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Tolerance to cannabinoid response on the myenteric plexus of guinea-pig ileum and human small intestinal strips

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1 We studied tolerance to cannabinoid agonist action by comparing the *in vitro* inhibition of electrically evoked contractions of longitudinal muscle from small intestine of human and guinea-pig (myenteric plexus preparations) after 48-h incubation with the synthetic agonist (+) WIN 55,212-2. We also investigated the intrinsic response to the selective cannabinoid CB_1 receptor antagonist rimonabant in control and tolerant strips.

2 (+) WIN 55,212-2 inhibited guinea-pig (IC_{50} 4.8 nM) and human small intestine (56 nM) contractions with similar potency before or after 48-h incubation in drug-free conditions; this effect was competitively antagonized by rimonabant (pA_2, 8.4, 8.2). A 48-h preincubation with (+) WIN 55,212-2, but not with (−) WIN 55,212-3, completely abolished the acute agonist response in both tissue preparations. The opiate K-receptor agonist U69593 inhibited human small intestine contractions with a similar potency in control and strips tolerant to (+) WIN 55,212-2, IC_{50} 39 and 43 nM.

3 Unlike human tissue, in guinea-pig small intestine, which has a high level of endocannabinoids, rimonabant alone increased the twitches induced by the electrical field stimulation (EC_{50} 100 nM) with a maximal effect of 123%.

4 In strips tolerant to (+) WIN 55,212-2, rimonabant markedly increased (155%) the electrical twitches in human ileum and in guinea-pig myenteric plexus smooth muscle (133%).

5 This study shows tolerance can be induced to the cannabinoids' action in intestinal strips of human and guinea-pig by long *in vitro* incubation with the agonist (+) WIN 55,212-2.
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Abbreviations: 2-AG, 2-arachidonoyl glycerol; AEA, anandamide; DMSO, dimethylsulfoxide; OEA, oleoylethanolamide

Introduction

The extensive recreational use of marijuana and its promotion for the treatment of emesis, pain and loss of appetite, are drawing increasing attention to the adverse consequences of chronic exposure, including tolerance and dependence. Tolerance and dependence to the cannabinoid action, with the underlying molecular events, have been studied in different organs and systems of many animal species, particularly the central nervous system of small rodents (for reviews see: Dewey, 1986; Pertwee, 1991; 1995; Tanda & Goldberg, 2003).

The gastrointestinal system is one of the cannabinoids' preferred target organs and many experimental and clinical studies have looked into the action of cannabimimetic drugs and endogenous cannabinoids on this system, but only a few have tackled the question of tolerance after chronic administration and the consequences of abrupt withdrawal on intestinal functions (Anderson *et al*., 1975; Basilico *et al*., 1999; and see for reviews: Pertwee, 2001; Hornby & Prouty, 2004). *In vivo* and *in vitro* experimental evidence indicates that cannabinoids reduce gastrointestinal transit in animals and relax intestinal smooth musculature, meaning they are important in regulating normal intestinal function and are probably involved in the pathogenesis of gut diseases (Izzo *et al*., 1999a; 2001; 2003; Mascolo *et al*., 2002; Pinto *et al*., 2002a; Kunos & Pacher, 2004; Massa *et al*., 2004). In addition, the pharmacological blockade of fatty acid amide hydroxylase, which catalyzes the hydrolysis of endocannabinoids and other bioactive amides inhibited gut motility through the activation of enteric cannabinoid CB_1 receptors (Capasso *et al*., 2005); this effect was antagonized by the selective cannabinoid CB_1 receptor antagonist rimonabant. Unlike CB_1 agonists, rimonabant increased gut peristalsis in mice, but tolerance to this effect rapidly developed after repeated treatments (Carai *et al*., 2004).

In previous studies on human innervated ileal and colonic preparations, we found that the synthetic cannabinoid agonist (+) WIN 55,212-2 reduced the neural electrically evoked contractions fully sensitive to atropine and tetrodotoxin (Croci *et al*., 1998; Manara *et al*., 2002). In the same studies, we also showed that (+) WIN 55,212-2 response was competitively inhibited by rimonabant. These results are consistent with the view that the most likely mechanism for the cannabinoid-mediated reduction in gut motility is the inhibition of release of

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excitatory neurotransmitters such as acetylcholine and substance P, from enteric neurons, through the stimulation of prejunctional cannabinoid receptors of the CB_1 subtype (Pertwee, 2001; Pinto *et al*., 2002b; Hornby & Prouty, 2004).

Intestinal signs of tolerance to marijuana and synthetic cannabinoids have been described in a few studies (for reviews see: Jones, 1993; Tanda & Goldberg, 2003). Thus, repeated treatment with Δ^9-tetrahydrocannabinol reduced the inhibitory effects of a challenge dose of this cannabinoid on gastrointestinal transit in mice and on electrically-evoked contractions of a myenteric plexus–longitudinal muscle preparation of guinea-pig ileum *in vitro* (Anderson *et al*., 1975; Pertwee, 2001). Crosstolerance between (+) WIN 55,212-2 and morphine was also described for the inhibition of electrically evoked contractions of the guinea-pig ileum preparations (Basilico *et al*., 1999).

