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Rapid tolerance to the intestinal prokinetic effect of cannabinoid CB_1 receptor antagonist, SR 141716 (Rimonabant)

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Abstract

The cannabinoid CB_1 receptor antagonist, SR 141716 (Rimonabant), has been reported to stimulate, when acutely administered, intestinal motility in mice. The present study was aimed at determining whether tolerance develops to its repeated administration. Mice were treated twice a day for up to 8 consecutive days with 0, 3 and 5.6 mg/kg SR 141716 (i.p.). On days 1, 3, 5 and 8, separate groups of mice were treated intragastrically with a non-absorbable colored marker (carmine). The distance traveled by the head of the marker in the small intestine was recorded. On day 1, SR 141716 markedly activated intestinal peristalsis, but complete tolerance to this effect developed within the third day of treatment. The results may have some relevance to the proposed future clinical use of SR 141716.

Keywords: Cannabinoid CB_1 receptor antagonist; SR 141716 (Rimonabant); Intestinal motility; Chronic treatment and tolerance development; Mouse

1. Introduction

A role for the cannabinoid CB_1 receptor in the modulation of intestinal motility has been recognized (see Izzo et al., 2001; Pertwee, 2001). Cannabinoid CB_1 receptors have been localized in the enteric nervous system (Lynn and Herkenham, 1994; Griffin et al., 1997; Kulkarni-Narla and Brown, 2000), and their activation by endogenous as well as synthetic agonists has been found to inhibit gastrointestinal motility. Thus, the administration of cannabinoid CB_1 receptor agonists delayed gastric emptying (Izzo et al., 1999b; Landi et al., 2002) and depressed intestinal peristalsis (Calignano et al., 1997; Colombo et al., 1998b; Izzo et al., 1999a; Krowicki et al., 1999). In vitro cannabinoid CB_1 receptor agonists have been shown to inhibit contractions of longitudinal and circular smooth muscles in isolated strips of ileal tissue of humans (Croci et al., 1998) and laboratory rodents (Pertwee et al., 1992a, 1996).

The selective antagonist of the cannabinoid CB_1 receptor, SR 141716 (Rimonabant) (Rinaldi-Carmona et al., 1994), has been reported to prevent the inhibitory effects of cannabinoids on intestinal motility (see above for reference), and to exert opposite effects when given alone. With regard to the latter effects, the addition of SR 141716 to in vitro preparations enhanced electrically evoked acetylcholine release from myenteric nerves (Coutts and Pertwee, 1997) and electrically evoked contractions of myenteric plexus longitudinal muscle (Pertwee et al., 1996; Izzo et al., 1998; Coutts et al., 2000) obtained from guinea pigs. Further, the acute administration of SR 141716 has been reported to stimulate the passage of a non-absorbable meal through the small intestine (Calignano et al., 1997; Colombo et al., 1998b; Izzo et al., 1999a) and defecation (Izzo et al., 1999a) in mice. These results have been interpreted as suggesting the existence of a cannabinoidergic tone that normally inhibits peristalsis or, alternatively, as reflecting of the inverse agonism activity of SR 141716 at the cannabinoid CB_1 receptor.

Despite this large body of evidence, to our knowledge no study has investigated the effect of repeated administration of SR 141716 on gastrointestinal propulsion. Accordingly, the present study was aimed at determining whether SR 141716 given to mice for up to 8 consecutive days would maintain its prokinetic activity.

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2. Materials and methods

2.1. Animals

Male CD1 mice (Charles River, Calco, LC, Italy), weighing 30–35 g, were used. Mice were housed 20 per cage in standard plastic cages with wood chip bedding under a 12-h/12-h artificial light–dark cycle (lights on at 7:00 a.m.), at a constant temperature of 22 ± 2 °C and relative humidity of approximately 60%. Tap water and standard laboratory rodent chow (Mucedola, Settimo Milanese, MI, Italy) were provided ad libitum.

2.2. Procedure

The present study employed the Upper Gastrointestinal Transit procedure, based on evidence that a non-absorbable marker (namely, carmine) travels approximately 50% along the small intestine when infused intragastrically, 20 min before sacrifice, to undrugged mice (Nagakura et al., 1996; Carai et al., 2002).

Mice were divided into 12 groups of $n = 7–10$ and treated with 0, 3 and 5.6 mg/kg SR 141716 acutely or for 3, 5 and 8 consecutive days. In the mouse groups of the repeated treatment, SR 141716 was given twice a day (9:00 a.m. and 7:00 p.m.). SR 141716 (Sanofi-Synthelabo, Montpellier, France) was suspended in saline with 0.1% Tween 80 and injected intraperitoneally in a 12.5-ml/kg volume. On the test day, carmine was infused intragastrically via a stainless steel, 3.8-mm long gavage, 30 min after SR 141716 injection. In the mouse groups receiving the repeated treatment, the test was conducted after the morning injection of SR 141716. Carmine (Sigma-Aldrich, Milan, MI, Italy) was suspended at the concentration of 6% (w/v) in distilled water containing 0.5% methylcellulose and administered at the dose of 0.3 ml/mouse. Twenty minutes after carmine administration, mice were sacrificed by cervical dislocation and intestines were removed from the pylorus to the cecum. The distance covered by the head of carmine was measured and expressed as percent of the total length of the small intestine.

