Pharmacology Biochemistry and Behavior, Vol. 60, No. 2, pp. 559–566, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(98)00012-4

The Enhancement of Morphine Antinociception in Mice by Δ^9-Tetrahydrocannabinol

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Received 8 August 1997; Revised 2 December 1997; Accepted 16 December 1997

SMITH, F. L., D. CICHEWICZ, Z. L. MARTIN AND S. P. WELCH. *The enhancement of morphine antinociception in mice by* Δ^9-tetrahydrocannabinol. PHARMACOL BIOCHEM BEHAV **60**(2) 559–566, 1998.—We have previously reported that intracerebroventricular or intrathecal administration of inactive doses of Δ^9-tetrahydrocannabinol (THC) greatly enhance the antinociceptive potency of morphine in the mouse tail-flick test. Experiments were conducted to test the hypothesis that morphine's potency would be enhanced in mice receiving THC and morphine by conventional per os (PO) and subcutaneously (SC) routes of administration. Antinociception was measured in the tail-flick test of radiant heat after administration of different combinations of THC and morphine PO and SC Subcutaneous administration of THC (4 and 25 mg/kg) increased the potency of SC morphine 8.5- and 22.3-fold, respectively, while SC THC (25 mg/kg) increased the potency of PO morphine 3.1-fold. Per os administration of THC (10 and 20 mg/kg) increased the potency of SC and PO morphine 11.4-fold and 7.6-fold, respectively. Thus, morphine's potency was significantly increased regardless of the enteral and parenteral routes of THC and morphine administration. The synthetic receptor selective cannabinoid CP-55, 940 (0.1 mg/kg, SC) also enhanced morphine's potency. Finally, the ability of the CB1 receptor antagonist SR141716A to antagonize the enhancement of morphine by THC indicates that THC was acting through a cannabinoid receptor mechanism. © 1998 Elsevier Science Inc.

Morphine antinociception Cannabinoids Radiant heat nociception

INTENSE investigation has lead to the identification and cloning of two distinct cannabinoid receptors: one that is predominantly in the central nervous system (19) and one that is found in splenic macrophages (23). In addition, anandamide is the first endogenous mammalian-derived arachidonic acid metabolite that binds with high affinity to cannabinoid receptors (8). Δ^9-Tetrahydrocannabinol (THC), the active constituent of marihuana, also binds with high affinity to cannabinoid receptors (6,40). Cannabinoids have been shown to elicit antinociception in mice and rats through both spinal and supraspinal mechanisms (17,18,33,34,43,45,46). Antinociception arising from intracerebroventricular THC injection can occur through spinal norepinephrine release and activation of alpha-2 adrenergic receptors (18). Additionally, intrathecal THC administration releases spinal endogenous opioids that stimulate kappa and delta receptors (28,29,35,43,44). In animals, cannabinoid administration IV, IP, SC, and PO also elicits antinociception, along with diverse behavioral, physiological, and pharmacological effects [for review, see (27)].

We have recently reported that nonantinociceptive doses of THC greatly enhance the antinociceptive potency of morphine. Intrathecal and intracerebroventricular administration of THC enhances the potency of morphine administered by the same route (29,45,46). These findings led us to speculate that THC might enhance opioid antinociception when mice received both drugs by conventional enteral and parenteral routes. Therefore, the hypothesis was tested that SC and PO THC administration would enhance the antinociceptive po-

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tency of morphine administered SC and PO. Our results reveal that any combination of two routes of THC and morphine administration enhance the potency of morphine.

METHOD

Animals

Male ICR mice (Harlan Laboratories, Indianapolis, IN), weighing 25–30 g, were housed five to a cage in the animal care quarters maintained at 22 ± 2°C on a 12 L:12 D cycle. Food and water were available ad lib. The mice were brought to a test room (at 22 ± 2°C on a 12 L:12 D cycle), marked for identification and allowed 24 h to recover from transport and handling. All experiments were conducted according to guidelines established by the Institutional Animal Care and Use Committee of the Medical College of Virginia.

The Tail-Flick Test

The tail-flick test used to assess for antinociception in mice was developed by D'Amour and Smith (7) and modified by Dewey et al. (9). Control reaction times were 2 to 4 s, and a cutoff time of 10 s was employed. Antinociception was quantified as the percentage of maximum possible effect (%MPE) as developed by Harris and Pierson (12) using the following formula: %MPE = [(test − control)/(10 − control)] × 100.

