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Characterization of Δ^9-Tetrahydrocannabinol and Anandamide Antinociception in Nonarthritic and Arthritic Rats

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SMITH, F. L., K. FUJIMORI, J. LOWE AND S. P. WELCH. *Characterization of Δ^9-tetrahydrocannabinol and anandamide antinociception in nonarthritic and arthritic rats.* PHARMACOL BIOCHEM BEHAV **60**(1) 183–191, 1998.—Little is known about the effectiveness of Δ^9-tetrahydrocannabinol (THC) and anandamide in blocking mechanical nociception. Even less is known about their antinociceptive efficacy in chronic inflammatory arthritis induced by Freund's complete adjuvant. The hypothesis was tested that THC and anandamide elicit antinociception in the paw pressure test, and that arthritic rats would exhibit a different response. In nonarthritic rats, THC- and anandamide-induced antinociception lasted 90 min and 15 min, respectively, while antinociception lasted 90 min and 30 min, respectively, in arthritic rats. Area under the curve calculations revealed no effect of arthritis on THC- and anandamide-induced antinociception. Another hypothesis was that paw pressure thresholds in arthritic rats reflect chronic cannabinoid receptor stimulation due to elevations in free anandamide levels. Yet, the CB1 receptor antagonist SR141716A failed to alter paw pressure thresholds in either nonarthritic or arthritic rats. Further investigation revealed that SR141716A significantly blocked THC antinociception, with no effect on anandamide. Thus, anandamide's effects did not result from CB1 receptor stimulation, and any potential contribution of endogenous anandamide in arthritis was not revealed. Finally, THC and anandamide appear to release an as yet unknown endogenous opioid, because naloxone significantly blocked their effects. This study indicates that anandamide and THC may act at different receptor sites to modulate endogenous opioid levels in mechanical nociception. © 1998 Elsevier Science Inc.

Cannabinoid antinociception in rats Freund's adjuvant arthritis Mechanical nociception

INTENSE investigation has lead to the identification and cloning of two distinct cannabinoid receptors: one that is predominantly in the central nervous system (24), and one that is found in splenic macrophages (28). In addition, anandamide is the first endogenous mammalian-derived arachidonic acid derivative that binds with high affinity to cannabinoid receptors (10). Δ^9-Tetrahydrocannabinol (THC), the active constituent of marihuana, also binds with high affinity to cannabinoid receptors (5,44). Surprisingly little is known about the antinociceptive properties of THC and anandamide against mechanical nociception. An early study by Sofia et al. (40) established in rats that THC (PO) is effective in the paw pressure test. Similarly, Herzberg et al. (16) have shown that the synthetic cannabinoid, WINN 55,212-2 alleviates the pain associated with sciatic nerve constriction in rats. Anandamide has not been previously assessed using pressure as the nociceptive stimulus, although an endogenous anandamide-like palmitoylethanolamide has been shown to reduce carrageenan-induced hyperalgesia in tests of mechanical nociception (25). Therefore, we evaluated the ability of THC and anandamide to elicit antinociception in the paw-pressure test in rats.

Even less is known about the efficacy of THC and anandamide against nociception arising from chronic inflammation. Experiments were conducted to test the hypothesis that Freund's adjuvant-treated arthritic rats would exhibit an altered antinociceptive response to THC and anandamide compared to nonarthritic rats. This hypothesis was based on reports of enhanced opioid antinociception in Freund's adjuvant-treated rats (41). An examination of the literature reveals that Freund's adjuvant treatment causes chronic inflammation, edema, and

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hyperalgesia in rats (3,7,27). There is good evidence that Freund's adjuvant treatment is associated with higher levels of arachidonic acid (30,31). Many of the mediators of inflammation in this model have been identified as derivatives of arachidonic acid. Levels of prostaglandins and leukotrienes increase 2–3 weeks after the administration of Freund's adjuvant (26). Anandamide is also a derivative of arachidonic acid, and the hypothesis remains to be tested whether endogenous anandamide levels are also elevated in Freund's adjuvant arthritis. We predicted that the antinociceptive effects of THC and anandamide could be affected in arthritic rats, if endogenous anandamide levels were elevated. Thus, antinociception might be enhanced because of higher levels, or reduced because of cannabinoid receptor desensitization by anandamide. In addition, low levels of anandamide have been shown to block the antinociceptive effects of THC, an effect not related to desensitization (14,48). Our data indicate that THC and anandamide were active in the paw-pressure test in both nonarthritic and arthritic rats, but no greater or lesser antinociception was observed in arthritic rats. THC and anandamide also appear to elicit antinociception through separate mechanisms that converge to modulate endogenous opioids, based on studies with the CB1 receptor antagonist SR141716A and naloxone.