To gain more knowledge of the mechanisms of tolerance to the motor inhibitory effects of cannabinoids in the intestine, we compared the contractile response to electrical stimulation of guinea-pig and human isolated small intestine after *in vitro* prolonged exposure to the cannabinoid agonist (+) WIN 55,212-2, a compound that showed crosstolerance with other cannabimimetic drugs (Pertwee *et al*., 1993; Fan *et al*., 1994). The withdrawal reaction in these tolerant preparations was studied by examining the increase in contractions precipitated by the cannabinoid CB_1 receptor antagonist rimonabant. This selective antagonist (Rinaldi-Carmona *et al*., 1995) precipitates a withdrawal syndrome *in vivo* in rats chronically treated with Δ^9-tetrahydrocannabinol (Tsou *et al*., 1995; Aceto *et al*., 1996). The drug has also been reported to have an intrinsic effect in nontolerant guinea-pig tissues, related to its putative inverse agonist properties rather than to the inhibition of an endocannabinoid tonus (Pertwee, 2001).

To investigate this, we assessed the tissues levels of several endocannabinoids in myenteric plexus smooth muscle of guinea-pig and human ileum. Comparing the functional response of human intestinal segments chronically exposed to cannabinoids with the guinea-pig ileum longitudinal muscle is of particular interest in view of the difficulties of obtaining reliable information on the mechanisms underlying the intestinal effects of chronic cannabinoids in man by clinical studies.

Methods

Human tissue

Healthy specimens of ileum and distal jejunum were obtained from a macroscopically normal region of patients undergoing surgery for intestinal malignancy at San Raffaele Hospital, Milan, Italy. Patients had not received radiotherapy or been treated chronically with steroids, opioids or chemotherapy. Specimens were available at the operating theatre, each consisting of a whole ileum or jejunum segment; they were washed in saline and immediately placed in cold (4°C) preaerated (95% O_2, 5% CO_2) Krebs solution (composition, mM: NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.7) and transported to Sanofi-aventis laboratories within about 30 min. Mucosa and submucosa were gently removed and longitudinal muscular regions were cut into strips approximately 3 mm wide (total length of each preparation: 1.5 cm).

This study was approved by the ethics committee of the San Raffaele Hospital, Milan, Italy.

Guinea-pig ileum

Male guinea-pigs (HA SPF, Harlan) weighing 250–350 g were housed in a room with controlled temperature (22±1°C), humidity (55±10%) and 12-h light–dark cycle for at least 7 days before being used. Food and water were available *ad libitum*. Food was withdrawn 18 h before the animals were killed by a blow on the neck and exsanguination. Animal care and killing were in accordance with the Sanofi-aventis international ethical code and the international principles governing the care and treatment of laboratory animals (E.E.C. Council Directive 86/609, DJL358, 1, 12 December 1987).

A 10-cm ileum segment, excised 10 cm proximally to the ileocaecal valve, was soaked and maintained in a Krebs solution of the above composition. The longitudinal muscle, including the attached myenteric plexus, was carefully separated from the underlying circular muscle, taking care not to damage the neuronal network and to maintain its functional integrity.

Incubation of muscle strips

Innervated smooth muscle strips were either used immediately or incubated at 18°C for 48 h in aerated (95% O_2, 5% CO_2) Dulbecco's modified Eagle's medium, containing 10% fetal calf serum, 10 U ml^{-1} penicillin and 10 μg ml^{-1} streptomycin. The solution was changed every day. This modified medium was used for all the incubations with human and guinea-pig tissues. According to the protocol of the experiment, incubation was carried out in the presence of 10 μM (+) WIN 55,212-2, 10 μM (−)WIN 55,212-3, 0.1 μM rimonabant or vehicle (0.3% dimethylsulfoxide (DMSO)).

Experimental conditions

Experiments were carried out with 8–16 muscle strips of human ileum or jejunum or guinea-pig myenteric plexus–longitudinal muscle, dissected from different specimens. After incubation, muscle strips were washed several times and mounted in a 20-ml organ bath containing aerated (95% O_2, 5% CO_2) Krebs solution at 37°C, under a tension of 1 g. After washing, 10 μM choline (precursor of acetylcholine) and 10 μM indomethacin (prostaglandins synthetase inhibitor) were added to the medium, to reduce spontaneous phasic contractions.

Isotonic contractions were evoked by electrical field stimulation (EFS). Two platinum wire electrodes were placed on the top and bottom of the organ bath and electric field stimulation was elicited by a Power Lab stimulator (AD Instruments Pty Ltd, Castle Hill, Australia) coupled to a multiplexing pulse booster (Ugo Basile, Varese, Italy). Supramaximal stimulation was applied to elicit maximal contractions (20 Hz; 2 ms pulsewidth; trains of 10 s every 2 min, about 100 mA for human small intestine or 4 s every 10 s, about 300 mA for the myenteric plexus of guinea-pig ileum). Then, the current was reduced to obtain submaximal

stimulation (20–50% reduction of the maximal contractile response). Under these experimental conditions, we can study drugs that either decrease or increase the electrically evoked twitches. Contractions were monitored by computer with a data recording and analysis system (Power Lab, Chart 5) linked to isotonic transducers (Ugo Basile, Varese, Italy) *via* preamplifiers (Octal Bridge Amp).