The Paw-Withdrawal Test

Antinociception was assessed in the hind paw using a modification of methods previously developed in this laboratory (32). A standard radiant heat tail-flick apparatus was used to stimulate the dorsal skin of the mouse hind paw. The intensity was adjusted to yield baseline withdrawal latencies of 2 to 4 s. A 10-s cutoff time was used to prevent tissue damage in the paw. Testing was performed by gently restraining the animal in a small towel and passively placing the hind paw on the stage so the animal was free to withdraw its paw from the stimulus without restriction. Antinociception was quantified as the percentage of maximum possible effect (%MPE) as developed by Harris and Pierson (12) using the preceding formula for the tail-flick test.

Subcutaneous and per os THC and Morphine Administration Protocol

Vehicle (emulphor, ethanol, saline; 1:1:18) or THC were given SC immediately before SC administration of isotonic saline or morphine. The response latency in the tail-flick test was measured 30 min later. Other animals received vehicle or THC SC immediately before PO administration of saline or morphine. The response latency was measured 30 min later. As described in the Results section, THC had relatively poor antinociceptive efficacy in the tail-flick test. Therefore, 30 min pretreatment time was chosen because THC produced a maximum −2.2°C change in rectal temperature. Rectal temperature decreases in parallel with antinociception when THC is administered by different routes (33).

Other animals received vehicle or THC PO 30 min before SC or PO administration of saline or morphine. The response latency was measured 30 min after administration of morphine. Time course studies indicate that PO THC had limited activity for up to 2 h. The greatest enhancement of morphine antinociception occurred when THC was administered 30 min before morphine.

Statistical Analyses

Dose–response curves were generated using at least four doses of test drug. Efficacy values were calculated for THC because of its relatively poor antinociceptive properties. Efficacy was calculated from double reciprocal analysis (1/dose vs. 1/%MPE) to yield a theoretical maximum effect (E_{max}), as

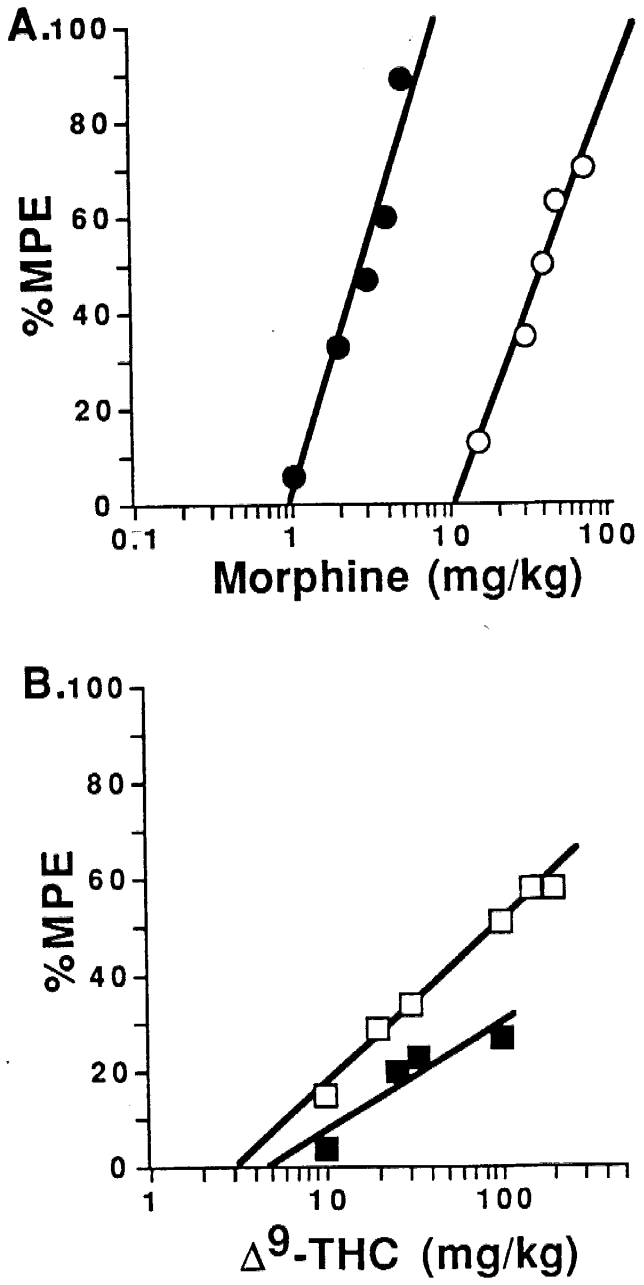


FIG. 1. (A) Antinociception elicited by SC and PO morphine in mice. Morphine was administered SC (●) or PO (○) 30 min before measuring the response latency in the tail-flick test. Each curve represents data from 30 mice. (B) Antinociception elicited by SC and PO THC in mice. THC was administered SC (■) or PO (□) 30 or 60 min, respectively, before measuring the response latency in the tail-flick test. Each curve represents data from 24 to 36 mice.