METHOD

Animals

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN), which weighed 250–300 g, were housed 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 rats were brought to a test room (at 22 ± 2°C) on the day of testing. All experiments were conducted according to guidelines established by the Institutional Animal Care and Use Committee of the Medical College of Virginia.

Freund's Adjuvant Treatment

A volume of 0.5 ml of vehicle (85:15 paraffin oil:Arlacel A) or Freund's complete adjuvant (heat-killed *Mycobacterium butyricum*; 0.5 mg) were injected intradermally into the plantar aspect of the rat paw. The animals remained in their cages for 18 days and were tested on day 19. Inflammation that begins within 24 h proceeds into a generalized polyarthritis within 19 days (3,7,27). Paw-pressure baseline measurements on day 19 indicated that arthritic rats were more sensitive to mechanical nociception than nonarthritic rats (Table 1).

Paw-Pressure Test

The paw-pressure test consisted of gently holding the body of the rat while the hind-paw was exposed to increasing mechanical pressure. The Analgesy-Meter (Ugo-Basile, Varese, Italy) is designed to exert a force on the paw that increases at a constant rate, in a manner similar to the Randall-Selitto (35) test of mechanical nociception. The force was applied to the hind paw that was placed on a small plinth under a cone-shaped pusher with a rounded tip. The operator depressed a pedal-switch to start the mechanism that exerted force. The force in grams at which the rat struggled was defined as the paw-pressure threshold. The baseline paw pressure was measured before injecting vehicle or drug. Antinociception was quantified as the paw pressure (g), with each repeated measures time point representing the mean response of eight rats. The upper limit of 500 g was imposed for the experiments.

TABLE 1

EFFECT OF FREUND'S ADJUVANT-INDUCED ARTHRITIS ON THE PAW PRESSURE BASELINE RESPONSE IN RATS

Treatment	Paw Pressure (g)
THC experiments	
Nonarthritic	160 ± 13
Arthritic	118 ± 4*
Anandamide experiments	
Nonarthritic	154 ± 13
Arthritic	112 ± 3*

Rats were administered vehicle or Freund's adjuvant 19 days before measurement of paw-pressure baselines as detailed in the Method Section.

*$p < 0.05$, compared to nonarthritic group.

Drug Administration Protocol

Following measurement of baseline paw-pressure thresholds, the animals were tested 15, 30, 60, 90, and 120 min after the IP administration of vehicle or THC (5 mg/kg). Other rats received vehicle or anandamide (10 and 40 mg/kg, IP) and were tested in manner identical to the THC group. In other experiments, rats received the vehicle or the cannabinoid receptor antagonist SR141716A (10 mg/kg, IP) (37) and were tested in manner identical to the THC group. The CB1 receptor selectivity for THC and anandamide antinociception was tested by administering SR141716A 15 min before THC (5 mg/kg, IP) or anandamide (40 mg/kg, IP). The rats were tested 15 min after administration of the cannabinoid. Finally, the participation of endogenous opioids to antinociception was tested by administering naloxone (5 mg/kg, SC) 5 min before cannabinoid.

Statistical Analyses

The time course of antinociception was analyzed using two factor (2 × 6) repeated-measures analysis of variance. Within group variability (i.e., MS_{error}) is not calculated in repeated-measures designs; therefore, no SEM values were presented. Post hoc analysis of simple effects were conducted using the Tukey's test. The absence of three-way interactions for THC and anandamide when the factor "pretreatment" was added (i.e., Freund's vehicle vs. Freund's adjuvant pretreatment), led us to calculate the area under the time course curves to determine whether the amount of drug-induced antinociception differed between nonarthritic and arthritic rats. The area calculation included only the period of apparent antinociception for THC (0 to 90 min) and anandamide (0 to 60 min). The trapezoidal rule was used because antinociception was measured over unequal time intervals, as described in Procedure 25 by Tallarida and Murray (43). Data for SR141716A and naloxone antagonism of THC and anandamide were analyzed with ANOVA followed by the Fisher's LSD test.

