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The Selective Cannabinoid Antagonist SR 141716A Blocks Cannabinoid-Induced Antinociception in Rats

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LICHTMAN, A. H. AND B. R. MARTIN. *The selective cannabinoid antagonist SR 141716A blocks cannabinoid-induced antinociception in rats.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 7–12, 1997.—The purported CB_1 cannabinoid antagonist SR 141716A has proven to be a useful tool in the investigation of cannabinoid pharmacology. This antagonist was employed in the present study to investigate the antinociceptive and cataleptic effects of cannabinoids after either systemic or intracerebroventricular (ICV) administration. The antinociceptive potency of systemically administered Δ^9-tetrahydrocannabinol (Δ^9-THC) was decreased 18-fold by SR 141716A, from an ED_{50} value of 0.3–5.1 mg/kg. Similarly, it completely blocked the antinociceptive effects of Δ^9-THC and CP 55,940, a potent bicyclic cannabinoid, after ICV administration. In addition, it prevented cannabinoid-induced catalepsy when given by either route of administration. In contrast, SR 141716A failed to antagonize the antinociceptive effects of morphine, indicating its selectivity for cannabinoid receptors. These findings indicate that the antinociceptive and cataleptic effects of Δ^9-THC and CP 55,940 are mediated through CB_1 cannabinoid receptors. © 1997 Elsevier Science Inc.

Δ^9-tetrahydrocannabinol (Δ^9-THC) CP 55,940 catalepsy Intracerebroventicular Marijuana Antinociception Morphine Cannabinoid Cannabis SR 141716A

OF the many pharmacologic effects elicited by the cannabinoids, their antinociceptive action has been one of the most extensively investigated (7,16) and may have therapeutic potential for the treatment of pain in humans (22). Two lines of evidence indicate that cannabinoid-induced antinociception is mediated, in part, by a supraspinal component of action. First, spinal transection only partially blocks the antinociceptive effects of cannabinoids suggesting both spinal and supraspinal contributions (11). Second, administration of cannabinoids into either the lateral ventricle or the periaqueductal gray (PAG) produces antinociception (9,18).

Currently, two cannabinoid receptors have been isolated and sequenced: the CB_1 and CB_2 receptors. Whereas the CB_1 receptor is present throughout the CNS and the periphery (19), the CB_2 receptor appears to be present in only the periphery (21). The recent discovery of SR 141716A, an antagonist that is selective for CB_1 cannabinoid receptors (28), has been a valuable tool in the investigation of cannabinoid pharmacology. In mice, this drug blocks the antinociceptive, cataleptic, hypothermic, and other motor effects of cannabinoids (5,27,28). In addition, SR 141716A antagonizes turning behavior elicited by unilateral intrastriatal injections of cannabinoid agonists (29). In rats, it antagonizes the hypothermic (28), hypotensive (32), and memory-impairing (13) effects of cannabinoids as well as the discriminative stimulus cues of several cannabinoids in the drug discrimination paradigm (34,35). SR 141716A has also been found to precipitate a withdrawal syndrome in rats chronically treated with Δ^9-THC (1,31). Finally, SR 141716A prevents the inhibitory effects of cannabinoids on adenylyl cyclase accumulation, cerebellar cGMP, twitch contractions of the mouse vas deferens, and long-term potentiation of hippocampal neurons (4,27,28). This antagonism by SR 141716A suggests that many of the pharmacologic effects of cannabinoids in both in vivo and in vitro tests are mediated by CB_1 cannabinoid receptors.

Although SR 141716A has been found to antagonize cannabinoid-induced antinociception in mice (5,28), it is unknown

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whether it is also effective in antagonizing cannabinoid-induced antinociception in rats. The primary purpose of the present investigation, therefore, was to evaluate whether the antinociceptive effects of the cannabinoid agonists Δ^9-THC and CP 55,940 in rats are mediated through CB_1 receptors in the brain using the cannabinoid antagonist SR 141716A. Rats were administered all drugs through either systemic or ICV routes of administration, and the tail-flick test to radiant heat was used to assess nociception. Because cannabinoids are known to produce a great variety of effects, subjects were also evaluated for catalepsy. Finally, to assess the specificity of ICV-administered SR 141716A to cannabinoid-induced antinociception, the effect of this antagonist on morphine-induced antinociception was evaluated.

METHODS

Subjects

Sprague–Dawley (Harlan, Indianapolis, IN) male rats served as subjects and were individually housed in a temperature-controlled (20–22°C) environment with a 12 L:12 D cycle. Food (Prolab; Agway, Richmond, VA) and water were available ad lib. Each subject was used only once.