After 2 h stabilization, (+)WIN 55,212-2 and its enantiomer (−) WIN 55,212-3 were tested on drug or vehicle preincubated preparations. When possible, cumulative concentration–response curves to these molecules were plotted, with and without the antagonist rimonabant, leaving each drug concentration in contact with the preparation for 15–20 min. The intrinsic effect of rimonabant (contact time 1 h) was assessed immediately or after 48 h incubation.

Atropine (1 μM), which inhibited the electrically evoked contractions almost completely (95–100%), was added to the incubation bath at the end of the experiment and was used for each strip as reference for rating the responses to the test substances. At least one preparation for each specimen was used as control (tissue incubated with the vehicle only).

(+)WIN 55,212-2, (−)WIN 55,212-3, naloxone, U69593 and rimonabant were stored at −20°C as a 1 mM stock solution in DMSO and dilutions were made in distilled water containing 30% DMSO. All the other drugs were dissolved in distilled water, if not otherwise specified.

Endocannabinoid tissue levels

Tissue levels of endocannabinoids anandamide (AEA) 2-arachidonoyl glycerol (2-AG), oleoylethanolamide (OEA) were measured according to the method of De Lago *et al.* (2005), with slight modifications. Mucosa and submucosa were gently removed, tissues were weighed, homogenized, extracted and submitted to LC-MS/MS analysis using electrospray ionization in the 'positive mode'. The amount of endocannabinoids are expressed as pmol per gram of wet tissue extracted.

Calculation and statistical analysis

The agonist concentration with 50% maximal effect (IC_{50}) was calculated using a four-parameter logistic model according to Ratkowsky & Reedy (1986), with adjustment by nonlinear regression using the Levenberg–Marquard algorithm in RS/1 software. The pA_2 for antagonists, as defined by Arunlakshana & Schild (1959), was obtained from linear regression of the mean of the log (DR-1) against the negative logarithm of the antagonist concentration. The negative logarithm of the dissociation constant (pK_B) was calculated using the Cheng–Prusoff equation, as reported by Kenakin (1997). Computer analysis was carried out as described by Tallarida & Murray (1987). Differences between means were determined by analysis of variance (ANOVA) followed by Bonferroni's test. Values of $P<0.05$ were considered statistically significant.

Chemicals

Rimonabant-SR141716 (*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4methyl-pyrazole-3-carboxamide) was synthesized at Sanofi-aventis Recherche, (Montpellier, France). Indomethacin, choline, atropine sulfate, naloxone, U69593, (+)WIN 55,212-2 and (−)WIN 55,212-3 were purchased from Sigma-Aldrich Corp. (St Louis, MO, U.S.A.). AEA, 2-AG and OEA were purchased from Tocris (Northpoint, U.K.).

Results

Acute (+)WIN 55,212-2 response

(+)WIN 55,212-2 (10 nM–10 μM) concentration-dependently inhibited the electrically induced twitch-like contractions of the myenteric plexus–longitudinal muscle strips from guinea-pig ileum and human small intestine, with similar efficacy (100% of the maximal effect of atropine) but with about 10 times higher potency in guinea-pig than in human tissue; the EC_{50} (95% confidence limits) were 4.8 (4.3–6.0) and 56 (51–94) nM, respectively. Rimonabant inhibited (+)WIN 55,212-2 response with similar potency in guinea-pig and human small intestinal strips. The pA_2 calculated from the respective Schild plots were (mean ± s.e.m.) 8.4 ± 0.4 and 8.2 ± 0.1, with slopes not significantly different from unity, 1.2 ± 0.2 and 1.1 ± 0.1 (Figure 1a and b).

(−)WIN 55,212-3, the optical isomer of (+)WIN 55,212-2, was markedly less potent in inhibiting contractions in intestinal strips from both species (Figure 2a and b).

Tolerance studies

To study tolerance to the smooth muscle relaxing action of cannabinoid agonists, the intestinal strips were incubated for 48 h, either with or without 10 μM (+)WIN 55,212-2, and then, after several washings, they were challenged with increasing concentrations of the same cannabinoid agonist. After 48-h preincubation in a drug-free solution, the inhibitory response of (+)WIN 55,212-2 was unchanged and similar to its response with no preincubation; the IC_{50} (95% confidence limits) was 7.9 (6.7–9.4) nM in the guinea-pig and 57 (50–92) nM in human tissue. By contrast, the 48-h preincubation with (+)WIN 55,212-2 almost completely abolished the challenger response to this cannabinoid agonist in both guinea-pig and human strips (Figure 3a and b). The inhibitory response to (+)WIN 55,212-2 was still present, although weaker, in small intestinal strips preincubated with the less active isomer (−)WIN 55,212-3: IC_{50} (95% confidence limits) guinea-pig 30 (22–41) nM, human 500 (300–700) nM.

The nonselective opiate receptor antagonist naloxone had no significant effect on electrical twitches in human preparations preincubated for 48 h with 10 μM (+)WIN 55,212-2 or its vehicle (data not shown).

In human strips, after 48-h incubation with (+)WIN 55,212-2 or its vehicle, the selective opiate K-receptor agonist U69593 inhibited the electrical twitches with a similar IC_{50} (95% confidence limits) 39 (28–41) nM and 43 (31–59) nM.

Rimonabant intrinsic effect

The intrinsic effect of the cannabinoid CB_1 receptor antagonist rimonabant on guinea-pig and human intestinal segments is reported in Figures 4a, b and 5a, b.