Drugs

Freund's adjuvant vehicle was composed of 85:15 paraffin oil:Arlacel A (Sigma Chemical Co., St. Louis, MO). Freund's complete adjuvant contained heat-killed *Mycobacterium butyricum* (Difco Laboratories, Detroit, MI). Delta-9-tetrahydrocannabinol (THC) obtained from the National Institute on

Drug Abuse was dissolved in 1:1:18 emulphor:ethanol:isotonic saline. Anandamide obtained from Raj Razdan (Organix, Inc., Woburn, MA) was dissolved in the same vehicle as THC. The vehicle control was composed of 1:1:18. SR141716A obtained from John Lowe (Pfizer Pharmaceuticals Inc., Groton, CT) was dissolved in 1:2:17 emulphor:ethanol:isotonic saline. The vehicle control of similar composition was used for the experiments with SR141716A. Naloxone (Sigma Chemical Co.) was dissolved in sterile isotonic saline for injection SC.

RESULTS

Experiments were conducted to test the hypothesis that THC elicits antinociception in the paw-pressure test in nonarthritic and arthritic rats. Before conducting the THC experiments, it was necessary to demonstrate that the treatment with Freund's complete adjuvant caused a significant reduction in paw pressure threshold (Table 1). These results indicate that the arthritic rats were significantly more sensitive to mechanical nociception than nonarthritic rats. In nonarthritic rats (Fig. 1A), THC (5 mg/kg, IP) elicited antinociception as indicated by a significant treatment-by-time interaction, $F(1, 5) = 5.81$, $p = 0.0002$. Post hoc analyses of simple effects revealed that THC-induced antinociception lasted 90 min. In arthritic rats, THC also elicited a significant antinociceptive effect to mechanical nociception that lasted 90 min [treatment × time interaction, $F(1, 5) = 5.80$, $p = 0.0002$] (Fig. 1B). Area under the curve values were calculated to determine whether THC effects differed in nonarthritic and nonarthritic animals (Fig. 3A). The results indicate that THC elicited a similar amount of antinociception in nonarthritic and arthritic rats.

Experiments were conducted to test the hypothesis that anandamide elicits antinociception in nonarthritic and arthritic rats. For the anandamide experiments, paw-pressure baseline measurements indicated that Freund's complete adjuvant caused a significant reduction in paw-pressure threshold (Table 1). In nonarthritic rats (Fig. 2A), the 10 mg/kg IP dose of anandamide was inactive; however, the 40 mg/kg dose elicited significant antinociception [treatment × time interaction, $F(1, 5) = 2.79$, $p = 0.023$]. Post hoc analyses revealed that anandamide-induced antinociception lasted 15 min. In arthritic rats, the 10 mg/kg dose of anandamide was also inactive, but the 40 mg/kg dose elicited significant antinociception that lasted 30 min [treatment × time interaction, $F(1, 5) = 3.19$, $p = 0.012$] (Fig. 2B). Calculation of area under the curve values revealed that anandamide elicited a similar degree of antinociception in nonarthritic and arthritic rats (Fig. 3B).

Experiments were conducted to test the hypothesis that paw-pressure thresholds in arthritic rats reflect chronic cannabinoid receptor stimulation due to elevations in endogenous anandamide levels. The effects of the CB1 receptor antagonist SR141716A (33) (10 mg/kg, IP) on the paw-pressure threshold were measured over time. Previous work has shown that this dose is effective in blocking THC-induced antinociception (4). The results indicate that SR141716A was ineffective in altering the paw pressure threshold in both nonarthritic (Fig. 4A) and arthritic (Fig. 4B) rats. Yet studies in this laboratory as well as another group indicate that SR141716A is unable to antagonize the antinociceptive effects of anandamide (B. Martin, submitted for publication). Therefore, experiments were conducted to determine whether SR141716A would block the antinociceptive effects of THC and anandamide. Pretreatment with SR141716A (10 mg/kg, IP) blocked THC- but not anandamide-induced antinociception (Fig. 5A and B). These results indicate that THC and anandamide may act through different receptor mechanisms in eliciting antinociception. Therefore, even if anandamide levels were elevated in arthritis, SR141716A could not reveal anandamide-mediated effects in arthritic rats.

