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Dopamine D_2 receptor antagonists prevent Δ^9-tetrahydrocannabinol-induced antinociception in rats

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Abstract

Δ^9-Tetrahydrocannabinol (1 and 5 mg/kg, i.p.) produced, dose-dependently, antinociceptive effects using hot plate and tail flick tests in rats. These effects were suppressed not only by the cannabinoid CB_1 receptor antagonist SR 141716A (0.5 mg/kg; i.p.) but also by the dopamine D_2 receptor antagonists *S*(−)-sulpiride (5 and 10 mg/kg; i.p.) and S(−)-raclopride (1.5 and 3 mg/kg; i.p.). Conversely, Δ^9-tetrahydrocannabinol antinociceptive effects were potentiated by the dopamine D_2 receptor agonists (−)-quinpirole (0.025 mg/kg, s.c.) and (+)-bromocriptine (0.5 and 1 mg/kg; i.p.). Our results indicate that the antinociceptive effects of Δ^9-tetrahydrocannabinol are mediated by the concomitant activation of cannabinoid CB_1 and dopamine D_2 receptors and that dopamine D_2 receptor agonists may be useful in improving the analgesic effects of cannabinoids.

Keywords: Analgesia; Δ^9-Tetrahydrocannabinol; SR 141716A; *S*(−)-Sulpiride; *S*(−)-Raclopride; (−)-Quinpirole; (+)-Bromocriptine; Dopamine

1. Introduction

Many anedoctal reports indicate that marijuana produces analgesic effect in humans. Moreover, Δ^9-Tetrahydrocannabinol, the major psychoactive constituent of *Cannabis sativa*, as well as different synthetic and endogenous cannabinoids have been shown to produce antinociceptive effects in different animal species (Lichtman and Martin, 1991; Calignano et al., 1998). Cannabinoids produce their antinociceptive effects through CB_1 cannabinoid receptors at spinal and supraspinal sites (Lichtman and Martin, 1991). Moreover, recent evidence indicates an involvement of both cannabinoid CB_1 and CB_2 receptors at sites of tissue injury controlling pain initiation (Calignano et al., 1998). On the other hand, dopaminergic mechanisms have been shown to be involved in the control of nociception (Tricklebank et al., 1984). Specifically, dopamine agonists such as apomorphine, d-amphetamine and cocaine produce analgesia in the formalin test in rats, and the dopamine D_2 receptor agonist (−)-quinpirole increases the antinociceptive effect of morphine (Zarrindast et al., 1999). Conversely, a large body of evidence suggests that cannabinoids activate meso-cortico-limbic dopamine neurons (Diana et al., 1998; Gessa et al., 1998) and increase dopamine release (Chen et al., 1990). These findings suggest that brain dopamine might play a role in cannabinoid-induced antinociceptive effects.

The purpose of the present study was to determine whether Δ^9-Tetrahydrocannabinol antinociceptive effects were modified by the blockade or the stimulation of dopamine D_2 receptors.

2. Materials and methods

In male Sprague–Dawley rats (200–250 g, Charles River, Como, Italy) the antinociceptive effects were evaluated using the hot plate (D'Amour and Smith, 1941) and tail flick tests (Eddy and Liembach, 1953). In the hot plate, kept at a temperature of 55 ± 0.5°C and delimited by a glass cylinder (60 cm high, 30 cm diameter), two responses were examined: paw-lick and jumping latencies, respectively. Cut-off times were set at 40 and 240 s, respectively. In the tail flick test the time required to respond to a thermal stimulus on the tail was measured. Animals were restrained and at mid-tail level an optic

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source of heat kept at a temperature of 52 ± 0.5°C was applied. Cut-off time was set at 10 s. Antinociception was expressed as percentage of the maximum possible effect (%MPE) in both tests and were calculated as follows:

$$\%\text{MPE} = [(\text{test latency} - \text{control latency}) / (\text{cut-off time} - \text{control latency})] \times 100.$$

