (0, 0.05, 0.1, 0.5, 1 and 5 mg/kg, corresponding to 0, 0.095, 0.19, 0.95, 1.9 and 9.5 μmol/kg) was administered i.p. 20 min before the i.g. administration of the marker (0.3 ml/mouse carmine). 20 min later, mice were killed by cervical dislocation and intestines were removed from the pylorus to the cecum. The distance covered by the head of the marker was measured and expressed as percent of the total length of the small intestine.

In experiment 2, SR 141716A (0, 0.62, 1.25, 2.5, 5 and 10 mg/kg, corresponding to 0, 1.344, 2.687, 5.375, 10.75 and 21.5 μmol/kg) was administered i.p. 20 min prior to the i.g. administration of the marker (0.3 ml/mouse carmine). 20 min later, mice were killed and intestines were removed as described above.

In experiment 3, SR 141716A (0 and 0.62 mg/kg) was administered i.p. 10 min prior to the i.p. injection of WIN 55,212-2 (0 and 0.5 mg/kg). The marker (0.3 ml/mouse carmine) was administered i.g. 20 min afterwards. 20 min later, mice were killed and intestines were removed as described above.

2.3. Drugs

SR 141716A (donated by Sanofi Recherche, Montpellier, France) and *R*(+)-WIN 55,212-2 mesylate (Research Biochemical International, Natick, MA) were suspended in saline with 0.1% Tween 80 and injected in a 12.5 ml/kg volume. Carmine (Sigma Chemical Co, St. Louis, MO) was dissolved at the concentration of 6% (w/v) in tap water containing 0.5% methylcellulose.

2.4. Data analyses

In each experiment, statistical evaluation of the upper gastrointestinal transit, expressed as percentage of the distance travelled by the head of the marker over the total length of the small intestine, was performed by a one-way analysis of variance, followed by the Newman–Keuls test for post-hoc comparisons.

3. Results

In experiment 1, administration of WIN 55,212-2 (0.05–5 mg/kg, i.p.) resulted in a dose-dependent reduction, up to approximately 60% in comparison to vehicle-treated mice, of the propulsive activity in the mouse small intestine ($F(6, 104) = 26.33$, $P < 0.01$) (Fig. 1).

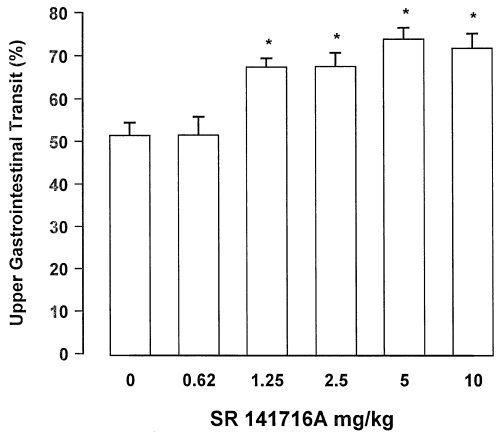

Fig. 2. Effect of the cannabinoid CB_1 receptor antagonist, SR 141716A, on propulsive activity in the mouse small intestine. SR 141716A was administered i.p. 20 min prior to the i.g. administration of the non-absorbable marker, carmine. 20 min later, mice were killed and the distance travelled by the head of the marker, between the pylorus and the cecum, was measured and expressed as percent of total length of the small intestine. Each bar is the mean ± S.E.M. of 15 mice. $^{*}P < 0.01$ with respect to vehicle-treated mice (Newman–Keuls test).

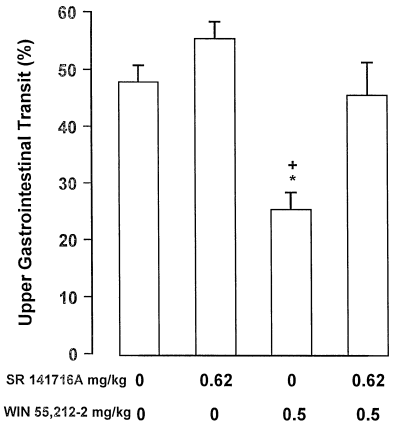

Fig. 3. Effect of the combination of SR 141716A and WIN 55,212-2 on propulsive activity in the mouse small intestine. SR 141716A was administered i.p. 10 min prior to the i.p. injection of WIN 55,212-2. 20 min later, the non-absorbable marker, carmine, was administered i.g. Mice were killed 20 min after the marker administration; the distance travelled by the head of the marker, between the pylorus and the cecum, was measured and expressed as percent of total length of the small intestine. Each bar is the mean ± S.E.M. of 15 mice. $^{*}P < 0.01$ with respect to 0 mg/kg SR 141716A + 0 mg/kg WIN 55,212-2-treated mice (Newman–Keuls test); $^{+}P < 0.01$ with respect to 0.62 mg/kg SR 141716A + 0.5 mg/kg WIN 55,212-2-treated mice (Newman–Keuls test).

Administration of SR 141716A (0.62–10 mg/kg, i.p.) (experiment 2) exerted an opposite action, dose-dependently stimulating the propulsion of the small intestine up to approximately 45% with respect to control mice ($F(5, 89) = 7.66$, $P < 0.01$) (Fig. 2).

Finally, injection of 0.62 mg/kg SR 141716A, which did not affect per se the upper gastrointestinal transit ($P > 0.05$, Newman–Keuls test), resulted in the blockade of the inhibitory action of 0.5 mg/kg WIN 55,212-2 on the propulsive activity of the mouse small intestine ($F(3, 59) = 11.06$, $P < 0.01$) (experiment 3; Fig. 3).

4. Discussion

The results of the present study demonstrate that cannabinoid CB_1 receptors are involved in the regulation of the intestinal propulsion in the mouse small intestine. Indeed (a) the cannabinoid receptor agonist WIN 55,212-2 dose-dependently inhibited the transit of an orally administered non-absorbable marker using the 'upper gastrointestinal transit' test conceived by Nagakura et al. (1996) and (b) the selective cannabinoid CB_1 receptor antagonist, SR 141716A, blocked the WIN 55,212-2-induced inhibition of intestinal propulsion at a dose that, per se, did not affect the marker transit.

Interestingly SR 141716A, when administered alone, dose-dependently increased the marker transit in the mouse small intestine. This prokinetic action might be due to SR 141716A (a) antagonizing an endogenous cannabinoid tone that normally inhibits the intestinal propulsive activity or (b) behaving as an inverse agonist at the cannabinoid CB_1 receptor.

The results of the present study are in agreement with the electrophysiological evidence of (a) cannabinoid agonists inhibiting and (b) SR 141716A stimulating the electrically-evoked contractions in myenteric muscle preparations from guinea-pigs (Pertwee et al., 1996). Furthermore, WIN 55, 212-2-induced inhibition of the chyme transit is consistent with several anedoctal reports suggesting the efficacy of marijuana in alleviating dysentery (see Grinspoon and Bakalar, 1993).

The results of this study suggest that the cannabinoid ligands may have a potential therapeutic relevance for intestinal motility disorders.

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