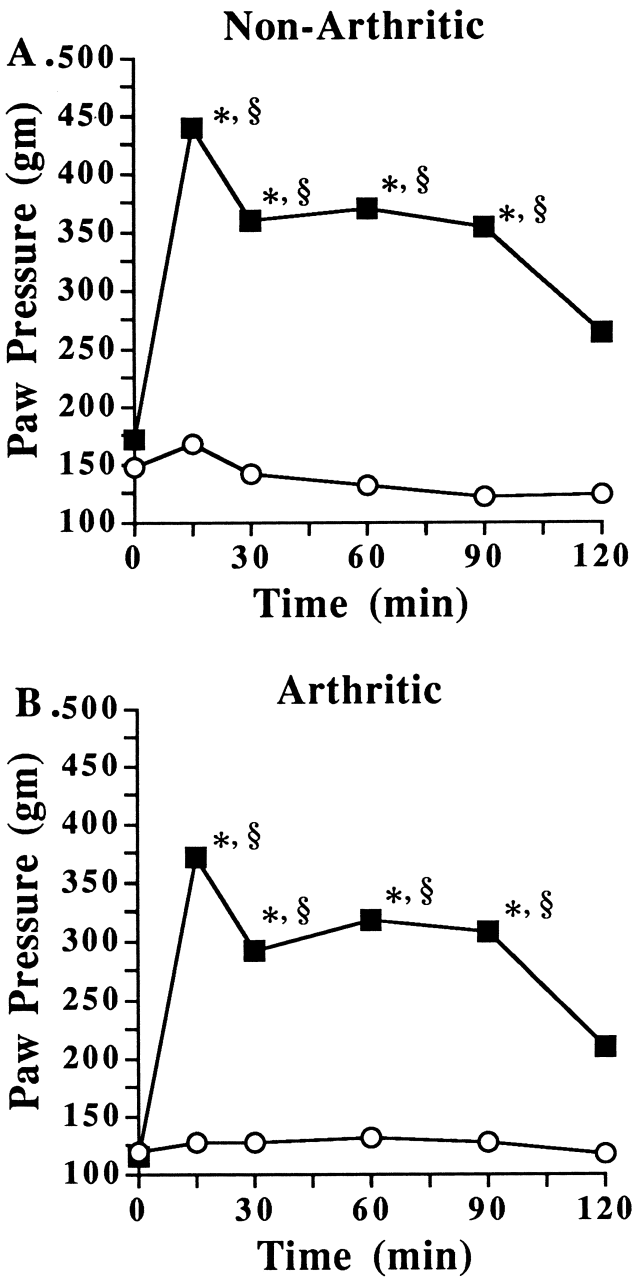

FIG. 1. (A) THC-induced antinociception in nonarthritic rats. Paw-pressure threshold was measured before and after the administration of vehicle (○) or THC (■). Each treatment represents a group of eight rats. (B) THC-induced antinociception in arthritic rats. Paw-pressure threshold was measured before and after the administration of vehicle (○) or THC (■). Each treatment represents a group of eight rats. Details on the induction of arthritis are detailed in the Method section. $*p < 0.05$ compared to baseline, $§p < 0.05$, compared to corresponding vehicle time.

Finally, experiments were conducted to test the hypothesis that endogenous opioids mediate the antinociceptive effects