Δ^9-Tetrahydrocannabinol (RBI, Italy) solutions were prepared from vials containing 10 mg of the drug in 1 ml of absolute ethanol. Vials were evaporated under nitrogen and the residue dissolved in two drops of Tween 80 and diluted in saline. SR 141716A (Sanofi, Recherche, Montpellier, France), a CB_1 receptor antagonist, was dissolved in two drops of Tween 80 and then diluted in saline. The dopamine D_2 receptor agonists (−)-quinpirole hydrochloride (RBI, Italy) and (+)-bromocriptine methanesulfonate (RBI, Italy) and the D_2 dopamine receptor antagonists *S*(−)-sulpiride (RBI, Italy) and *S*(−)-raclopride L-tartrate were dissolved in saline. (−)-Quinpirole was given subcutaneously (s.c.) in a volume of 2 ml/kg, while the other drugs were administered intraperitoneally in a volume of 3 ml/kg. Control rats were treated with the vehicle used to dissolve the active ingredient.

Between-group comparisons were performed by analysis of variance (ANOVA) for repeated measures. Post-hoc comparisons were performed by a Student–Newman–Keuls tests. Significance was held at $P < 0.01$.

The study reported in this manuscript was carried out in accordance with the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals. All experiments were approved by the local ethics committee.

3. Results

In line with previous reports, Δ^9-tetrahydrocannabinol (5 mg/kg) produced significant increase in latency to paw-lick, jumping and tail flick (Figs. 1 and 2). The antinociceptive effects of Δ^9-tetrahydrocannabinol were fully antagonized not only by the cannabinoid CB_1 receptor antagonist SR 141716A (0.5 mg/kg) but also by the dopamine D_2 receptor antagonists *S*(−)-sulpiride (5 and 10 mg/kg) and (*S*)(−)-raclopride (1.5 and 3 mg/kg) (Figs. 1 and 2). Conversely, administration of the dopamine D_2 receptor agonists (−)-quinpirole (0.025 mg/kg) and (+)-bromocriptine (1 mg/kg) maximally potentiated the