In guinea-pig tissues, rimonabant increased the twitch-like contractions with an EC_{50} (95% confidence limits) of 100

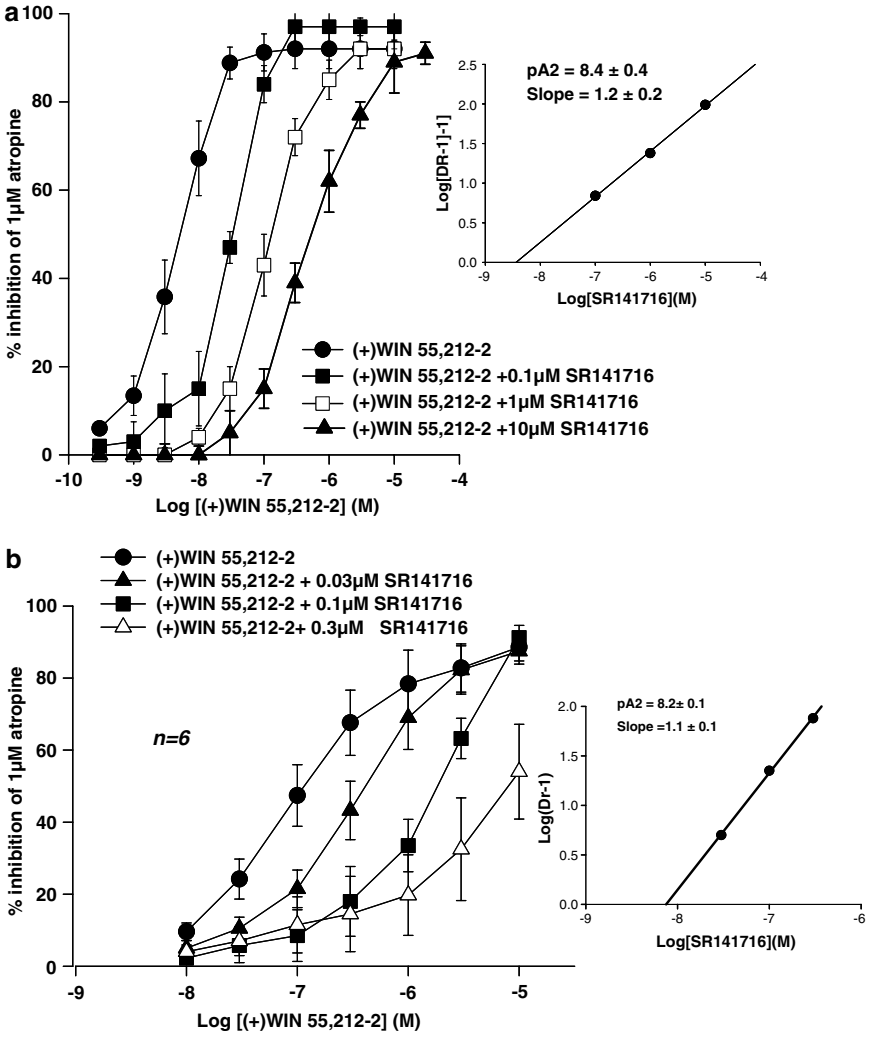

Figure 1 Logarithm concentration–response curves of (+)WIN55,212-2 in the absence or presence of increasing concentrations of rimonabant (SR 141716) on EFS-induced contractions of longitudinal muscle strips from guinea-pig (a) and human (b) small intestine. Each curve represents the mean±s.e.m. of at least six strips from different specimens. The response is expressed as a percentage of the maximal effect of 1 μM atropine. The Schild plot was calculated as reported in the Methods (Panel b already published in Croci *et al*., 1998).

(80–105) nM, by contrast it had no significant effect on human small intestine (Figure 5a and b). With no or 48-h preincubation in drug-free conditions, rimonabant (1 μM) showed a maximal increase of the twitches of guinea-pig ileal myenteric plexus–longitudinal muscle by (mean±s.e.m.) 110±9 and 123±28%, respectively. At the same concentration, rimonabant increased the twitches of both guinea-pig and human strips made tolerant by 48-h incubation with 10 μM (+)WIN 55,212-2 (mean±s.e.m.) by 133±36 and 155±19%, respectively (Figure 5a and b). Incubation with (−)WIN 55,212-3 did not substantially affect the guinea-pig intestinal strips' response to rimonabant and only marginally increased that of human strips, which were completely unresponsive to rimonabant in normal conditions. Finally, the contracting response to rimonabant was significantly lower in guinea-pig ileal strips incubated for 48 h with this cannabinoid CB_1 antagonist, while the human strips in the same experimental conditions remained insensitive to its action, like the controls.

Endocannabinoid tissue levels

In order to investigate the role of endocannabinoids on the intrinsic effect of rimonabant, we measured their levels in human and guinea-pig ileum (Figure 6a–c). The levels of 2-AG, but not of AEA, were about five times higher in guinea-pig than human strips. OEA levels were higher in guinea-pig than human tissue, but the difference did not reach statistical significance.

Discussion

The present study found important similarities in the function of cannabinoid receptors in the guinea-pig and human small intestine but also some differences that may have implications in interpreting the effects of cannabimimetic drugs and cannabinoid antagonists in experimental and clinical studies.