TABLE 1

EFFECTIVE DOSE-50 VALUES AND POTENCY RATIO VALUES IN THE TAIL-FLICK TEST FOR MORPHINE ALONE AND IN COMBINATION WITH Δ-9-TETRAHYDROCANNABINOL IN MICE

Pretreatment	Morphine ED_{50} mg/kg (95% C.L.)	Potency Ratio
(SC + SC)		
Vehicle + morphine	2.81 (2.24 to 3.53)	—
Δ^9THC (4 mg/kg) + morphine	0.29 (0.04 to 1.94)*	8.5
Δ^9THC (25 mg/kg) + morphine	0.12 (0.05 to 0.28)*	22.3
CP-55940 (0.1 mg/kg) + morphine	0.44 (0.34 to 0.57)*	6.8
(PO + SC)		
vehicle + morphine	4.00 (1.60 to 10.03)	—
Δ^9THC (10 mg/kg) + morphine	0.23 (0.05 to 0.95)*	11.4
(SC + PO)		
Vehicle + morphine	40.2 (37.3 to 43.3)	—
Δ^9THC (25 mg/kg) + morphine	13.2 (10.6 to 16.4)*	3.1
(PO + PO)		
vehicle + morphine	31.7 (22.4 to 44.9)	—
Δ^9THC (20 mg/kg) + morphine	2.8 (2.0 to 3.9)*	7.6

Mice received vehicle, THC, or CP-55940 SC or PO along with morphine SC or PO before measuring antinociception in the tail-flick test. Pretreatment times are described in the Method section.

*Significantly different from respective vehicle + morphine control.

described in Procedure 8 by Tallarida and Murray (38). Effective dose-50 (ED_{50}) values and 95% confidence limits were calculated using unweighted least squares linear regression for the log-dose–response curves as described in Procedures 8 and 9 by Tallarida and Murray (38). Before calculation of relative potency ratios (Procedure 11), tests for parallelism between curves were calculated using Procedure 6 by Tallarida and Murray (38). Experiments with the cannabinoid receptor antagonist SR141716A were analyzed using ANOVA followed by post hoc comparisons using the Tukey's test.

Drugs

Delta-9-tetrahydrocannabinol (THC) obtained from the National Institute on Drug Abuse was dissolved in 1:1:18 emulphor:ethanol:isotonic saline. Vehicle or THC were administered SC or PO in mice. SR141716A obtained from John Lowe (Pfizer Pharmaceuticals Inc., Groton, CT) was dissolved in 1:2:17 emulphor:ethanol:isotonic saline. Morphine sulfate pentahydrate (Research Biochemicals International, Natick, MA) was dissolved in sterile pyrogen-free isotonic saline for SC or PO administration.

RESULTS

Antinociceptive Properties of Morphine and THC

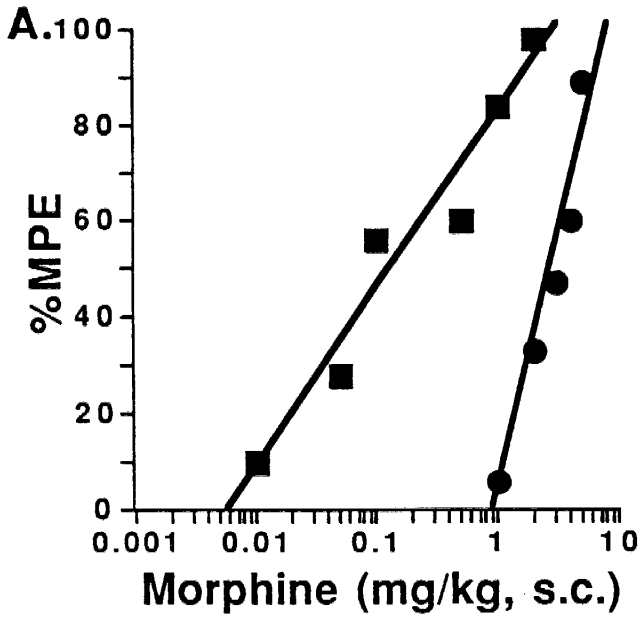
Initial studies were conducted to examine the antinociceptive properties of morphine and THC alone. Morphine administered SC or PO elicited dose-dependent antinociception (Fig. 1A). Morphine administered SC was 14.4-fold more potent than PO morphine. Although PO morphine was less potent, it was just as efficacious as SC morphine (E_{max} = 100 %MPE SC and PO).