Drug Preparation and Administration

CP 55,940 and SR 1417816A were obtained from Pfizer Pharmaceuticals (Groton, CN), and Δ^9-THC and morphine sulfate were provided by the National Institute on Drug Abuse (Bethesda, MD). For each systemic injection, Δ^9-THC and SR 141716A were dissolved in a 1:1 mixture of absolute ethanol and alkamuls-620 (formerly called emulphor-620; Rhone-Poulenc, Princeton, NJ) and diluted with saline. The vehicle ratio for Δ^9-THC was 1:1:18 (ethanol:alkamuls:saline), and was 1:1:8 (ethanol:alkamuls:saline) for SR 141716A. Because the antinociceptive effects of systemically administered Δ^9-THC have been reported to be almost 50-fold more potent via intravenous (IV) administration than subcutaneous (SC) injection (15), we elected to administer Δ^9-THC via the IV route of administration. In contrast, the intraperitoneal (IP) route of administration of SR 141716A appears to antagonize the pharmacologic effects of cannabinoid agonists in mice (28) as well as in rats (35). Consequently, Δ^9-THC was administered intravenously into a tail vein and SR 141716A was given by the IP route of administration. Each of the cannabinoids was dissolved in 100% dimethylsulfoxide (DMSO) for the ICV injections to obtain a sufficient concentration of drug; saline was the vehicle for morphine sulfate. The volumes for the ICV injections and the systemic injections were 5 μl and 1 ml/kg, respectively.

ICV Implantation and Injections

Each animal was anesthetized with sodium pentobarbital (60 mg/kg) and a 23-ga stainless-steel guide cannula was implanted above the right lateral ventricle using a stereotaxic technique with bregma as the reference point (24). The coordinates for the cannulae placements were: A/P −0.9 mm, L +1.3 mm, and D/V −3.4 mm. A 29-ga stainless-steel needle was inserted into each cannula and advanced 1.8 mm beyond the tip of the guide cannula into the lateral ventricle. The injections were 1 min in duration, and the needles were removed 1 min after infusion. Each cannula was kept patent with a stainless-steel obturator. At the conclusion of the study, each animal was given a lethal dose of sodium pentobarbital (100 mg/kg) and 5 μl of brilliant blue was microinjected through the cannula to confirm whether the injection was in the ventricles. The brain was removed for histologic examination; the injection site was considered successful if the ventricles contained the dye. Data were included in the statistical analyses only from subjects in which the cannula tips were in the lateral ventricle.

Behavioral Testing

Antinociception. The tail-flick response to radiant heat (6) was used to assess antinociception. The intensity of the heat stimulus was fixed to yield control latencies of 3–4 s, and an automatic 8-s cutoff was used to prevent tissue damage.

Catalepsy. A ring-test procedure that has been used to evaluate catalepsy in mice (25) was modified and automated to assess catalepsy in rats (17). The apparatus and procedure have been described elsewhere (9,10).

Protocol. In the systemic as well as the ICV studies, subjects were administered the cannabinoid antagonist or appropriate vehicle, followed 10 min later by an injection of Δ^9-THC, CP 55,940, or the vehicle. SR 141716A administered 10 min before the agonist has been reported to antagonize the antinociceptive effects of Δ^9-THC in mice (5) as well as the discriminative stimulus properties of Δ^9-THC and CP 55,940 in rats (34,35). The subjects were evaluated in the tail-flick test at 30 min and for catalepsy at 50 min after the first injection. Cannabinoid agonists have been previously demonstrated to produce optimal effects at these time points (9). In the morphine experiment, the same parameters were used, except the subjects were only assessed for antinociception. For each of the experiments, subjects were only used once.

Statistical Analyses

Analysis of variance (ANOVA) was used to analyze the data, and posthoc analyses were conducting using the Tukey test when appropriate. Differences were considered significant at the $p < 0.05$ level. Catalepsy data were expressed as the mean immobility time in seconds. Tail-flick response latencies were expressed as percentage of the maximum possible effect (%MPE) by the following equation: %MPE = 100 × [(Test latency − Control latency)/(Cutoff time − Control latency)]. The ED_{50} values were calculated for graded data (30).

RESULTS

Systemic Administration

The antagonistic effects of an IP injection of SR 141716A (0, 3, 10, or 30 mg/kg) given before 3 mg/kg of Δ^9-THC (IV) in the tail-flick and catalepsy tests are presented in Fig. 1. SR 141716A significantly blocked the antinociceptive [$F(3,20) = 8.5, p < 0.05$)] and cataleptic [$F(3, 20) = 7.1, p < 0.05$)] effects of Δ^9-THC. Posthoc analysis revealed that the 10- and 30-mg/kg doses of SR 141716A significantly reduced the antinociceptive effects compared with the vehicle and 3-mg/kg groups. In contrast, all three doses of SR 141716A significantly reduced the cataleptic effects of Δ^9-THC.