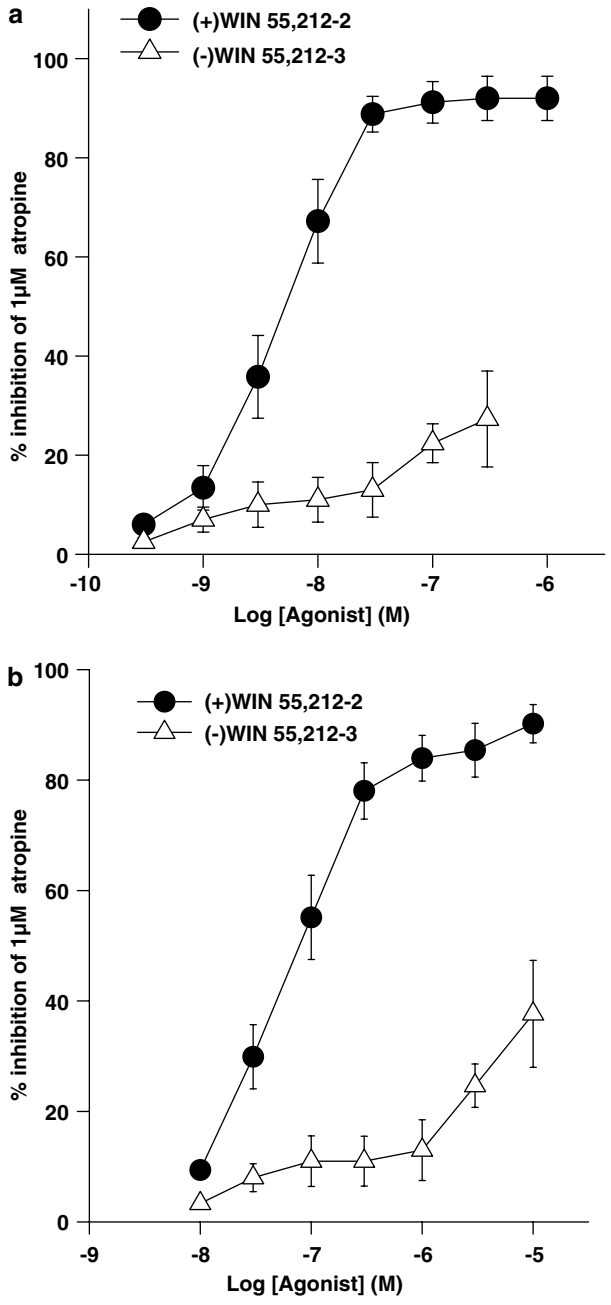

Figure 2 Logarithm concentration–response curves to (+)WIN 55,212-2 and (−) WIN 55,212-2 on EFS-induced contractions of longitudinal muscle strips from guinea-pig (a) and human (b) small intestine. Each curve represents the mean±s.e.m. of at least six strips from different specimens. The response is expressed as a percentage of the maximal effect induced by 1 μM atropine.