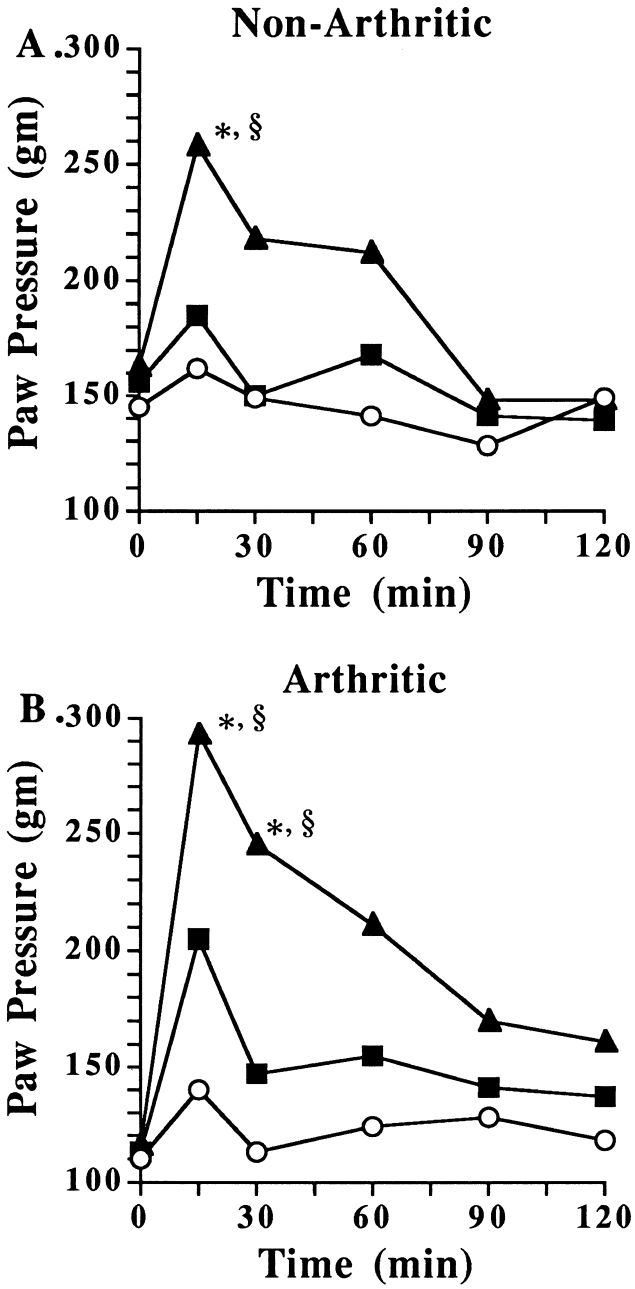

FIG. 2. (A) Anandamide-induced antinociception in nonarthritic rats. Paw-pressure threshold was measured before and after the administration of vehicle (○) or anandamide (■, 10 mg/kg; ▲, 40 mg/kg, IP). Each treatment represents a group of eight rats. (B) Anandamide-induced antinociception in arthritic rats. Paw-pressure threshold was measured before and after the administration of vehicle (○) or anandamide (■, 10 mg/kg; ▲, 40 mg/kg, IP). Each treatment represents a group of eight rats. *$p < 0.05$ compared to baseline, §$p < 0.05$, compared to corresponding vehicle time point.