Alternatively, THC elicited relatively poor antinociception when administered SC and PO in mice. The efficacy of PO THC was limited to 66% MPE, whereas the calculated E_{max} value of SC THC was only 31% MPE (Fig. 1B). THC administered SC was less efficacious than PO THC in the tail-flick test. Thus, the route of administration can influence both the potency and efficacy of drugs, as in the case of morphine and THC, respectively.

Enhancement of the Potency of Morphine by THC

The hypothesis was tested that inactive doses of THC would enhance the potency of morphine. For these experiments, THC and morphine were administered SC and PO in different combinations. The first combination examined the effect of SC THC on the potency of SC morphine. Doses of 4 and 25 mg/kg THC did not elicit significant antinociception above the vehicle response, although the 25 mg/kg dose was selected to elicit a maximum nonsignificant response. The 4 and 25 mg/kg doses increased the potency of SC morphine by 8.5- and 22.3-fold, respectively (Fig. 2A and Table 1). These curves did not differ significantly from parallel according to Procedure 6 by Tallarida and Murray (38). The expectation that THC was acting through a cannabinoid receptor was supported in experiments with the selective cannabinoid receptor agonist CP55,940. By itself, CP55,940 SC elicited potent antinociception, with an ED_{50} value of 0.31 mg/kg (95% C.L. 0.18 to 0.52). However, SC injection of an inactive dose of CP55,940 (0.1 mg/kg) increased the potency of morphine 6.8-fold (Table 1).

Other combinations of SC and PO THC and morphine were tested. THC (10 mg/kg) administered PO enhanced the potency of SC morphine 11.4-fold (Fig. 2B and Table 1). THC (25 mg/kg) administered SC enhanced the potency of PO morphine 3.1-fold (Fig. 3A and Table 1). THC (20 mg/kg, SC) did not elicit significant antinociception, although the dose was selected to elicit a maximum nonsignificant response. THC (20 mg/kg) administered PO enhanced the potency of PO morphine 7.6-fold (Fig. 3B and Table 1). Thus, THC enhanced the potency of morphine when both drugs were given by any combination of two routes of administration.



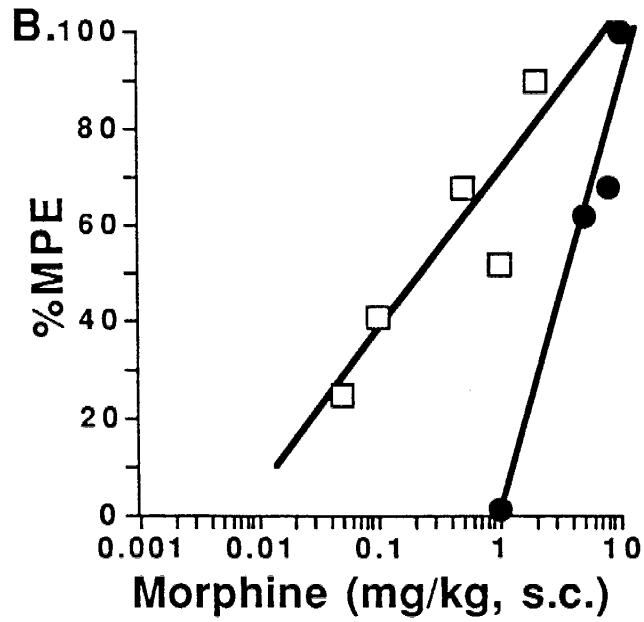
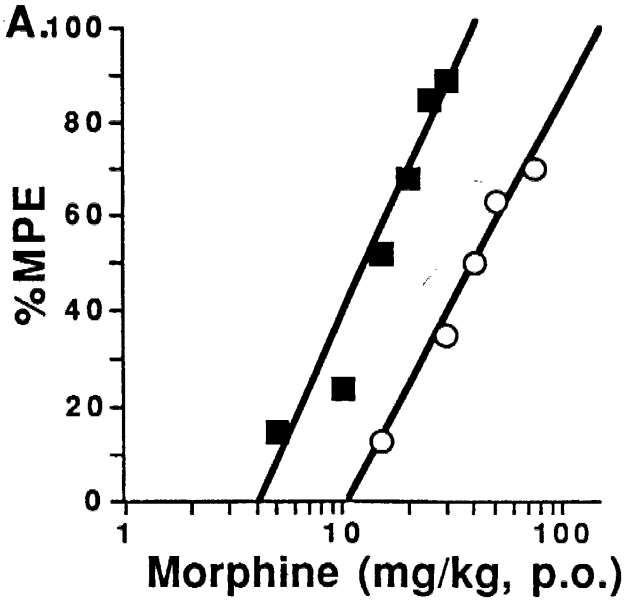


