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Modulation of gastric emptying and gastrointestinal transit in rats through intestinal cannabinoid CB_1 receptors

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Abstract

We studied the delay in gastric emptying and gastrointestinal transit induced by the cannabinoid receptor agonists (+)-WIN 55,212-2 (*R*(+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate) and CP 55,940 ((−)-*cis*-3[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol), as prevented by the selective cannabinoid CB_1-receptor antagonist SR141716 ((*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide)) in rats after systemic or central drug administration. Oral SR141716 showed comparable potency (ID_{50} range 1.0–3.9 mg/kg) in antagonizing gastric emptying and gastrointestinal transit delay by (+)-WIN 55,212-2 or CP 55,940. Gastric emptying and gastrointestinal transit delay after intracerebroventricular (i.c.v.) (+)-WIN 55,212-2 was prevented by oral or i.c.v. SR141716, but i.c.v. SR141716 did not significantly reduce the effect of i.p. (+)-WIN 55,212-2. Pertussis toxin prevented the delaying action of i.c.v. (+)-WIN 55,212-2 on both gastric emptying and gastrointestinal transit, but had no effect on (+)-WIN 55,212-2 i.p. These findings are consistent with a primary role of peripheral cannabinoid CB_1 receptor mechanisms in gastrointestinal transit delay by specific agonists.

Keywords: Gastric emptying; Gastrointestinal transit; Pertussis toxin; Cannabinoid CB_1 receptor

1. Introduction

For centuries, hashish and marijuana, both derived from Indian hemp, *Cannabis sativa* L., have been used for their medicinal and psychomimetic effects. Cannabinoids exert their biological function through receptor-mediated mechanisms (Howlett, 1995; Pertwee, 1997) and two subtypes of the cannabinoid receptors, CB_1 and CB_2, have been identified. The amino acid sequence of the cannabinoid CB_1 receptor was found to be consistent with a tertiary structure typical of the G-protein coupled receptors. Indeed, the cannabinoid receptor agonists, through cannabinoid CB_1 receptor activation, inhibit adenylate cyclase activity in a dose-dependent, stereoselective, pertussis-toxin sensitive manner (Matsuda et al., 1990). This receptor was initially characterized in rat brain (Devane et al., 1988) and designated as the central cannabinoid receptor. However, cannabinoid CB_1 binding sites (Pertwee, 1997) and immunoreactivity (Kulkarni-Narlan and Brown, 2000) have been found in peripheral tissues, where cannabinoid CB_1 transcripts have also been reported (Bouaboula et al., 1993; Galiegue et al., 1995).

In vitro animal studies have now established that cannabinoids inhibit electrically induced contractions of guinea-pig myenteric plexus-longitudinal muscle preparations by activating cannabinoid CB_1 receptors (Pertwee et al., 1992, 1996; Coutts and Pertwee, 1997) for which SR141716 ((*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide)) is a highly potent and selective antagonist (Rinaldi-Carmona et al., 1994). Croci et al. (1998) provided in vitro functional evidence of prejunctional cannabinoid CB_1 receptors in the human ileum longitudinal smooth muscle. Cannabinoid agonists inhibit gut motility in vivo in several animal species

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(Chesher et al., 1973; Shook and Burks, 1989; Colombo et al., 1998; Izzo et al., 1999; Krowicki et al., 1999) and in man (Sridhar et al., 1984). The cannabinoid CB_2 receptor has been found mainly in tissues responsible for cannabinoids' effects on immune functions (Herkenham, 1995; Felder and Glass, 1998), where responses mediated by this receptor are selectively and competitively antagonized by the cannabinoid CB_2 receptor antagonist, SR144528 ((*N*-[(1*S*)-1,3,3-trimethylbicyclo(2.2.1)-hept-2-endo-yl)-5-(4-chloro-3-methylphen-yl)-1-(4-methylbenzyl) pyrazole-3-carboxamide)) (Rinaldi-Carmona et al., 1998).