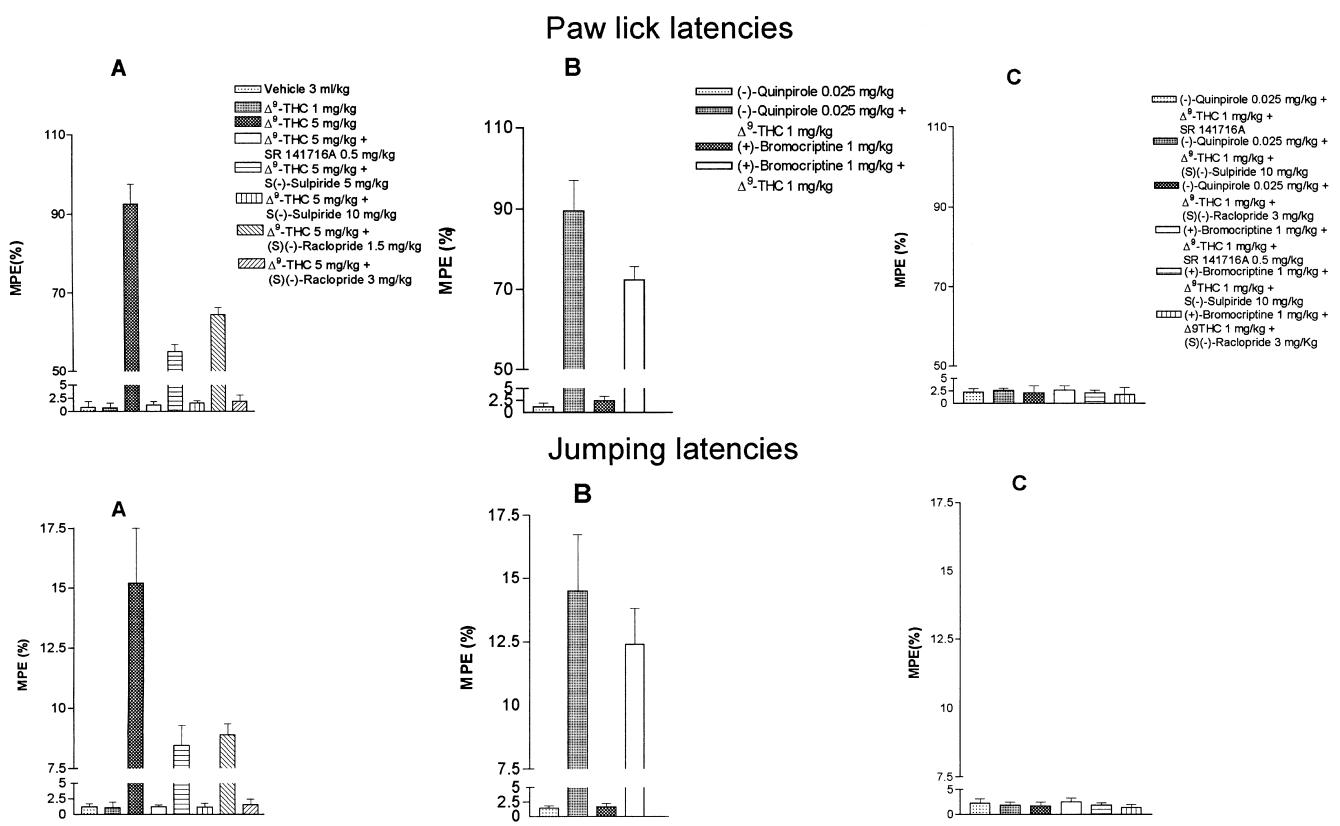

Fig. 1. (A) Blockade of the antinociceptive effects of Δ^9-tetrahydrocannabinol by SR 141716A, *S*(−)-sulpiride and (*S*)(−)-raclopride in the hot plate test. (B) Potentiation of Δ^9-tetrahydrocannabinol effect by (−)-quinpirole and (+)-bromocriptine and (C) reversal by SR 141716A, *S*(−)-sulpiride and (*S*)(−)-raclopride. Δ^9-tetrahydrocannabinol was given 60 min before the test, while (−)-quinpirole, (+)-bromocriptine, SR 141716A, *S*(−)-sulpiride and (*S*)(−)-raclopride were given 20 min before Δ^9-tetrahydrocannabinol. Antinociception is expressed as the percent of maximum possible effect (%MPE) in the hot plate test. $n = 6$ rats per column. All statistical comparisons (Student–Newman–Keuls test) revealed significant differences with $P < 0.0001$.