FIG. 2. (A) Enhancement of the antinociceptive potency of SC morphine with SC THC. Vehicle (●) or THC (25 mg/kg, ■) was administered immediately before injection of morphine. The response latency was measued 30 min after morphine administration. (B) Enhancement of the antinociceptive potency of SC morphine with PO THC. Vehicle (●) or THC (10 mg/kg, □) was administered 30 min before incremental doses of morphine. The response latency was measured 30 min after morphine administration.



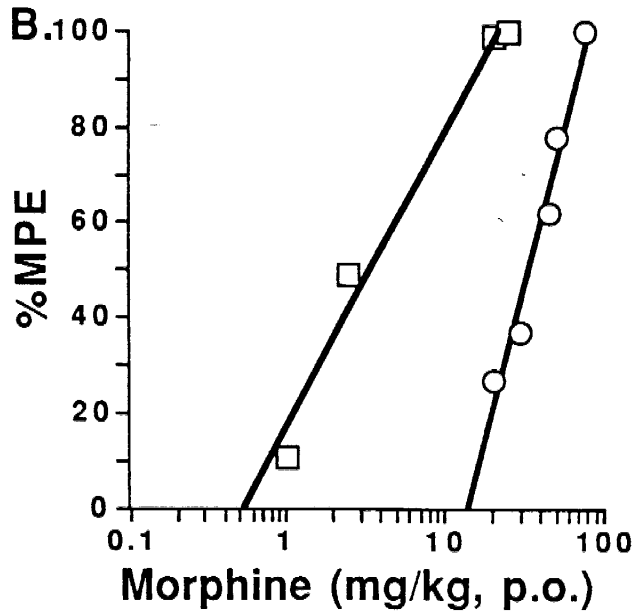


FIG. 3. (A) Enhancement of the antinociceptive potency of PO morphine with SC THC. Vehicle (○) or THC (25 mg/kg, ■) was administered immediately before injection of morphine. The response latency was measured 30 min after morphine administration. (B) Enhancement of the antinociceptive potency of PO morphine with PO THC. Vehicle (○) or THC (20 mg/kg, □) was administered 30 min before injection of morphine. The response latency was measured 30 min after morphine administration.

We also wanted to demonstrate that the enhancement of antinociception was not limited to the tail. Thus, antinociception was measured in the hind paw using paw-withdrawal assay as described in the Method section. Baseline paw-withdrawal latencies to radiant heat were obtained before injecting both THC and morphine SC THC (4 mg/kg, SC) increased the potency of SC morphine 4.7-fold in the paw. THC reduced the ED_{50} value of morphine in the paw from 2.19 mg/kg (95% C.L. 0.83 to 5.78) to 0.50 mg/kg (95% C.L. 0.36 to 0.69).

Enhancement of the Efficacy of THC by Morphine

Finally, the bidirectional properties of enhancement were investigated by determining whether SC morphine would en-