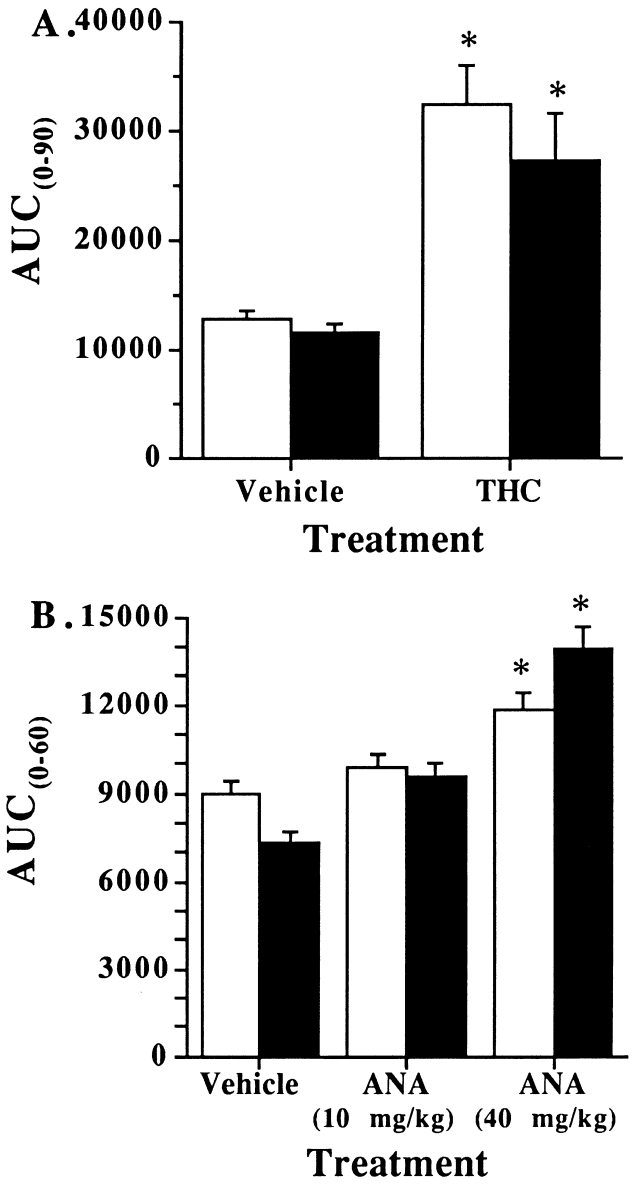

FIG. 3. Area under the curve of THC-induced antinociception in nonarthritic and arthritic rats. The area under the curve of antinociception from 0 to 90 min was calculated for nonarthritic and arthritic rats treated with vehicle or THC. (B) Area under the curve of anandamide-induced antinociception in nonarthritic and arthritic rats. The area under the curve of antinociception from 0 to 60 min was calculated for nonarthritic and arthritic rats treated with vehicle or anandamide. *$p < 0.05$ compared to corresponding vehicle group.

of THC and anandamide. A dose of naloxone (5 mg/kg, SC), which is sufficient to block mu, kappa, and delta opioid effects, significantly antagonized both THC and anandamide in nonarthritic rats (Fig. 6A and B). These results indicate that both THC and anandamide may release endogenous opioids albeit through different mechanisms.

DISCUSSION

The results of this study indicate that THC and anandamide elicited significant antinociception in the paw-pressure test in nonarthritic rats. THC had a rapid onset and long duration of action. Early studies by Sofia et al. (40) also estab-