Figure 2 shows the effect of IP administration of either vehicle or SR 141716A (30 mg/kg) on the dose–response relationship of Δ^9-THC-induced antinociception. The Δ^9-THC doses were 0, 0.1, 0.3, 0.56, 1, or 3 mg/kg for the rats pretreated with vehicle and were 0, 1, 3, 10, or 20 mg/kg for the subjects pretreated with SR 1417116A. The ED_{50} values of Δ^9-THC (IV) after vehicle pretreatment and SR 141716A pretreatment were 0.3 and 5.1 mg/kg, respectively. Δ^9-THC produced sig-

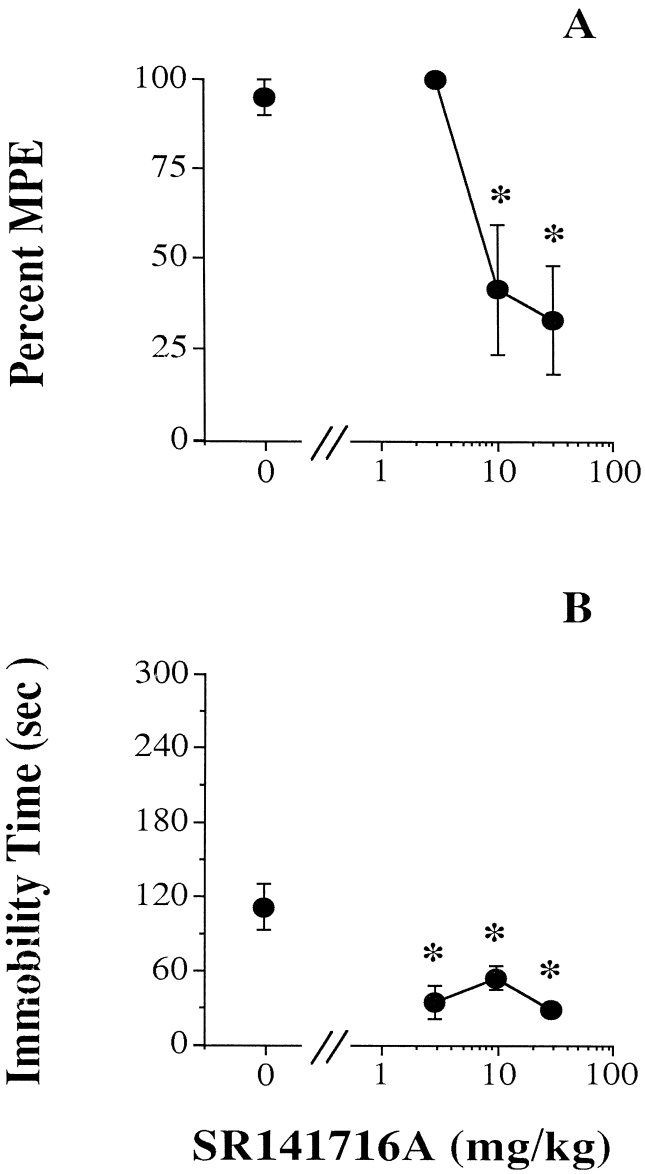

FIG. 1. An IP injection of the cannabinoid antagonist SR 141716A blocked the effects of IV-administered Δ^9-THC (3 mg/kg) in: (A) the tail-flick test and (B) the ring-immobility test. The doses of SR 141716A administered were 3, 10, or 30 mg/kg. The results are presented as means ± SEM, n = 6/group. *Significantly different from the vehicle pretreatment group, $p < 0.05$.

nificant antinociceptive effects in subjects pretreated with vehicle [$F(6, 38) = 8.6$, $p < 0.05$)] as well as those pretreated with SR 141716A [$F(4, 31) = 4.8$, $p < 0.05$)]. In the subjects pretreated with vehicle, the 0.5-, 1-, 2-, and 4-mg/kg doses of Δ^9-THC significantly differed from the vehicle–vehicle group. In the subjects pretreated with SR 141716A, the only dose that significantly differed from the controls was 10 mg/kg of Δ^9-THC.

ICV Administration

The impact of SR 141716A (0, 30, 100, or 300 μg) on the antinociceptive and cataleptic effects of a 50-μg injection of

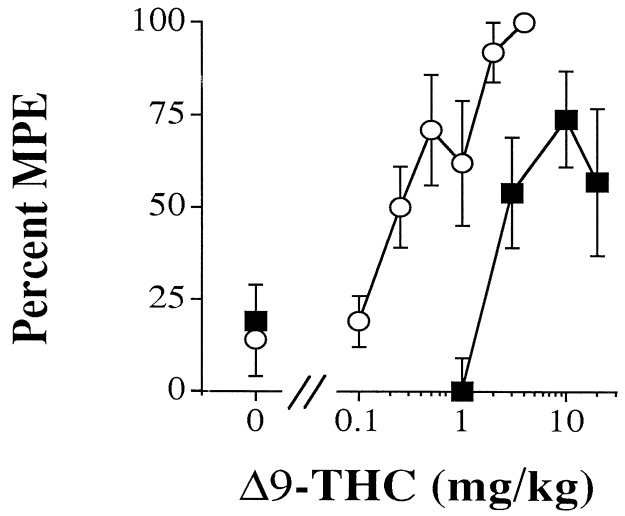

FIG. 2. The effects of pretreatment with either vehicle (○) or 30 mg/kg of SR 141716A (■) on the dose–response relationship of Δ^9-THC-induced antinociception. Δ^9-THC and SR 141716A were administered through the IV and IP routes of administration, respectively. In the vehicle-pretreated rats the doses of Δ^9-THC were 0.1, 0.3, 0.56, 1, and 3 mg/kg, and the Δ^9-THC doses in the SR 141716A-pretreated subjects were 1, 3, 10, and 20 mg/kg. The results are presented as means ± SEM; n = 6–8 rats/group.