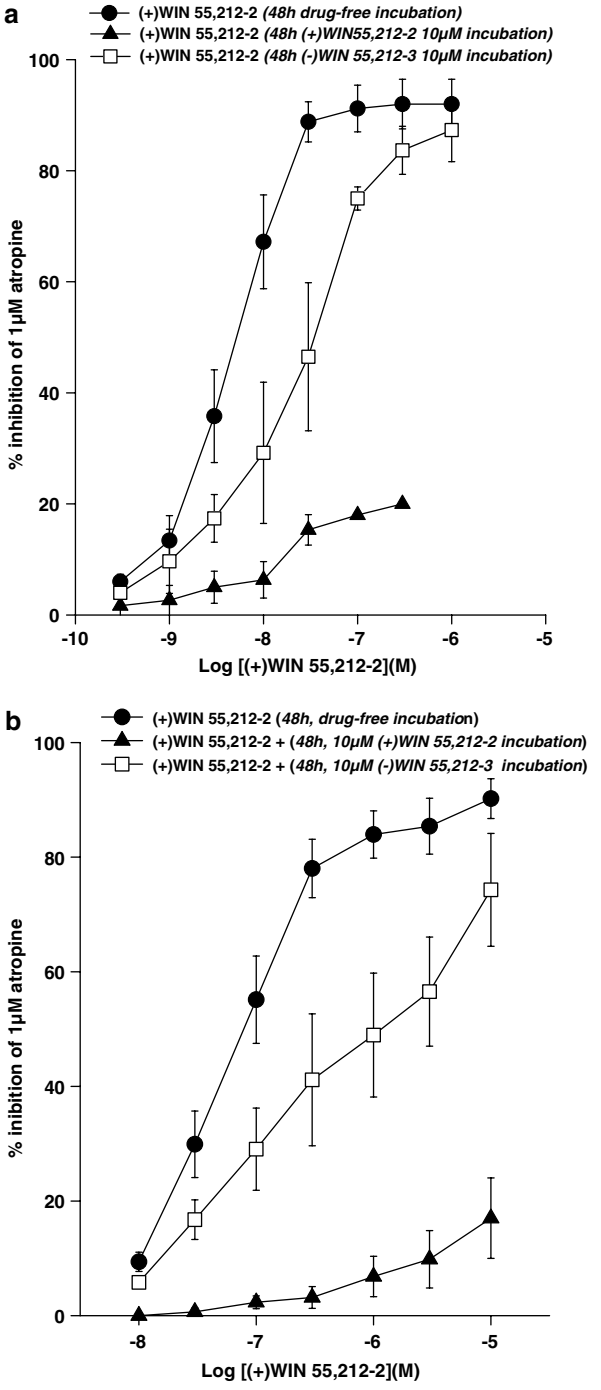

Figure 3 Logarithm concentration–response curves to (+)WIN 55,212-2 after 48-h incubation in drug-free solution or in the presence of either (+)WIN 55,212-2 or (−)WIN 55,212-2 on EFS-induced contractions of longitudinal muscle strips from guinea-pig (a) and human (b) small intestine. Each curve represents the mean±s.e.m. of at least six strips from different specimens. The response on the ordinate is expressed as a percentage of the maximal effect induced by 1 μM atropine.

Our results are consistent with the view, supported by previous *in vitro* (Pertwee *et al*., 1996; Coutts & Pertwee, 1997; Croci *et al*., 1998; Izzo *et al*., 1998; 2000) and *in vivo* (Colombo *et al*., 1998; Izzo *et al*., 1999b; Krowicki *et al*., 1999) studies, that stimulation of prejunctional cannabinoid CB_1 receptors inhibits muscle contraction by reducing the release of acetylcholine from enteric neurons, in both the guinea-pig and human small intestine.

The synthetic cannabinoid agonist (+)WIN 55212-2 inhibited the electrically induced contractions of both human and guinea-pig small intestine muscle strips, but it was at least 10 times less potent in the former than the latter preparation. In these tissues, the selective cannabinoid CB_1-receptor antagonist rimonabant induced a similar right-ward shift of the log concentration–response curves to (+)WIN 55212-2,

with virtually identical pA_2. This indicates that rimonabant has the same affinity for human and guinea-pig cannabinoid CB_1 receptors. Thus, the lower sensitivity of the human than the guinea-pig small intestine to (+)WIN 55212-2 indicates that in humans these receptors have a lower density and/or are less efficiently coupled to the effector system(s). The lower potency and efficacy of the (−) isomer of WIN 55,212-2 indicates a similar stereoselective modulation of the cannabinoid CB_1 receptor in both tissues.

Rimonabant had different intrinsic effects in the two species: it had no effect in human strips but increased the contractions by more than 100% in the guinea-pig ileum myenteric plexus. This effect in the guinea-pig ileum has already been described and can be ascribed either to an antagonism of the endocannabinoid-sustained physiological inhibitory tone (Pertwee *et al*., 1996; Coutts & Pertwee, 1997; Izzo *et al*., 1998) or to the inverse agonist property of rimonabant (see the recent review by Bouaboula *et al*., 1997; Landsman *et al*., 1997; Mac Lennan *et al*., 1998; Manara *et al*., 2002; Pertwee, 2005).

Finally, we found that this intrinsic effect rimonabant is probably due to the antagonism of endocannabinoid tonic activation of CB_1 receptors and not to rimonabant's inverse agonist properties. In fact, high levels of 2-AG were detected in guinea-pig ileum myenteric plexus preparations but not in human small intestine.

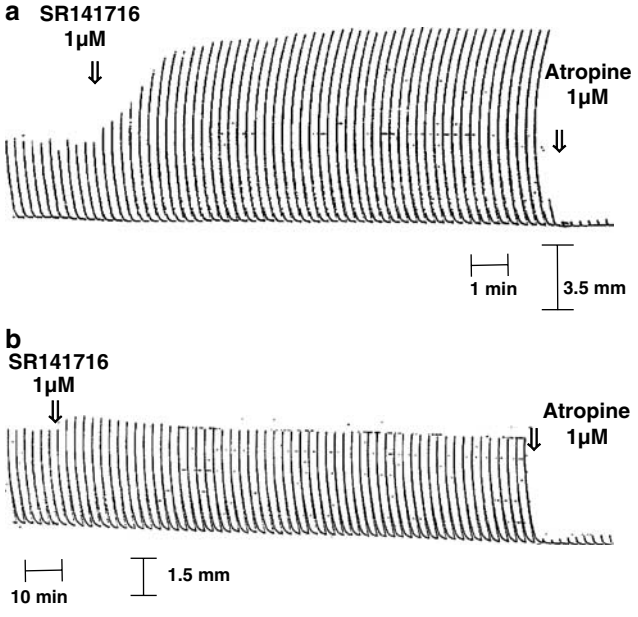

Figure 4 Representative tracings showing the intrinsic effect of SR 141716 (1 μM) on the EFS-induced contractions of longitudinal muscle strips from guinea-pig (a) and human (b) small intestine.