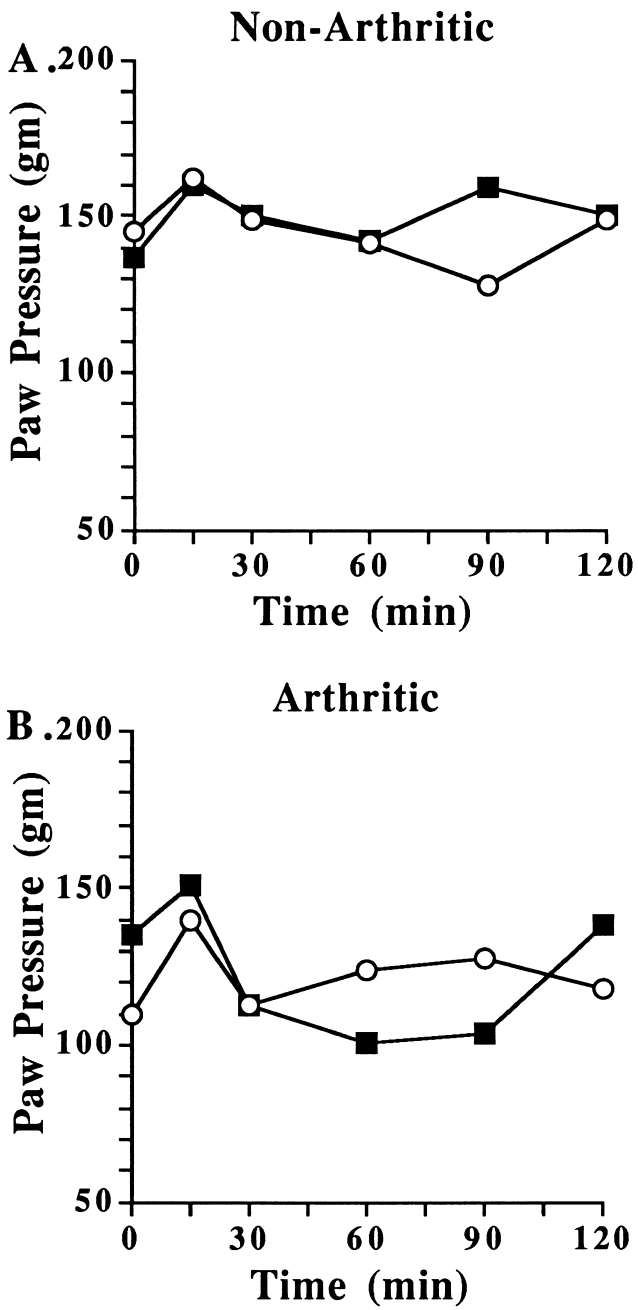

FIG. 4. (A) SR141716A-induced antinociception in nonarthritic rats. Paw-pressure threshold was measured before and after the administration of vehicle (○) or SR141716A (■, 10 mg/kg). Each treatment represents a group of six and eight rats, respectively. (B) SR141716A-induced antinociception in arthritic rats. Paw-pressure threshold was measured before and after the administration of vehicle (○) or SR141716A (■, 10 mg/kg). Each treatment represents a group of eight and nine rats, respectively.

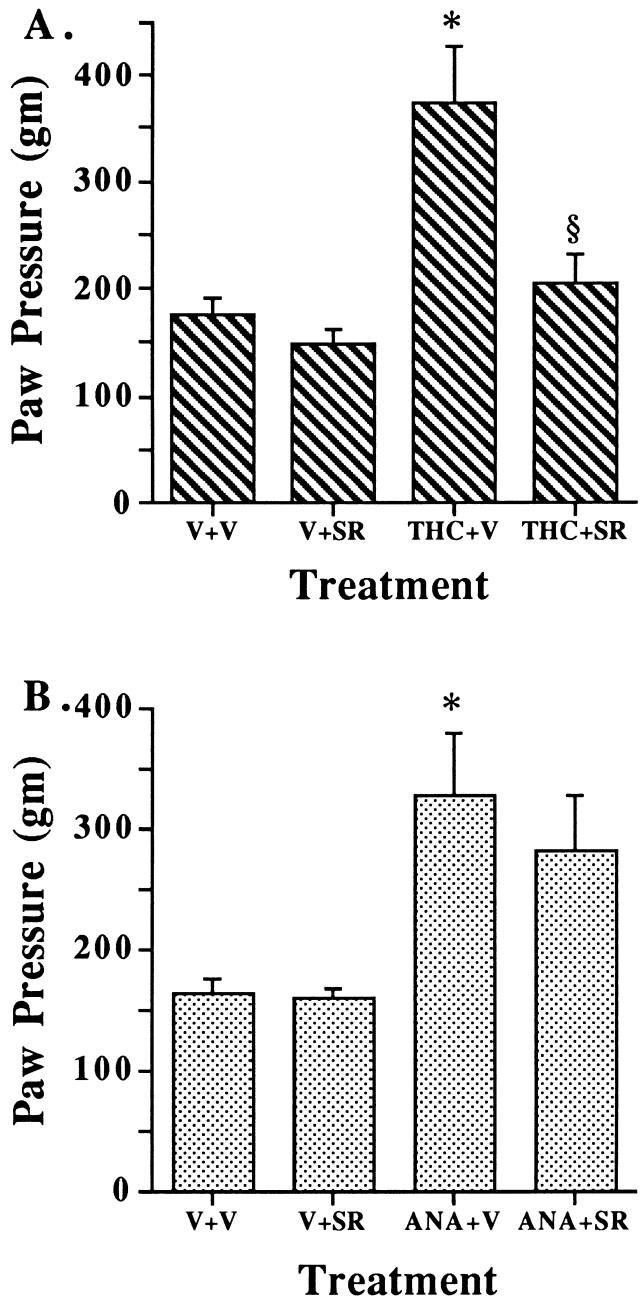

FIG. 5. (A) SR141716A antagonizes THC-induced antinociception in nonarthritic rats. SR141716A (10 mg/kg, IP) was administered 15 min before THC (5 mg/kg, IP). The paw-pressure test was conducted 15 min after the administration of THC. (B) Failure of SR141716A to antagonize anandamide-induced antinociception in nonarthritic rats. SR141716A (10 mg/kg, IP) was administered 15 min before anandamide (40 mg/kg, IP). The paw-pressure test was conducted 15 min after the administration of anandamide. *$p < 0.05$ compared to veh + veh, §$p < 0.05$, compared to respective cannabinoid + veh treatment.