In the present study, we investigated the inhibitory effect on gastrointestinal propulsion of the cannabinoid receptor agonists (+)-WIN 55,212-2 (*R*(+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate) or CP 55,940 ((−)-*cis*-3[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol). Our aim was to ascertain, in an in vivo animal model of the progression of a test meal along different sections of the upper gut, whether peripheral cannabinoid CB_1 receptors by themselves only have substantial effects on intestinal motor function, not involving a centrally elicited component. In the same model, we also challenged the reported ability of SR141716 to elicit on its own effects opposite to those of the cannabinoid agonists, in view of our previous negative findings in the human isolated intestine (Croci et al., 1998). A preliminary partial account of this work was presented at the joint meeting of the American and French Pharmacological Societies, Boston, MA, USA, June 4–8, 2000 (see reference Manara et al., 2000).

2. Materials and methods

2.1. Animals

Male Crl:CD BR rats (Charles River, Italy) weighing 250 ± 30 g were used, according to internationally accepted principles for care of laboratory animals (E.E.C. Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987). The experimental protocol has been approved at corporate level by the "Comité Expérimentation Animale" (Animal Care and Use Committee) of Sanofi-Synthelabo Recherche. Animals were housed for at least 7 days before the experiment, under controlled environmental conditions (22 ± 1 °C, 55 ± 15% relative humidity, 12 h light, 6 h30–18 h30) and were given a pellet diet (4RF 21, Mucedola, Italy) and water ad libitum.

2.2. Experimental conditions

Rats were anesthetized with pentobarbital (60 mg/kg, i.p.) and prepared for intracerebroventricular (i.c.v.) microinjection according to Altaffer's procedure (Altaffer et al., 1970). Briefly, anesthetized rats were fixed in a stereotaxic apparatus and the right lateral ventricle was located using a stereotaxic atlas (Paxinos and Watson, 1982). A permanent polyethylene cannula (PE10) was implanted so as to penetrate the ventricle 4.5 mm from the top of the skull, to which it was fixed with dental cement (Heraus Kulzer, type Paladur®). The animals were then placed in individual cages and allowed to recover for 7 days. At the end of the experiment, 10 μl Evans blue dye (0.5%) was injected through the cannula. The brains were then removed, cut, and examined macroscopically to verify cannula placements and dye distribution.

We assessed gastric emptying (Croci et al., 1995), small intestinal and gastrointestinal transit in 24 h fasted rats. To assess gastric emptying, a bolus (1 ml/100 g body weight) of a standard meal ($BaSO_4$, Prontobario Esofago, Bracco, Italia; 75% W/V with 0.5% Na-carboxymethylcellulose (CMC) was given by gavage and the rats were euthanized by cervical dislocation immediately ($t=0$) or 60 min (15 min only in the experiment reported in Fig. 4) after the meal. The pylorus and the esophageal junction were clamped before excision of the stomach. The gastric content was assessed by weighing the stomachs before and after removing their contents and then cutting, rinsing and blotting them on dry tissues. At each time, the stomach content remaining after drug treatment was compared to that of control drug-free rats, euthanized at $t=0$. The percentage inhibition of gastric emptying was calculated for each rat: %gastric emptying=(content of treated rats/mean content of control rats) × 100.

Gastrointestinal and small intestinal transit were assessed 20 min after a standard charcoal meal (distilled water suspension containing 10% gum arabic, 10% vegetable charcoal and 20% starch), given respectively by gavage (2 ml/rat) or intraduodenally (i.d., 0.4 ml/rat) through a cannula (Silmedic L602-205, Lepetit, Italia) implanted 2 cm from pylorus 7 days earlier. Rats were euthanized and the percentage of intestine traversed by the front of the test meal was measured.