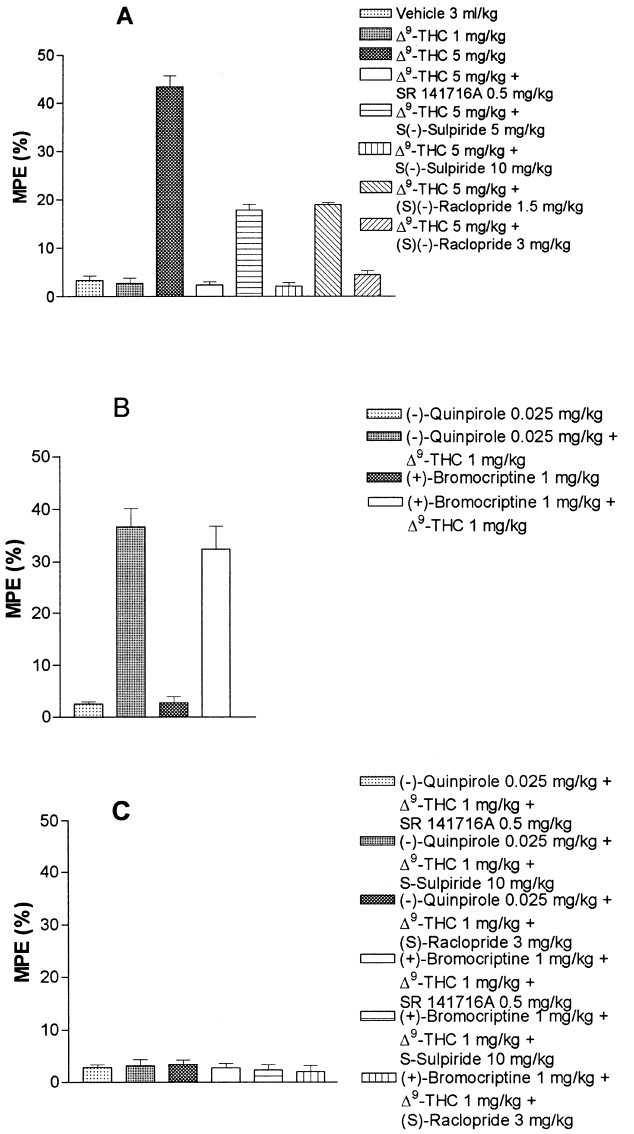

Fig. 2. (A) Blockade of the antinociceptive effects of Δ^9-tetrahydrocannabinol by SR 141716A, *S*(−)-sulpiride and (*S*)(−)-raclopride in the tail flick test. (B) Potentiation of Δ^9-tetrahydrocannabinol effect by (−)-quinpirole and (+)-bromocriptine and (C) reversal by SR 141716A, *S*(−)-sulpiride and (*S*)(−)-raclopride. Δ^9-Terahydrocannabinol was given 60 min before the test, while (−)-quinpirole, (+)-bromocriptine, SR 141716A, *S*(−)-sulpiride and (*S*)(−)-raclopride were given 20 min before Δ^9-Terahydrocannabinol. The figure shows only the highest doses of the dopamine D_2 receptor agonists tested. Antinociception is expressed as the percent of maximum possible effect (%MPE) in the hot plate test. $n = 6$ rats per column. All statistical comparisons (Student–Newman–Keuls test) revealed significant differences with $P < 0.0001$.

analgesic effect of an ineffective dose of Δ^9-tetrahydrocannabinol (1 mg/kg) (Figs. 1 and 2). The effects of the drug combinations were totally antagonized by the cannabinoid CB_1 receptor antagonist SR 141716A and by the dopamine D_2 receptor antagonists *S*(−)-sulpiride and (*S*)(−)-raclopride (Figs. 1 and 2). At the dose tested, SR 141716 A, *S*(−)-sulpiride, (*S*)(−)-raclopride, (−)-quinpirole and (+)-bromocriptine when given alone, failed to modify paw lick, jumping and tail flick latencies.

4. Discussion

These results confirm the action of Δ^9-tetrahydrocannabinol in the modulation of nociceptive responses. Moreover, our findings suggest that Δ^9-tetrahydrocannabinol antinociceptive effects are mediated by the concomitant activation of cannabinoid CB_1 and dopamine D_2 receptors, the latter being stimulated by endogenously released dopamine after the cannabinoid administration. Interestingly, since the hot plate test elicited an acute pain involving supraspinal processes, whereas tail flick responses are considered to be a spinal reflex we might suppose the involvement of the cannabinoid CB_1 and dopamine D_2 receptors both at supraspinal and spinal sites controlling cannabinoid-induced antinocipetive effects. With regard to the molecular mechanism involved we might suggest that the concomitant activation of both receptors produces a degree of cyclic AMP inhibition sufficient for Δ^9-tetrahydrocannabinol effect to occur (Raffa et al., 1999). On the other hand, stimulation of either receptor alone appears to be insufficient to produce the analgesic effects. Our results raise the important question whether dopamine D_2 receptors play a permissive role in other pharmacological effects of cannabinoids besides analgesia, and suggest that D_2 receptor agonists may be useful in potentiating the analgesic effects of cannabinoids for clinical purposes.

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