CP 55,940 is shown in Fig. 3. Significant effects were found for both antinociception [$F(3, 21) = 7.8$, $p < 0.05$)] and catalepsy [$F(3, 21) = 6.0$, $p < 0.05$)]. The 30- and 300-μg doses of SR 141716A significantly blocked both pharmacologic effects of CP 55,940.

The effects of SR 141716A (0, 100, or 300 μg) on the dose–response relationship of CP 55,940-induced antinociception are depicted in Fig. 4A. In the subjects pretreated with either vehicle or 100 μg of SR 141716A, the CP 55,940 doses were 0, 10, 25, 35, 50, or 100 μg. Subjects pretreated with the 300-μg dose of SR 141716A were administered either 0, 50, 100, or 300 μg of CP 55,940. The ED_{50} values of CP 55,940 after pretreatment with either vehicle or 100 μg of SR 141716A were 22 and 46 μg, respectively. The ED_{50} value could not be determined in rats pretreated with 300 μg of SR 141716A, because the antagonist completely blocked the pharmacologic effects of CP 55,940 at doses up to 300 μg. CP 55,940 produced significant antinociceptive effects in subjects pretreated with vehicle [$F(5, 41) = 8.5$, $p < 0.05$)] or 100 μg of SR 141716A [$F(5, 34) = 3.1$, $p < 0.05$)]. In the vehicle-pretreated subjects, 50 and 100 μg of CP 55,940 produced significantly more antinociception than the vehicle–vehicle group. In the subjects pretreated with 100 μg of SR 141716A, only the 100-μg dose of CP 55,950 differed from the controls. As shown in Fig. 4B, a similar pattern of results occurred for the cataleptic effects of ICV administration of CP 55,940. Significant increases in catalepsy occurred in subjects pretreated with vehicle [$F(5, 39) = 14.1$, $p < 0.05$)] or 100 μg of SR 141716A [$F(5, 33) = 7.6$, $p < 0.05$)], but not 300 μg of the antagonist ($p = 0.36$) before administration of CP 55,940. In the vehicle-pretreated subjects, the cataleptic effects of the 50- and 100-μg doses of CP 55,940 were significantly higher than the controls; and in the subjects pretreated with 100 μg of SR 141716A, only the 100-μg dose of CP 55,940 differed from the controls.

SR 141716A (300 μg, ICV) pretreatment also completely

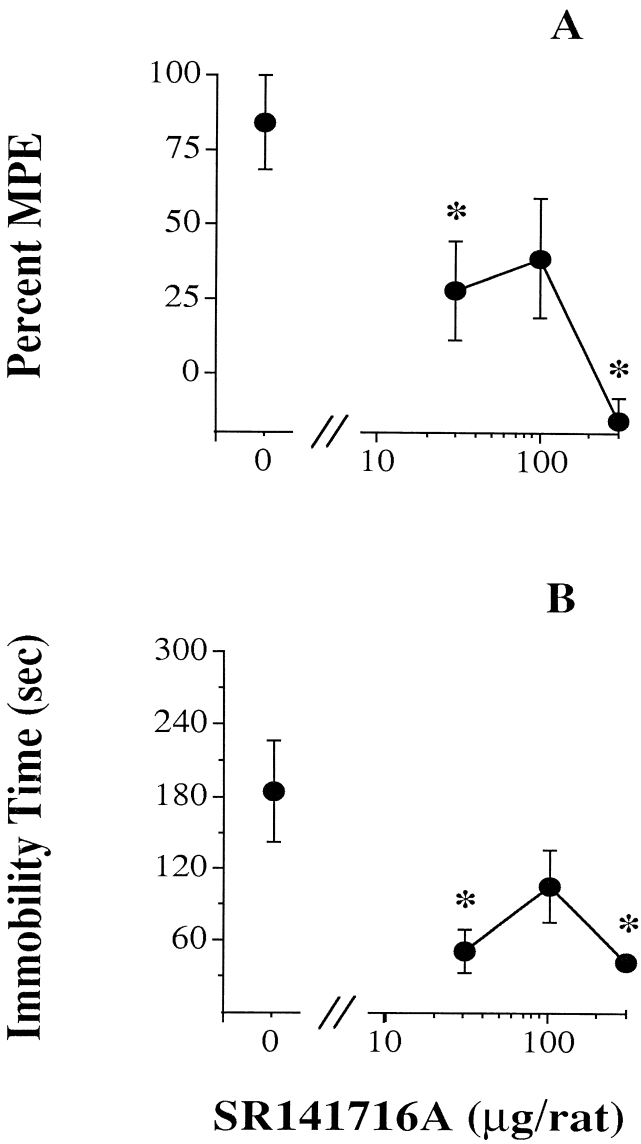

FIG. 3. SR 141716A pretreatment blocked the pharmacologic effects of CP 55,940 (50 μg) in: (A) the tail-flick test and (B) the ring-immobility test. All drugs were administered via the ICV route of administration, and SR 141716A was administered at doses of 30, 100, or 300 μg/rat. The results are presented as means ± SEM; n = 6/group. *Significantly different from the vehicle pretreatment group, $p < 0.05$).