Treatment was assigned using random tables. (+)-WIN 55,212-2 was dissolved in either 10% propylenglycol acid solution, 2 ml/kg, and injected intraperitoneally (i.p.) or dimethylsulfoxide (DMSO), 5 μl/rat, and injected i.c.v. immediately before the test meal in chronically implanted animals (7 days before the test). CP 55,940 was dissolved in acidic ethanol 10%, 2 ml/kg and injected i.p.; SR141716 was either suspended in 0.5% CMC (2 ml/kg), and given orally (p.o.) 60 min before the agonist or dissolved in pure DMSO (5 μl/rat, i.c.v.) and given immediately before the agonist; SR144528 was suspended in 0.5% CMC and given p.o. 60 min before the agonist (2 ml/kg). We also investigated the effect of (+)-WIN 55,212-2 on gastrointestinal transit or gastric emptying on day 4 after i.c.v. injection of pertussis toxin (0.5 μg/rat; Parolaro et al., 1990) or saline, because a previous study had shown that it takes 3 days for pertussis toxin to be ribosylated and for pertussis toxin-sensitive G proteins to become inactivated (Aghanjanian

and Wang, 1986). Drug-free control rats were treated with the appropriate vehicles, which had no effect on the end-points investigated compared to rats receiving no treatment other than the test meal; each experiment included controls receiving different solvents, as needed. The effect of (+)-WIN 55,212-2 (0.3 mg/kg, i.p.) on body temperature in conscious rats was assessed by a rectal probe (TM-36/S1, LSI, Settala, Milan, Italy) 1 and 2 h before, 0.5, 1, 2 and 3 h after i.p. injection of the agonist or its vehicle (Crawley et al., 1993).

2.3. Calculation and statistical analysis

Results are expressed as mean ± S.E.M. The ED_{50} and ID_{50} with their 95% confidence limits were obtained by using the four-parameter logistic model according to Ratkowsky and Reedy (1986). The adjustment was obtained by non-linear regression analysis using the Levenberg–Marquandt algorithm in RS/1 software (B.B.N., Cambridge, MA). The means were compared by completely randomized one-way analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons using RS/1 software. A probability of less than 0.05 was considered statistically significant.

Table 1
Half maximally effective intraperitoneal (i.p.) doses of the cannabinoid agonists (+)-WIN 55,212-2 and CP 55,940 for delaying gastric emptying, gastrointestinal and small intestinal transit in rats

	ED_{50}, μg/kg, i.p.		
	Gastric emptying	Gastrointestinal transit	Small intestinal transit
(+)-WIN 55, 212-2	243 (194–306)	205 (146–283)	137 (79–234)
CP 55,940	5 (3–11)	5 (3–8)	6 (4–11)

In parentheses 95% confidence intervals.
(+)-WIN 55,212-2 (10, 30, 100, 1000, 3000 μg/kg) and CP 55,940 (0.3, 1, 3, 10, 30, 100 μg/kg) were given immediately before the test meal.
$n = 7–15$ rats each dose.
The regression coefficient of the dose–response curves ranged from 0.9 to 0.96.

2.4. Chemicals

SR141716 and SR144528 were synthetized at Sanofi Synthelabo Recherche, Montpellier, France. The following