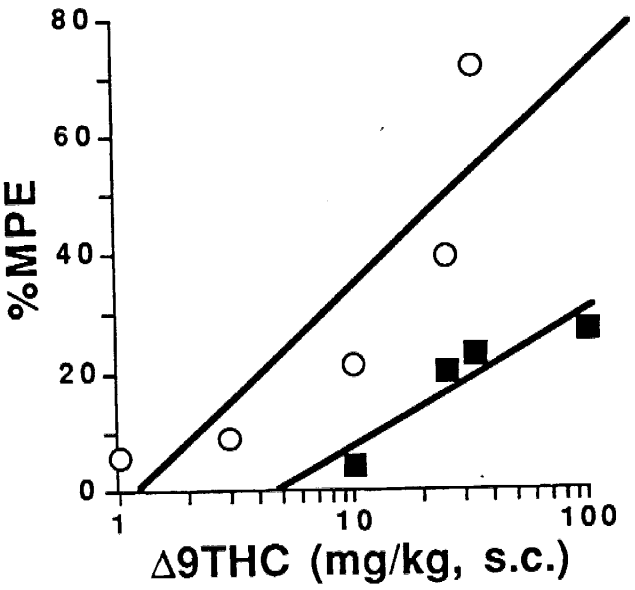
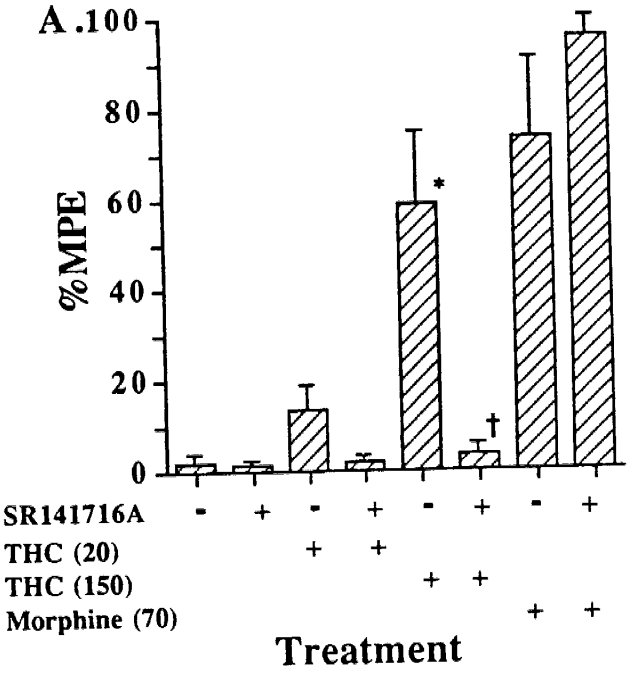


FIG. 4. Enhancement of the antinociceptive efficacy of SC THC with SC morphine. Vehicle (■) or morphine (0.1 mg/kg, ○) was administered immediately after incremental doses of THC. The response latency was measured 30 min later in the tail-flick test.

hance the efficacy of SC THC. As noted in Fig. 1B, the efficacy of SC THC was limited to 31% MPE. However, as seen in Fig. 4, an inactive dose of morphine (0.1 mg/kg, SC) increased the efficacy of THC to 79 %MPE.

Evidence for a Cannabinoid Receptor Mechanism

Experiments with SR141716A were conducted to demonstrate that THC's enhancement of morphine's potency was through CB1 receptor stimulation. Per os routes of THC and morphine administration were selected due to the potential that clinicians would utilize this route of administration for both drugs. The first step was to demonstrate the activity of SR141716A against THC. Previous work has shown that a 20 mg/kg dose is effective in blocking THC-induced antinociception (5). As seen in Fig. 5A, SR141716A (20 mg/kg, IP) blocked an active dose of THC (150 mg/kg, PO), but had no effect on morphine (70 mg/kg, PO). Thus, SR141716A selectively anatagonized THC. In another experiment, the administration of inactive PO doses of morphine (20 mg/kg) and THC (20 mg/kg) resulted in enhanced antinociception (Fig. 5B). This enhancement was significantly blocked by pretreatment with SR141716A (20 mg/kg, IP). These results indicate that PO THC acts through a CB1 receptor to enhance the potency of PO morphine.