Simply incubating human and guinea-pig intestinal strips for 48 h in drug-free solutions did not substantially change the potency (IC_{50}) of (+)WIN 55,212-2 in inhibiting the

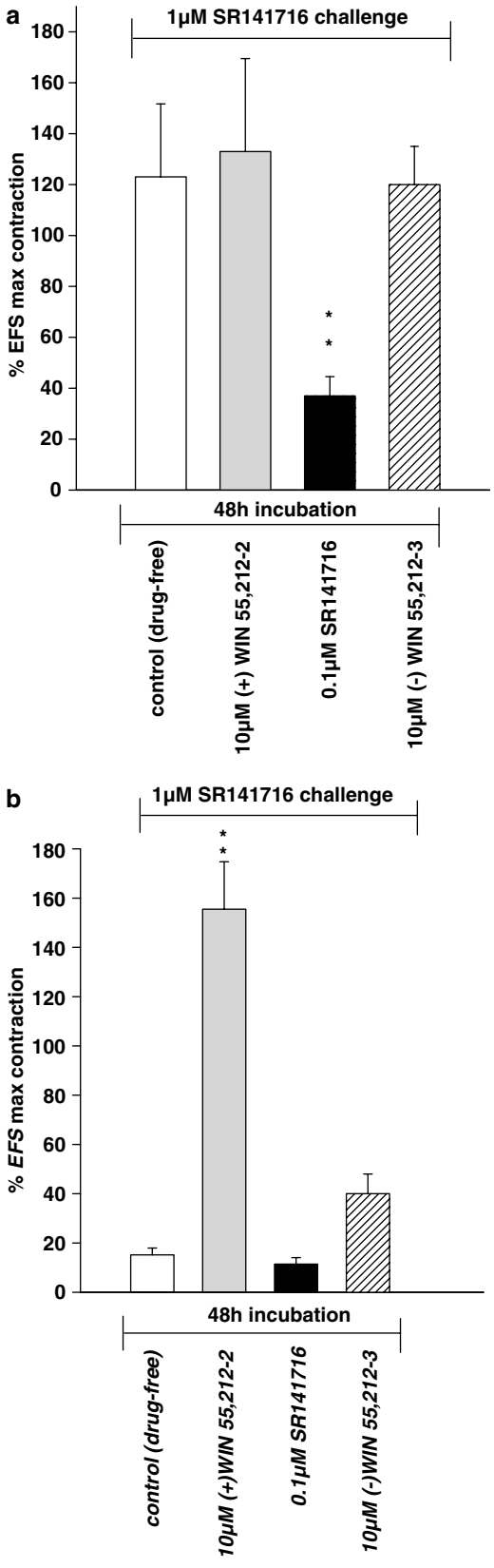

Figure 5 Effect of challenge with 1 μM rimonabant (SR 141716) on EFS induced contractions of longitudinal muscle strips from guinea-pig (a) and human (b) small intestine after 48-h incubation in control drug-free solution (open column), in the presence of (+)WIN 55,212-2, 10 μM (gray column), (−)WIN 55,212-2, 10 μM (striped column) or rimonabant, 0.1 μM (black column). The columns show mean ± s.e.m. of at least six strips from different specimens and are expressed as a percentage of maximal contraction before the addition of SR 141716 to the bath. $^{**}P<0.01$ vs control drug-free by ANOVA followed by Bonferroni's test.

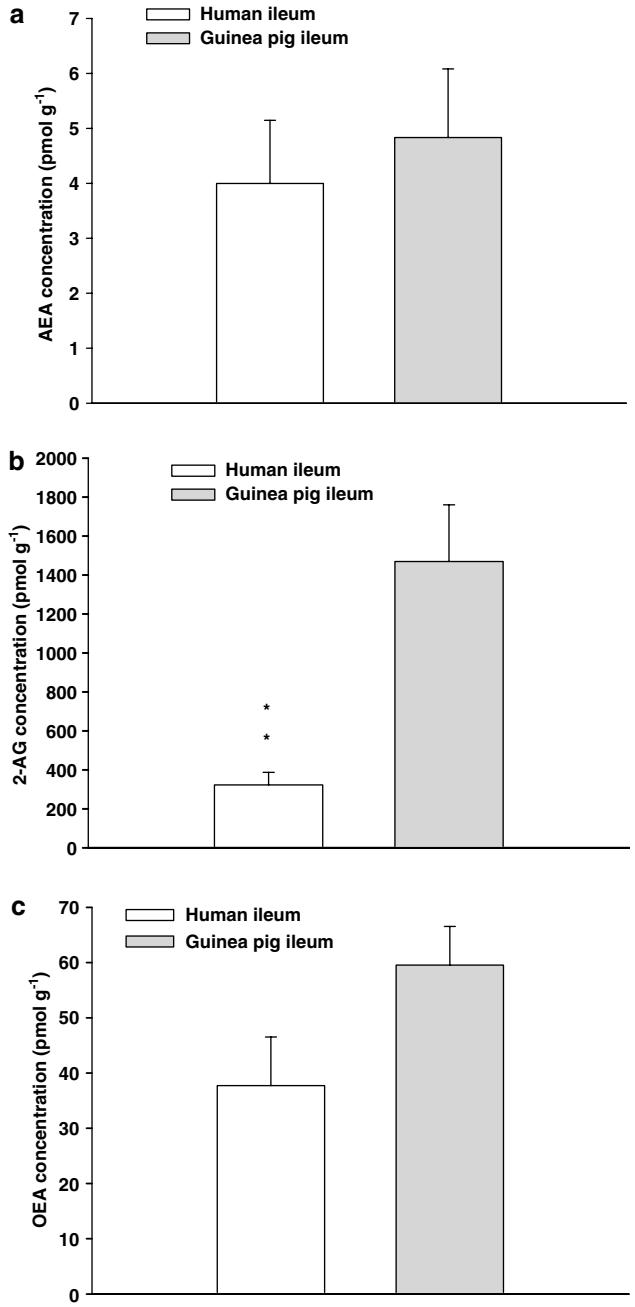

Figure 6 AEA (a), 2-AG (b) and OEA (c) in human and guinea-pig specimens. The columns show mean ± s.e.m. of four separate measurements and are expressed as pmol per gram of humid tissue. $^{**}P<0.01$ human vs guinea-pig by ANOVA followed by Bonferroni's test.