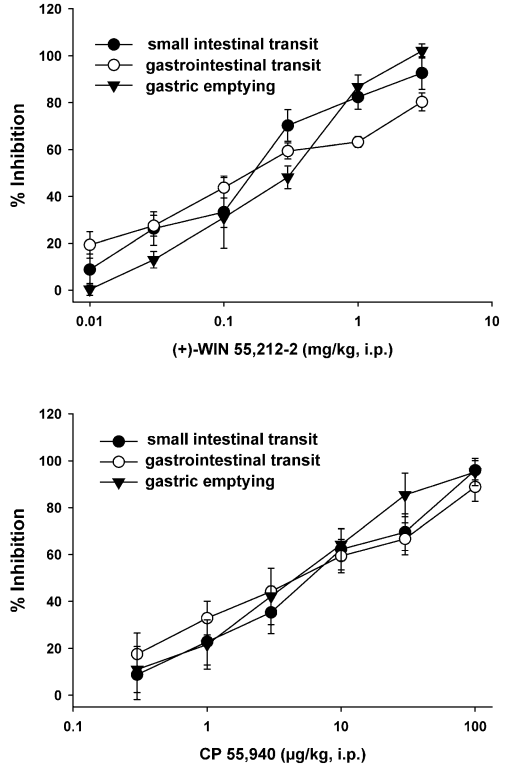

Fig. 1. Dose-related inhibition of gastric emptying, gastrointestinal and small intestinal transit by the cannabinoid receptor agonists, (+)-WIN 55,212-2 or CP55,940 after intraperitoneally (i.p.) administration. Rats received the agonists immediately before the meals (gastric emptying, $BaSO_4$; gastrointestinal and small intestinal transit, charcoal) and were euthanized after 60 min (gastric emptying) or 20 min (gastrointestinal transit and small intestinal transit). Each point represents the means ± S.E.M. of 7–15 animals for each experimental group.

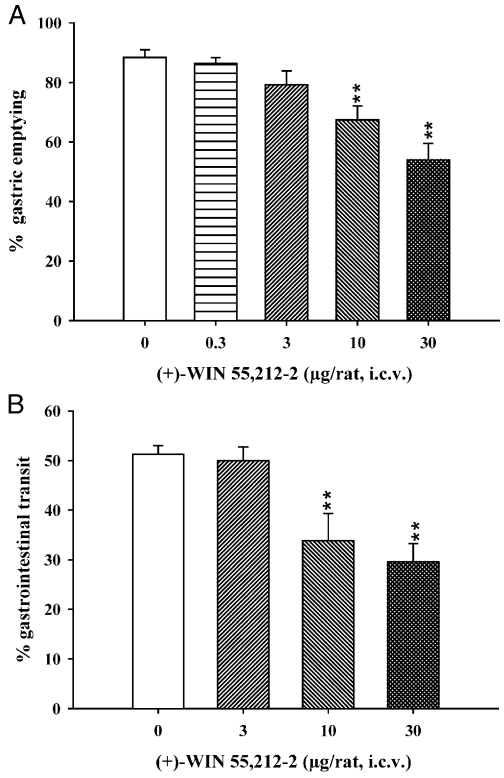

Fig. 2. Inhibition of gastric emptying (A) and gastrointestinal transit (B) after intracerebroventricular (i.c.v.) injection of the cannabinoid agonist (+)-WIN 55,212-2 (0.3–30 μg/rat). Rats were injected with either the vehicle or the drug immediately before the standard meals (gastric emptying, $BaSO_4$; gastrointestinal transit, charcoal) and were euthanized after 60 min (gastric emptying) or 20 min (gastrointestinal transit). In drug-free rats, stomach content was: $t = 0$ min, 4.35 ± 0.11 g. Data are means ± S.E.M., $n = 8–16$. $^{**}p < 0.01$ vs. controls (Duncan's test).

Table 2
Inhibition of (+)-WIN 55,212-2 and CP 55,940 effect on gastric emptying and gastrointestinal transit by oral SR141716

	SR141716, ID_{50}, mg/kg, p.o.	
	Gastric emptying	Gastrointestinal transit
(+)-WIN 55,212-2	1.3 (0.4–2.5)	1.0 (0.2–2.8)
CP 55,940	3.9 (2.1–9.0)	3.4 (0.7–10.5)

In parentheses 95% confidence intervals.
SR141716 (0.3, 1, 3, 10 mg/kg) was given 60 min before the agonist.
$n = 7–12$ rats each dose.
The regression coefficient of the dose–response curves ranged from 0.79 to 0.91.

chemicals were purchased from the commercial sources indicated: RBI (Natick, MA, USA), (+)-WIN 55,212-2, pertussis toxin; Tocris Cookson (Bristol, UK), CP 55,940.