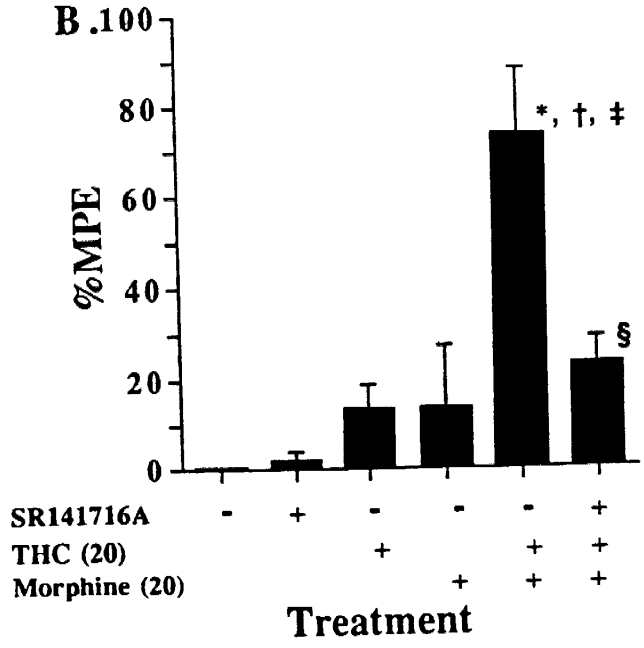


FIG. 5. (A) Blockade of the antinociceptive effects of THC, but not morphine, with the cannabinoid receptor antagonist SR141716A. SR141716A (20 mg/kg IP) was administered 30 min before PO administration of THC (20 or 150 mg/kg), or morphine (70 mg/kg). The animals were tested 30 min later. ANOVA was conducted followed by the Tukey's test. *$p < 0.05$, compared to vehicle/vehicle control; †$p < 0.05$ compared to vehicle/THC response. (B) The enhancement of antinociception by inactive doses of morphine and THC is blocked with SR141716A. SR141716A (20 mg/kg, IP) was administered 15 min before THC (20 mg/kg, PO) and 30 min before morphine (20 mg/kg, PO). The animals were tested 30 min later. ANOVA was conducted followed by the Tukey's test. *$p < 0.05$, compared to vehicle/vehicle/vehicle control; †$p < 0.05$, compared to vehicle/THC/vehicle; ‡$p < 0.05$, compared to vehicle/vehicle/morphine; §$p < 0.05$ compared to vehicle/THC/morphine.

DISCUSSION

The overall aim of this study was to determine whether enteral and parenteral THC administration would enhance the potency of morphine injected by the same routes. Initial experiments revealed that morphine administered SC was 14.4-fold more potent than PO morphine. Differences in potency are consistent with reports of the extensive first pass metabolism of PO morphine (39).

Alternatively, THC administered SC and PO had relatively poor activity in the tail-flick test. Regarding SC THC, Bloom et al. (2) also observed only 17% MPE with a large dose of THC (100 mg/kg), and Buxbaum (3) reported only a doubling of latency above baseline. However, others report that THC administered SC was sufficiently efficacious for calculation of ED_{50} values (20,22). Such discrepancies could be attributed to the mouse strains used, and differences in the methods of handling and injecting the animals. Regarding PO THC, Dewey et al. (9) also reported relatively poor antinociception by this route of administration. It is surprising that SC THC was less efficacious than PO THC. To our knowledge, no one has compared the kinetics of SC vs. PO THC that would account for the difference. Yet, THC administered PO is susceptable to first-pass metabolism by P450 microsomal enzyme hydroxylation of THC into the highly active 11-hydroxy-delta 9-THC in animals (24) and humans (42). We have shown that intrathecal 11-hydroxy-delta 9-THC is more potent than THC in the tail-flick test (45). Thus, PO THC may have been more efficacious than SC THC due to the combined activity of THC and 11-hydroxy-delta 9-THC.

Humans have also reported a reduction in pain perception following the administration of THC. Zeidenberg et al. (47) reported that PO THC was effective in blocking a painful thermal stimulus in volunteers receiving a 15-mg dose. In two other trials, patients with moderate continuous cancer pain given THC PO reported mild analgesia equivalent to the activity of codeine (25,26). A 10-mg dose was well tolerated, whereas higher doses were associated with somnolence, dizziness, ataxia, and blurred vision, which greatly limited the range of doses providing therapeutic value. In other words, lower doses had fewer side effects but were also less effective in reducing pain. Further limiting the clinical appeal of THC are conflicting reports that THC did not provide analgesia to volunteers exposed to noxious thermal and mechanical stimuli (4,30). Cannabinoid derivatives with potent analgesic properties have been tested in humans with the hope that CNS and other side effects would be reduced. Levonantradol was effective in blocking moderate to severe postoperative pain, yet 57% reported one or more CNS side effects associated with cannabinoid intake (15). A nitrogen-containing benzopyran (NIB) derivative of delta-9-trans-THC was as potent as codeine in cancer patients, yet was not useful clinically because of the frequency of side effects (36). Thus, although available cannabinoids may be analgesic, cannabinoids administered alone are unsuitable due to their CNS side effects.