contractile response. This indicates that the cannabinoid CB_1 receptor functional response is fully maintained up to 48 h. On the other hand, 48-h preincubation with (+)WIN 55,212-2 markedly reduced both the potency and the maximal contractile inhibitory effect on a challenging log concentration–response curve to the same agonist, indicating *in vitro* tolerance to the cannabinoid's action. The same preincubation with the (−) isomer of WIN 55,212-2 only slightly shifted the concentration–response curve to (+) WIN 55,212-2 with virtually no loss of maximal effect, indicating that full tolerance developed only after stereoselective activation of the cannabinoid CB_1 receptors. This specificity was further confirmed in human tissues, where a 48-h preincubation with (+)WIN 55,212-2 had no effect on the response of the potent opiate K-receptor agonist U69593. This result corresponds with the absence of naloxone effect in human preparations made tolerant to (+) WIN 55,212-2; it also indicates that no interaction occur between opiate and cannabinoid system, at least in human small intestine.

Previous studies have shown that tolerance to cannabinoids, in guinea-pig myenteric plexus–longitudinal muscle and in mouse vas deferens, can be induced by *in vivo* administration of Δ^9 tetrahydrocannabinol (Pertwee *et al*., 1993). Basilico *et al*. (1999) showed that tolerance to cannabinoids could be induced by *in vitro* incubation of the myenteric plexus with (+)WIN 55,212-2. Although the adaptive changes responsible for the lower sensitivity of the guinea-pig ileum to the inhibitory effect of (+)WIN 55,212-2 are not known, the fact that an isolated tissue can be made tolerant to (+)WIN 55,212-2 by *in vitro* incubation with this agonist strengthens the theory that cannabinoid tolerance is pharmacodynamic in nature and depends on the long-term interaction of the agonist with its tissue receptors (Pertwee, 1991). However, it remains to be established whether changes in the concentration–response curves for (+)WIN 55,212-2, such as a right-ward shift and reduction of maximal effect, involve alterations in receptor density and/or transduction processes rather than in postjunctional events affecting the smooth muscle sensitivity to neurotransmitters.

To assess whether tolerance to (+)WIN 55,212-2 is accompanied by an *in vitro* withdrawal response, we challenged the (+)WIN 55,212-2-incubated preparations with rimonabant. In human small intestine, this antagonist, which had no effect in nonincubated or vehicle-preincubated preparations, strongly potentiated the contractions in preparations made tolerant to (+)WIN 55,212-2, indicating a withdrawal effect. No similar withdrawal response was seen in tolerant guinea-pig preparations since the challenge concentration of rimonabant was already able to induce near-maximal contraction in nontolerant preparations, as mentioned before.

The response to rimonabant in tolerant guinea-pig ileum is different from that observed by Basilico *et al*. (1999), who reported that rimonabant, at the same concentration we used, did not induce contractions, either in preparations rendered tolerant to (+)WIN 55,212-2 or in those incubated without cannabinoids. The higher agonist concentration (about 200 times) and the longer incubation time (48 vs 5 h) we used to induce tolerance compared with Basilico's study may possibly explain the rimonabant-induced withdrawal contractions in our hands.

Finally, we tested the effect of 48-h preincubation with rimonabant (instead of the agonist) on the response to the antagonist itself. In the guinea-pig ileum, this incubation significantly reduced the contractile response to a challenging concentration of this antagonist. We speculate that the high level of endocannabinoids, physiologically present in guinea-pig tissues, might induce CB_1 receptor partial desensitization. Thus, rimonabant's prevention of endocannabinoid-induced tolerance (and the associated withdrawal response) might explain the reduced contractile response in this experimental condition. It is noteworthy that an altered neuronal control of

contractility involving high levels of endocannabinoid was reported in the colon of patients with diverticulitis. In this study, the high levels of AEA, and the rimonabant-induced intrinsic response suggest an apparent desensitization of the presynaptic CB_1 receptor (Guagnini *et al*., 2006). A similar dysfunction could also occur in other intestinal disorders with a common inflammatory basis such as few subtypes of irritable bowel syndrome or even ulcerative colitis and Crohn's (Guagnini *et al*., 2006).

In conclusion, we found that tolerance to the muscle relaxing action of cannabinoids can be induced by incubating guinea-pig and human intestinal smooth muscles with the synthetic agonist (+)WIN 55,212-2. Similar cannabinoid CB_1 receptor mechanisms are probably involved in the tolerance and withdrawal effects in these preparations. However, the CB_1 receptor-selective antagonist rimonabant disclosed some receptor functional differences between the two species since it potentiated the neurally evoked contractions in nontolerant guinea-pig ileum, but had no such effect in human small intestine. Endocannabinoid-sustained CB_1 receptor tonic activation and high receptor sensitivity in guinea-pig ileum might account for this difference.

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