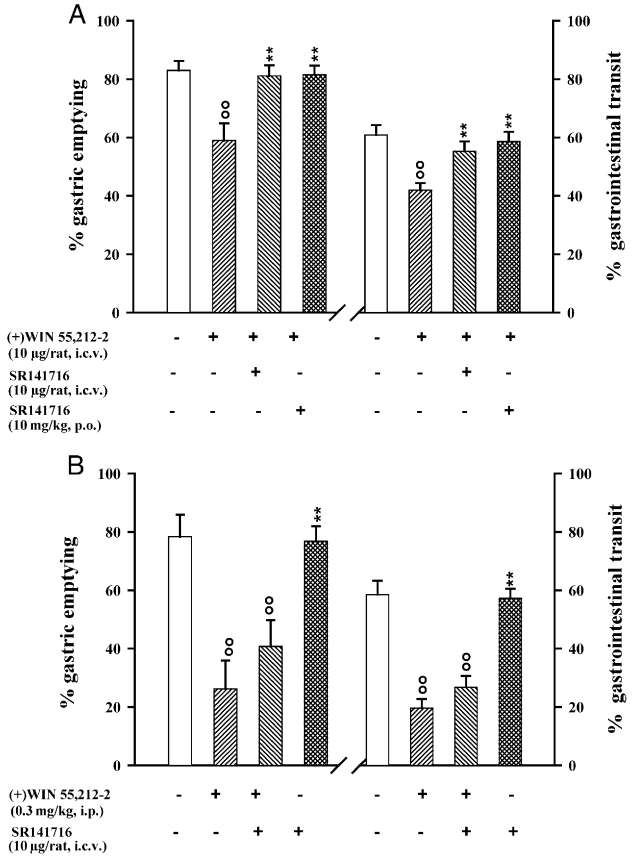

Fig. 3. Effects of the cannabinoid CB_1 antagonist SR141716 on gastric emptying and gastrointestinal transit inhibited by peripheral (i.p.) or central (i.c.v.) injection of (+)-WIN 55,212-2. (A) Rats were given the vehicle or SR141716 i.c.v. immediately, or p.o. 60 min before (+)-WIN 55,212-2 (i.c.v.). (B) Rats were given the vehicle or SR141716 i.c.v. immediately before (+)-WIN 55,212-2 (i.p.). Test meals were given immediately after (+)-WIN 55,212-2 and rats were euthanized after 60 min (gastric emptying) or 20 min (gastrointestinal transit). In drug-free rats, stomach content was: $t = 0$ min, 4.0 ± 0.17 g. Data are means ± S.E.M., $n = 7–15$. $^{**}p < 0.01$ vs. (+)-WIN 55,212-2 groups; $^{\circ\circ}p < 0.01$ vs. control groups (Duncan's test).

3. Results

As shown in Fig. 1, the intestinal progression of a non-absorbable marker given by gavage (gastric emptying and gastrointestinal transit) or i.d. (small intestinal transit) to rats was dose-dependently delayed by i.p. injection of the cannabinoid agonists (+)-WIN55,212-2 (10–3000 µg/kg) and CP 55,940 (0.3–100 µg/kg). Their ED_{50} for gastric emptying, gastrointestinal and small intestinal transit are shown in Table 1; CP 55,940 was 23–49 times more effective than (+)-WIN 55,212-2; each of the agonists showed similar potency in the different tests.

(+)-WIN 55,212-2 i.c.v. was effective in delaying gastric emptying and gastrointestinal transit also when administered i.c.v. (Fig. 2), but only at doses (10 and 30 µg/rat) producing unequivocal behavioral effects in all the animals, i.e. sedation, flat body posture and tremors, consistently with other central effects (antinociception at 5 to 10 µg/rat) reported by other investigators (Martin et al., 1995). Submaximal systemic doses of CP 55,940 or (+)-WIN 55,212-2 (10 and 300 µg/kg, i.p., respectively) had no behavioral or hypothermic effects (rectal temperature 0.5, 1, 2 and 3 h after drug treatment did not differ significantly from that of vehicle-treated rats; data not shown).