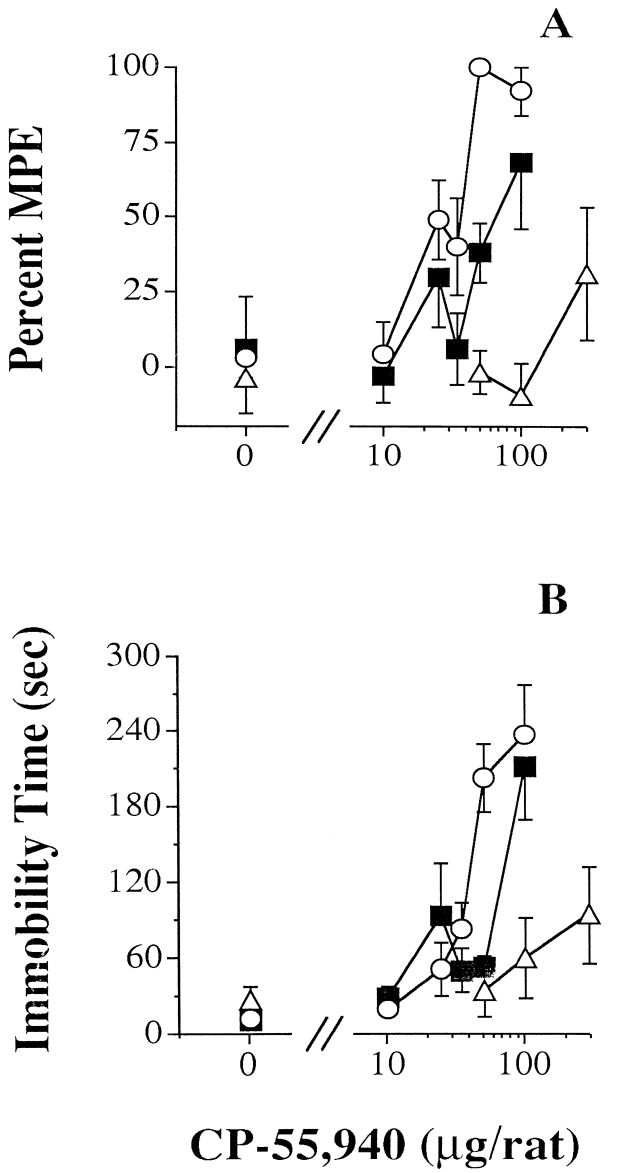

FIG. 4. The effects of either vehicle (○), or 100 μg (■) or 300 μg (△) of SR 141716A on the dose–response relationship of CP 55,940 in: (A) the tail-flick test and (B) the ring-immobility test. The doses of CP 55,940 in the rats pretreated with vehicle or 100 μg of SR 141716A were 10, 25, 35, 50, and 100 μg/rat; the doses of CP 55,940 in the rats pretreated with 300 μg of SR 141716A were 50, 100 and 300 μg/rat. All injections were given via the ICV route of administration, and the results are presented as means ± SE; n = 6–8/group.

blocked the ICV antinociception produced elicited by 250 μg of Δ^9-THC (Fig. 5A). Significant effects were found for SR 141716A [$F(1, 22) = 6.1$, $p < 0.05$)] as well as Δ^9-THC [$F(1, 22) = 6.6$, $p < 0.05$)]. In contrast, SR 141716A failed to ameliorate the antinociceptive effects of morphine (Fig. 5B). Although a significant main effect of morphine was found [$F(1, 24) = 5.3$, $p < 0.05$], neither the main effect of SR 141716A nor the Morphine × SR 141716A interaction achieved statistical significance.

DISCUSSION

The findings that SR 141716A antagonized cannabinoid-induced antinociception and catalepsy extend the results of previous reports in which it blocked these effects in mice (5,28). In addition, ICV administration of SR 141716A blocked both of these cannabinoid effects, suggesting the possibility that it may be microinjected into specific brain sites to elucidate the neural substrates of systemically administered cannabinoids. Similarly, ICV administration of SR 141716A has been reported to precipitate a cannabinoid withdrawal syndrome in rats chronically treated with Δ^9-THC (31). Finally, the failure of SR 141716A to block the antinociceptive effects of morphine after ICV administration indicates its selectivity for cannabinoid receptors.