Data were analyzed by a two-way (treatment × time) analysis of variance (ANOVA), followed by the Newman–Keuls test for post hoc comparisons.

The experimental procedures employed in the present study were in accordance with the European Communities Council Directive (86/609/EEC) and the subsequent Italian Law on the "Protection of animals used for experimental and other scientific reasons".

3. Results

Acute treatment with 3 and 5.6 mg/kg SR 141716 resulted in a 60–75% increase in gastrointestinal transit, with respect to vehicle-treated mice (Fig. 1). However, on continuing treatment, the stimulating effect of SR 141716 on intestinal propulsion rapidly decreased. In mice treated with 3 mg/kg SR 141716, on days 3, 5 and 8, the distance covered by the marker was not significantly higher than that in the corresponding vehicle-treated groups (Fig. 1). In the mouse groups treated with 5.6 mg/kg SR 141716, on days 3, 5 and 8, the distance traveled by the marker was (a) significantly lower than that monitored on day 1 in 5.6 mg/kg SR 141716-treated mice, and (b) not significantly higher than that recorded in the corresponding vehicle-treated groups (Fig. 1).

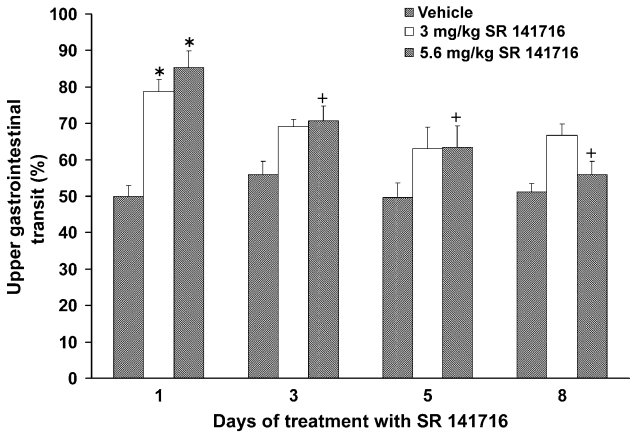

Fig. 1. Effect of acute (day 1) and repeated (days 3, 5 and 8) intraperitoneal administration of the cannabinoid CB_1 receptor antagonist, SR 141716, on propulsive activity in the mouse small intestine. In the mouse groups receiving the repeated treatment, SR 141716 was injected twice a day. On the test days, SR 141716 was administered 30 min before the intragastric administration of the non-absorbable marker, carmine. Twenty minutes later, mice were killed and the distance traveled 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. ANOVA results: $F_{treatment}(2;105) = 25.9390$, $P < 0.000001$; $F_{time}(3;105) = 6.8651$, $P < 0.0005$; $F_{interaction}(6;105) = 3.0319$, $P < 0.01$. Each bar is the mean ± S.E.M. of $n = 7–10$ mice. $^{*}P < 0.05$ with respect to vehicle-treated rats on day 1 (Newman–Keuls test); $^{+}P < 0.05$ with respect to mice treated with the same dose on day 1 (Newman–Keuls test).

4. Discussion

Our results confirm previous reports (Calignano et al., 1997; Colombo et al., 1998b; Izzo et al., 1999a) indicating that the acute administration of SR 141716 produces a marked stimulation of mouse small intestine peristalsis. However, tolerance to this effect rapidly developed after repeated treatments. Indeed, the stimulant effect of SR 141716 on the transit of the non-absorbable marker through the small intestine had already vanished on the third day of treatment with each of the tested dose.

To our knowledge, these results constitute the first evidence of the development of tolerance to the prokinetic effect of SR 141716. On the other hand, tolerance to the constipating effect of Δ^9-tetrahydrocannabinol in mice and rats was reported long time ago (Masur et al.,

1971; Anderson et al., 1975). More recently, tolerance to the inhibitory action of different cannabinoid receptor agonists in in vitro preparations of myenteric plexus longitudinal muscles of rodents has been observed (Pertwee et al., 1992b; Basilico et al., 1999). No data are presently available to understand the possible contribution of metabolic tolerance to SR 141716 to the observed phenomenon.

The results of the present study extend to the prokinetic effect of SR 141716 the development of tolerance to different in vivo effects of the drug, including hypophagia (Colombo et al., 1998a; Vickers et al., 2003), wet dog and head shakes, forepaw fluttering and facial rubbing (Rubino et al., 2000). Clarification of the mechanism of the rapid onset of tolerance to the prokinetic effect of SR 141716 might help to understand the physiological role of the cannabinoid CB_1 receptor in the control of intestinal motility and, more generally, the mechanisms involved in tolerance to cannabinoid agents. Such information appears to be timely because of the proposed introduction of SR 141716 in clinic for its ability to induce weight loss and smoking cessation (Le Fur et al., 2001).

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