As shown in Table 2, oral SR141716 (0.3–10 mg/kg) given 1 h before submaximal doses of (+)-WIN 55,212-2 (300 µg/kg, i.p.) or CP 55,940 (10 µg/kg, i.p.) dose-dependently prevented their delaying action on gastric emptying and gastrointestinal transit, with comparable potency. Oral SR141716 (10 mg/kg) also prevented the delay of gastric emptying and gastrointestinal transit (as well as behavioral and hypothermic effects by i.c.v. (+)-WIN 55,212-2 (10 µg/rat) (Fig. 3A). When given alone, either i.c.v. (10 µg/rat) (Fig. 3B) or orally (0.1–10 mg/kg) (Fig. 4), SR141716 did not alter gastric emptying or gastrointestinal transit.

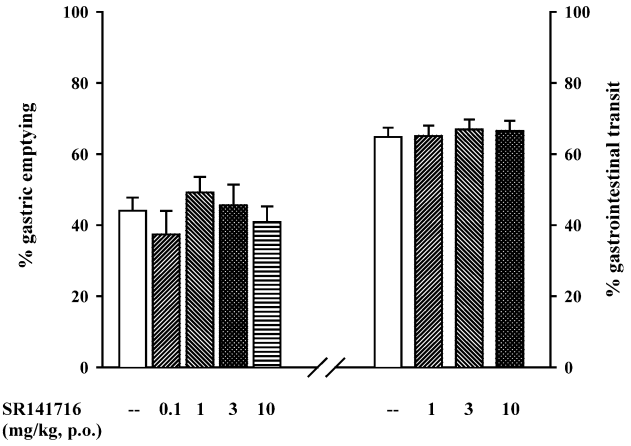

Fig. 4. Lack of effect of the cannabinoid CB_1 receptor antagonist, SR141716 on gastric emptying or gastrointestinal transit. Rats received either the vehicle (control, open bars) or SR141716 orally 60 min before the standard meals and were euthanized after 15 min (gastric emptying) or 20 min (gastrointestinal transit). Data are mean ± S.E.M., $n = 8$.

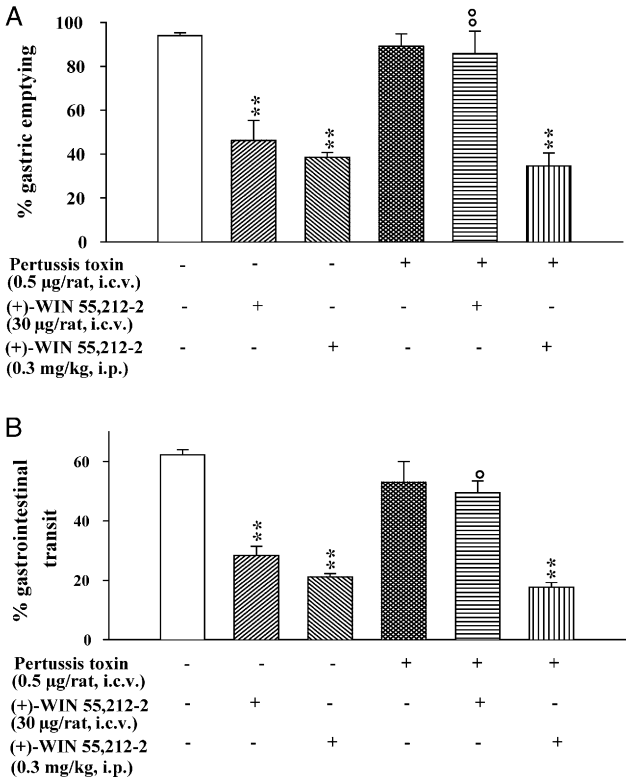

Fig. 5. Effect of pretreatment (96 h) with pertussis toxin (0.5 µg/rat, i.c.v.) or vehicle (saline) on gastric emptying (A) and gastrointestinal transit (B) inhibited by peripheral (i.p.) or central (i.c.v.) injection of (+)-WIN 55,212-2. Four days after pertussis toxin (or vehicle) rats were given the vehicle or (+)-WIN 55,212-2 (i.c.v. or i.p.) immediately before the test meals and were euthanized after 60 min (gastric emptying) or 20 min (gastrointestinal transit). Data are mean ± S.E.M., $n = 5–8$. $^{**}p < 0.01$ vs. controls; °, °° $p < 0.05$, 0.01 vs. respective pertussis toxin-free groups (Duncan's test).