The relatively poor antinociceptive properties of cannabinoids alone led us to examine whether THC would act as an augmenting agent to enhance the potency of morphine. In chronic pain conditions, inactive doses of THC might increase the potency of opioids and reduce the need to escalate opioid dose. In humans, cannabis extracts have been documented to potentiate the analgesic activity of morphine (11). In animals, cannabinoids also enhance opioid antinociception. Ghosh and Bhattacharya (11) were the first to report that an extract of Cannabis indica injected IP enhanced the antinociceptive properties of IP morphine in rats. Several years later, THC and Δ^6-THC administered PO were found to enhance the potency of PO codeine and morphine in mice (21). Our results with PO THC and PO morphine are consistent with those of Mechoulam (21). In addition, our data also indicate any combination of drug administration enhanced the potency of morphine. Furthermore, the enhancement is not limited to the PO and SC routes of administration. We have previously reported that intrathecal or intracerebroventricular injection of THC enhanced the activity of morphine given by the same route (45,46). Thus, the robust nature of this enhancement indicates that route of administration is not a consideration.

Considerable effort has been devoted to understanding the mechanism of interaction between THC and morphine. As mentioned earlier, THC injected intraventricularly releases norepinephrine from the spinal cord that stimulates alpha-2 adrenergic receptors (18). THC might enhance the antinociceptive potency of morphine by releasing spinal norepinephrine. Additionally, THC could enhance morphine by releasing endogenous opioids. Intrathecal administration of antibodies to dynorphin A (1–17) and dynorphin A (1–8) antagonized the antinociceptive properties of intrathecal THC (29). Furthermore, spinal cord perfusion of THC in artificial CSF in anesthetized rats has been shown in this laboratory to release dynorphin A (1–17) within 10 min (preliminary results). The link between THC-induced dynorphin A (1–17) release and kappa opioid receptor activation is close. Both nor-binaltorphimine (nor-BNI) and the selective kappa-1 antagonist naloxone benzoylhydrazone block the antinociceptive properties of THC (43,44). In addition, IT THC-induced antinociception was abolished in mice injected IT with antisense to the kappa-1 receptor (28). The story is further complicated by the finding that dynorphin A (1–17) released by THC may be converted into Leu-enkephalin, and that both peptides may enhance the potency of morphine (29). Spinal cord perfusion of THC in anesthetized rats causes an increase in Leu-enkephalin CSF levels by 30 min (preliminary results). The metabolism of dynorphins into Leu-enkephalin has been reported by others (10,13).

These results have led to the hypothesis that THC-induced endogenous opioid release enhances the potency of morphine. Evidence indicates that mu-kappa and mu-delta receptors exist in functionally coupled states (31,41). In the spinal cord, inactive doses of U50,588H (37), dynorphin A (1–17), or dynorphin A (1–8) (29) significantly enhance the potency of mu agonists. In addition, the delta receptor agonists DPDPE and Leu-enkephalin have both been shown to increase the potency of mu agonists (14,41). Therefore, THC may enhance opioid activity by releasing endogenous opioids that can act on delta or kappa receptors functionally coupled to mu receptors.

In summary, many patients could benefit from the combination of THC and opioids to treat chronic pain conditions. Selection of appropriate THC dose is an important consideration. In humans, PO doses greater than 10 mg are mildly analgesic but also elicit cannabinoid-like side effects. Currently, the PO THC preparation dronabinol (Marinol®) is indicated for the treatment of nausea and vomiting associated with cancer chemotherapy, and the treatment of anorexia associated with weight loss in patients with AIDS (1,16). Available commercial doses of 2.5, 5.0, and 10.0 mg could be combined with opioids. Recently, the HHS Agency for Health Care Policy and Research panel on cancer pain recommended morphine by patient-controlled administration and long-acting PO mor-

phine products such as MS Contin and OraMorph as the cornerstone for pain management (FDC Reports, p. 7–9, March 7, 1994). Our research data indicate that PO dronabinol might greatly enhance the potency of PO or injectable morphine in patients suffering from chronic pain.

ACKNOWLEDGEMENTS

This work was supported by the National Institute on Drug Abuse Grants: DA-05274, DA-03672, and K02-00186. We wish to thank Dr. Billy R. Martin, Department of Pharmacology and Toxicology, Medical College of Virginia, for his assistance on this project.

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