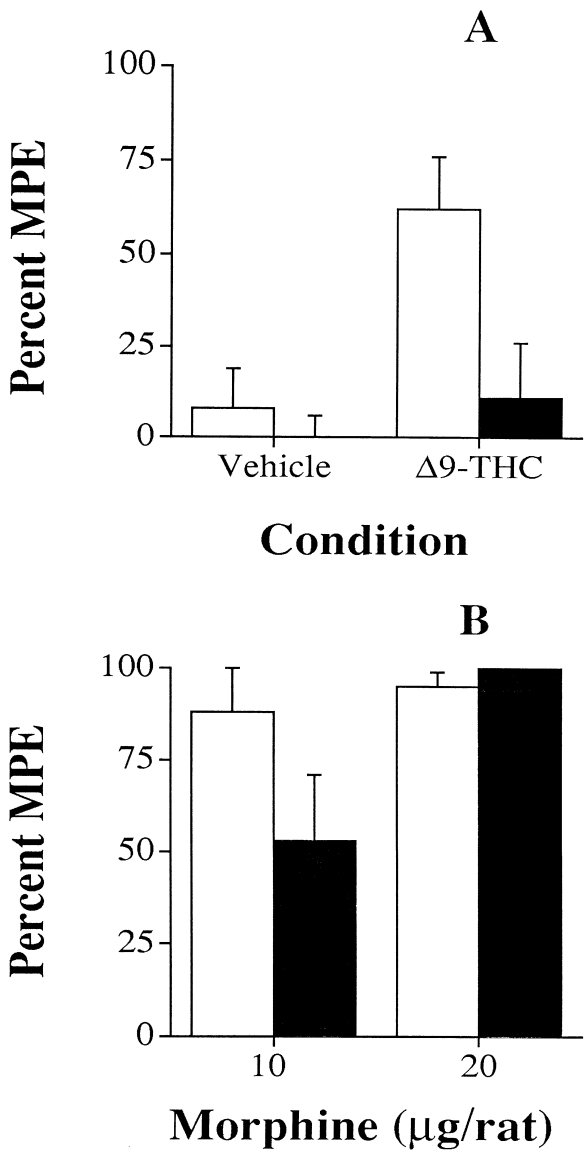

FIG. 5. SR 141716A pretreatment (A) blocked the antinociceptive effects of Δ^9-THC (250 μg) in the tail-flick test, (B) but failed to block the antinociceptive effects of morphine. Subjects were administered either vehicle (□) or 300 μg of SR 141716A (■) before either Δ^9-THC or morphine. All injections were given via the ICV route of administration, and the results are presented as means ± SE; n = 6–8/group.

It is noteworthy that cannabinoids and opioids share common sites of action in the CNS; each produces antinociception when given intrathecally (11,14,37,38), intracerbroventricularly (18,40), or into the PAG (9,39). Moreover, both classes of drugs appear to produce antinociception, in part, through the activation of descending monoaminergic systems (12,36). Finally, opioids and cannabinoids belong to a family of G protein–coupled receptors (20). Accordingly, pertussis toxin, a compound that inactivates both Gi and Go proteins by ADP-ribosylation (3), blocks the antinociceptive effects of both drug classes (2,9,23,26,33), suggesting a common signal transduction mechanism.

Despite the similarities between cannabinoids and opioids, they do not appear to affect nociceptive processing through a common serial pathway. Specifically, the opioid antagonist naloxone does not block cannabinoid-induced antinociception after intrathecal (37), ICV (33), or systemic (8) injections. Conversely, the failure of SR 141716A to block morphine-induced antinociception suggests that the antinociceptive effects of opioids are not mediated through the activation of cannabinoid systems.

Although SR 141716A antagonized Δ^9-THC–induced antinociception and catalepsy, higher doses were required to block these effects than those previously reported in mice (5,28) and rats (35). On the other hand, the doses of SR 141716A to block the effects of Δ^9-THC in the chemical-induced stretching test (5) as well as on memory impairment (13) were similar to those in the present study. The dose of antagonist required to block the pharmacologic actions of cannabinoids may depend on the specific effect of interest; however, it should be noted that in the present study subjects were challenged with doses of Δ^9-THC that produced almost 100% MPE. In fact, the antinociceptive potency of Δ^9-THC after IV administration in the present study was considerably greater than that previously reported (11), although the effects of Δ^9-THC and CP 55,940 after ICV administration were comparable to previous results (9).

A potential limitation of using SR 141716A intracerebrally, however, is the observation that high doses of it were required to block the pharmacologic effects of cannabinoids after ICV administration. SR 141716A has also been reported to elicit a much less severe precipitated abstinence syndrome in Δ^9-THC–treated rats when administered into the lateral ventricles than when given systemically (31). This apparent decrease in pharmacologic potency upon ICV injection also occurs with cannabinoid agonists, including Δ^9-THC and CP 55,940 (9,18). It is likely that the highly lipophillic nature of these drugs hinders their diffusion from the ventricles to the active sites in the brain. Consistent with this notion is the observation that considerably lower doses of CP 55,940 were required to produce pharmacologic effects when injected into the PAG than when infused into the ventricles (9).