Gastric emptying and gastrointestinal transit delay in rats given (+)-WIN 55,212-2 i.c.v. (10 µg/rat) was prevented by SR141716 i.c.v. (10 µg/rat) (Fig. 3A), which had no significant effect on gastric emptying and gastrointestinal transit after i.p. (+)-WIN 55,212-2 (Fig. 3B). In rats given pertussis toxin i.c.v. (0.5 µg/rat) 96 h before, the delaying effect of i.c.v. (+)-WIN 55,212-2 on gastric emptying and gastrointestinal transit, but not that of i.p. (+)-WIN 55,212-2, was significantly ($p < 0.01$) reduced (Fig. 5).

The selective cannabinoid CB_2-receptor antagonist SR144528, 3 mg/kg p.o. 1 h before (+)-WIN 55,212-2, 0.3 mg/kg, i.p., did not affect its delaying action on either gastric emptying or gastrointestinal transit; percentages of inhibition ± S.E.M. were: for (+)-WIN 55,212-2 control, SR144528 alone and (+)-WIN 55,212-2 plus SR144528, 63 ± 6, −4 ± 8 and 64 ± 9 in gastric emptying and 65 ± 2, 5 ± 7 and 67 ± 4, in gastrointestinal transit, as listed.

4. Discussion

Cannabinoids inhibit gastric emptying and gastrointestinal transit in rodents (Chesher et al., 1973; Shook and Burks, 1989; Colombo et al., 1998; Izzo et al., 1999; Pertwee, 2001) and in man (Sridhar et al., 1984). Their psychoactive properties and their ability to reduce gut motility when administered directly into the brain of laboratory animals (Shook and Burks, 1989; Shook et al., 1986) suggest a central site of intestinal action. However, Ross et al. (1998) obtained evidence of cannabinoid receptors in the guinea-pig myenteric plexus-longitudinal muscle preparation by showing that this tissue contains specific binding sites. In vitro motor responses to cannabinoid agonists of isolated intestinal segments from animals (Pertwee et al., 1996; Izzo et al., 1998) and man (Croci et al., 1998; Manara et al., 2002) and their prevention by the selective cannabinoid CB_1 antagonist SR141716 (Rinaldi-Carmona et al., 1994) provide functional evidence of these peripheral action sites.

The present in vivo animal study contributes original data for assessing: (a) the relative importance of central and peripheral cannabinoid CB_1 receptors in synthetic cannabinoids' effects on gastrointestinal transit; (b) the potentially similar role of putative endogenous cannabinoids; (c) the proposed ''inverse agonist'' properties of cannabinoid antagonists. Unprecedented observations were obtained in rats pre-treated i.c.v., either with a selective cannabinoid CB_1 receptor antagonist, or with the receptor coupling impairing agent pertussis toxin, in order to challenge the intestinal action of systemically and i.c.v. administered cannabinoids. Further original data come from investigation of gastrointestinal propulsion in rats given only the cannabinoid CB_1 receptor antagonist SR141716, whose negative results are consistent with those we obtained before in the human isolated intestine (Croci et al., 1998).

Thus, we have confirmed in rats with (+)-WIN 55,212-2 the earlier observation by other investigators in mice given Δ^9-tetrahydrocannabinol i.c.v., and the similarly modest agonist dose–effect pattern after central administration (Shook et al., 1986), implying the primary involvement of a peripheral action site. In our study, SR141716, given either orally or centrally, consistently prevented gastrointestinal transit delay by i.c.v. (+)-WIN 55,212-2, whereas when administered centrally it had virtually no effects against the gastrointestinal action of i.p. (+)-WIN 55,212-2.

We also showed that pretreatment of rats with i.c.v. pertussis toxin greatly reduces the inhibition of gastric emptying and the antitransit effects of centrally administered (+)-WIN 55,212-2, suggesting that the agonist effect depends on a G protein-mediated pertussis toxin-sensitive mechanism. On the other hand, i.c.v. pertussis toxin did not influence the effects of (+)-WIN 55,212-2 i.p., further indicating that they are not centrally mediated. Izzo et al. (2000) indicated an exclusively locally elicited intestinal effect of (+)-WIN 55,212-2 administered i.p. to mice, since this persisted in animals given hexamethonium, that renders i.c.v. (+)-WIN 55,212-2 ineffective.

These findings are consistent with recent data attesting to poor penetration in the mouse brain of synthetic agonists

such as (+)-WIN 55,212-2 and CP 55,940, compared to natural compounds like Δ^9-tetrahydrocannabinol (Petitet et al., 1999) and suggest that certain of them may affect digestive motility by acting mostly at peripheral sites. The opposite might be the case with synthetic cannabinoid agonists, which are readily available to the central nervous system after systemic administration. Indeed, a similar central and/or peripheral mode of the motor effects on the gut, most probably dependent on their pharmacokinetic properties, applies to opioids (Peracchia et al., 1984), whose intestinal effects are not necessarily accompanied and/or accounted for by behavioral effects or analgesia (Manara and Bianchetti, 1985).

The gastrointestinal effects of drugs delivered into the central nervous system provide reasonable evidence of specific action sites therein. But it seems logical to question the not uncommon practice of comparing such effects with those of systemically administered compounds in terms of the effective doses, and arguing about the relative contribution of centrally and peripherally located receptors in the effects of systemically administered drugs on the gut. In the present case, in fact, we noticed no hypothermia, sedation or behavioral effects in rats whose gastrointestinal transit was delayed by submaximal doses of i.p. (+)-WIN 55,212-2, whereas only ''sedative'' amounts of i.c.v. (+)-WIN 55,212-2 caused a similar delay. This is also consistent with the already mentioned report of poor penetration of (+)-WIN 55,212-2 in the CNS (Petitet et al., 1999).

The results we obtained by monitoring the progression of a test meal, administered either intragastrically or i.d. through different sections of the rat gut are consistent with the only previous study using the same approach (Shook and Burks, 1989). These findings substantiate the notion that the delaying action of cannabinoids may be exerted at multiple levels along the alimentary canal (Pertwee, 2001). In addition, since the agonists' action was prevented by the cannabinoid CB_1-selective antagonist SR141716, but not by SR144528, which is selective for cannabinoid CB_2 receptors (Rinaldi-Carmona et al., 1998), the latter are presumably not involved in these endpoints.

SR141716, beside antagonizing cannabinoid agonists, in line with our in vitro findings in the human gut (Croci et al., 1998), apparently had no effects on its own. Specifically, it did not increase gastric emptying or gastrointestinal transit. This does not support the contention by several other investigators who, under different experimental conditions, noted effects opposite to those of cannabinoid agonists after administration of SR141716 alone, and concluded it may have ''inverse agonist'' properties and/or may disclose a tonic action by endogenous agonists at cannabinoid CB_1 receptors (Pertwee et al., 1996; Pertwee, 2001; Santucci et al., 1996; Bouaboula et al., 1997; Izzo et al., 1998, 2000). These inconsistencies on the ability of SR141716 to elicit responses on its own may be related to species-dependent factors.

In conclusion, the findings in our animal model are indicative of a primary role of local intestinal cannabinoid CB_1 receptors in gastric emptying and gastrointestinal transit delay by specific agonists. This opens the way to designing compounds with no central actions, as new therapeutic agents for altered gastrointestinal motor function, all the more so since gut motility seems susceptible to endogenous cannabinoids (Calignano et al., 1997).

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