The complete blockade of cannabinoid-induced antinociception and catalepsy by ICV administration of SR 141716A suggests that it may be used to elucidate the neural substrates underlying the antinociceptive and other pharmacologic actions of the cannabinoids. For example, microinjection of CP 55,940 into the ventrolateral PAG, in the area of the dorsal raphe, produced antinociception and catalepsy (9), whereas its administration into the dorsal hippocampus impaired spatial memory (10) but failed to elicit any other cannabinoid effects. Intracerebral administration of the cannabinoid antagonist, SR 141716A may be used to evaluate the contribution of these brain sites and other CNS sites to the antinociceptive and other pharmacologic effects of systemically administered cannabinoids.

In summary, the results of the present study demonstrate that IP or ICV administration of the cannabinoid antagonist SR 141716A blocks the antinociceptive and cataleptic effects of cannabinoids. Conversely, SR 141716A failed to alter the antinociceptive effects of morphine when given by ICV injection, suggesting its selectivity for cannabinoids. These results suggest the potential usefulness of SR 141716A to elucidate the neural substrates of cannabinoid-induced antinociception and other pharmacologic actions of cannabinoids as well as the function of endogenous cannabinoid systems.

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REFERENCES

1. Aceto, M. D.; Scates, S. M.; Lowe, J. A.; Martin, B. R. Cannabinoid precipitated withdrawal by the selective cannabinoid antagonist, SR 141716A. Eur. J. Pharmacol. 282:R1–R2; 1995.
2. Bodnar, R. J.; Paul, D.; Rosenblum, M.; Liu, L.; Pasternak, G. W. Blockade of morphine analgesia by both pertussis and cholera toxins in the periaqueductal gray and locus coeruleus. Brain Res. 529:324–328; 1990.
3. Brown, A. M.; Birnbaumer, L. Ionic channels and their regulation by G protein subunits. Ann. Rev. Physiol. 52:197–213; 1990.
4. Collins, D. R.; Pertwee, R. G.; Davies, S. N. Prevention by the cannabinoid antagonist, SR 141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice. Br. J. Pharmacol. 115:869–870; 1995.
5. Compton, D. R.; Aceto, M. D.; Lowe, J.; Martin, B. R. In vivo characterization of a specific cannabinoid receptor antagonist (SR 141716A): Inhibition of Δ^9-tetrahydrocannabinol-induced responses and apparent agonist activity. J. Pharmacol. Exp. Ther. 277:586–594; 1996.
6. D'Amour, F. E.; Smith, D. L. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72:74–79; 1941.
7. Dewey, W. L. Cannabinoid pharmacology. Pharmacol. Rev. 38:151–178; 1986.
8. Ferri, S.; Cavicchini, E.; Romualdi, P.; Speroni, E.; Murari, G. Possible mediation of catecholaminergic pathways in the antinociceptive effect of an extract of *Cannabis sativa L*. Psychopharmacology (Berlin) 89:244–247; 1986.
9. Lichtman, A. H.; Cook, S. A.; Martin, B. R. Investigation of brain sites mediating cannabinoid-induced antinociception in rats: Evidence supporting periaqueductal gray involvement. J. Pharmacol. Exp. Ther. 276:585–593; 1996.
10. Lichtman, A. H.; Dimen, K. R.; Martin, B. R. Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. Psychopharmacology 119:282–290; 1995.
11. Lichtman, A. H.; Martin, B. R. Spinal and supraspinal components of cannabinoid-induced antinociception. J. Pharmacol. Exp. Ther. 258:517–523; 1991.
12. Lichtman, A. H.; Martin, B. R. Cannabinoid induced antinociception is mediated by a spinal α_2 noradrenergic mechanism. Brain Res. 559:309–314; 1991.
13. Lichtman, A. H.; Martin, B. R. Δ^9-Tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. Psychopharmacology 126:125–131; 1996.
14. Lichtman, A. H.; Smith, P. B.; Martin, B. R. The antinociceptive effects of intrathecally administered cannabinoids are influenced by lipophilicity. Pain 51:19–26; 1992.
15. Martin, B. R. Characterization of the antinociceptive activity of intravenously administered delta-9-tetrahydrocannabinol in mice. In: Harvey, D. J., ed. Marihuana '84: Proceedings of the Oxford Symposium on Cannabis. Oxford: IRL Press; 1985:685–692.
16. Martin, B. R. Cellular effects of cannabinoids. Pharmacol. Rev. 38:45–74; 1986.
17. Martin, B. R.; Prescott, W. R.; Zhu, M. Quantitation of rodent catalepsy by a computer-imaging technique. Pharmacol. Biochem. Behav. 43:381–386; 1992.
18. Martin, W. J.; Lsi, N. K.; Patrick, S. L.; Tsou, K.; Walker, J. M. Antinociceptive actions of cannabinoids following intraventricular administration in rats. Brain Res. 629:300–304; 1993.
19. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564; 1990.
20. Mountjoy, K. G.; Robbins, L. S.; Mortrud, M. T.; Cone, R. D. The cloning of a family of genes that encode the melanocortin receptors. Science 257:1248–1251; 1992.
21. Munro, S.; Thomas, K. L.; Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365: 61–65; 1993.
22. Noyes, J. R.; Brunk, S. F.; Avery, D. H.; Canter, A. The analgesic properties of delta-9-tetrahydrocannabinol and codeine. Clin. Pharmacol. Ther. 18:84–89; 1975.
23. Parenti, M.; Tirone, F.; Giagnoni, G.; Pecora, N.; Parolaro, D. Pertussis toxin inhibits the antinociceptive action of morphine in the rat. Eur. J. Pharmacol. 124:357–359; 1986.
24. Paxinos, G.; Watson, C.; The rat brain in stereotaxic coordinates. 2nd ed. Australia: Academic Press; 1986.
25. Pertwee, R. G. The ring test: A quantitative method for assessing the "cataleptic" effect of cannabis in mice. Br. J. Pharmacol. 46:753–163; 1972.
26. Przewlocki, R.; Costa, T.; Lang, J.; Herz, A. Pertussis toxin abolishes the antinociception mediated by opioid receptors in rat spinal cord. Eur. J. Pharmacol. 144:91–95; 1987.
27. Rinaldi-Carmona, M.; Barth, F.; Héaulme, M.; Shire, D.; Alonso, R.; Congy, C.; Soubrié, P.; Brelière, J. C.; Le Fur, G. Biochemical and pharmacological characterization of SR 141716A, the first potent and selective brain cannabinoid receptor antagonist. Life Sci. 56:1941–1947; 1995.
28. Rinaldi-Carmona, M., Barth, F., Héaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Néliat, G., Caput, D., Ferrara, P., Soubrié, P., Brelière, J. C., and Le Fur, G. SR 141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350:240–244; 1994.
29. Souilhac, J.; Poncelet, M.; Rinaldi-Carmona, M.; Fur, G. L.; Soubrié, P. Intrastriatal injection of cannabinoid receptor agonists induced turning behavior in mice. Pharmacol. Biochem. Behav. 51:3–7; 1995.
30. Tallarida, R. J.; Murray, R. B.; Manual of pharmacological calculations with computer programs. 2nd ed. New York: Springer-Verlag; 1987:26–31.
31. Tsou, K.; Patrick, S. L.; Walker, J. M. Physical withdrawal in rats tolerant to Δ^9-tetrahydrocannabinol precipitated by a cannabinoid receptor antagonist. Eur. J. Pharmacol. 280: R13–R15; 1995.
32. Varga, K.; Lake, K.; Martin, B. R.; Kunos, G. Novel antagonist implicates the CB_1 cannabinoid receptor in the hypotensive action of anandamide. Eur. J. Pharmacol. 278:279–283; 1995.
33. Welch, S. P.; Thomas, C.; Patrick, G. S. Modulation of cannabinoid-induced antinociception following intracerebroventricular vs. intrathecal administration to mice: Possible mechanisms for interaction with morphine. J. Pharmacol. Exp. Ther. 272:310–321; 1995.
34. Wiley, J. L.; Barrett, R. L.; Lowe, J.; Balster, R. L.; Martin, B. R. Discriminative stimulus effects of CP 55,940 and structurally dissimilar cannabinoids in rats. Neuropharmacology 34:669–676; 1995.
35. Wiley, J. L.; Lowe, J.; Balster, R. L.; Martin, B. R. Antagonism of the discriminative stimulus effects of Δ^9-THC in rats and rhesus monkeys. J. Pharmacol. Exp. Ther. 275:1–6; 1995.
36. Yaksh, T. L. Direct evidence that spinal serotonin and noradrenaline terminals mediate the spinal antinociceptive effects of morphine in periaqueductal gray. Brain Res. 160:180–185; 1979.
37. Yaksh, T. L. The antincociceptive effects of intrathecally administered levonantradol and desacetyllevonantradol in the rat. J. Clin. Pharmacol. 21:334S–340S; 1981.
38. Yaksh, T. L.; Rudy, T. A. Studies on the direct spinal action of narcotics in the production of analgesia in the rat. J. Pharmacol. Exp. Ther. 202:411–428; 1977.
39. Yaksh, T. L.; Yeung, J. C.; Rudy, T. A. Systematic examination in the rat of brain sites sensitive to the direct application of morphine: Observation of differential effects within the periaqueductal gray. Brain Res. 114:83–103; 1976.
40. Yeung, J. C.; Rudy, T. A. Multiplicative interaction between narcotic agonisms expressed at spinal and supraspinal sites of antinociceptive action as revealed by concurrent intrathecal and intracerebroventricular injections of morphine. J. Pharmacol. Exp. Ther. 215:633–642; 1980.