lished that THC (PO) is effective in the rat paw pressure test, as well as the Haffner tail-pinch test in mice. In another study, the synthetic cannabinoid WIN 55,212-2 in anesthetized rats effectively blocked the response of wide dynamic range dorsal spinal neurons to noxious mechanical pressure (17). Our results along with those of others indicate that THC can effectively block mechanical nociception in rats. Furthermore, the blockade of antinociception with the selective antagonist SR141716A (2,4,38,51) is consistent with another report indi-

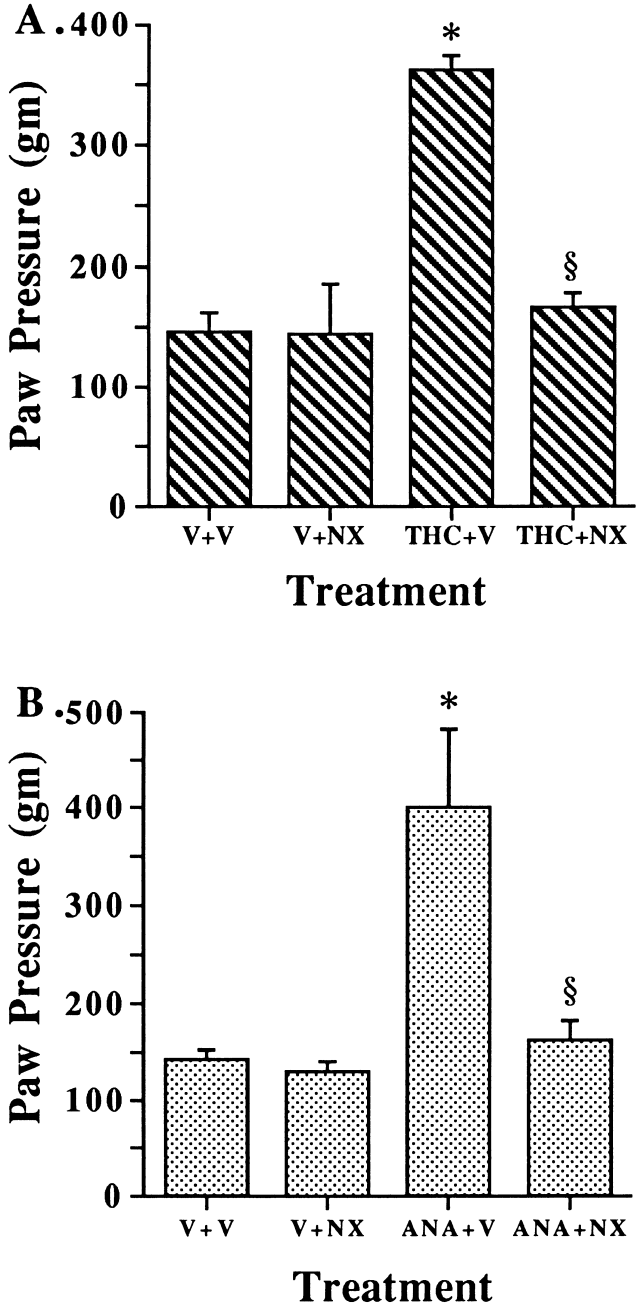

FIG. 6. (A) Naloxone antagonizes THC-induced antinociception in nonarthritic rats. Naloxone (5mg/kg, SC) was administered 5 min before THC (5 mg/kg, IP). The paw-pressure test was conducted 15 min after the administration of THC. (A) Naloxone antagonizes anandamide-induced antinociception in nonarthritic rats. Naloxone (5 mg/kg, SC) was administered 5 min before anandamide (40 mg/kg, IP). The paw-pressure test was conducted 15 min after the administration of anandamide. *$p < 0.05$ compared to veh + veh, §$p < 0.05$, compared to respective cannabinoid + veh treatment.

cating that THC-induced antinociception in mice is mediated through CB1 receptors (4).

Considerable effort has been devoted to understanding the mechanisms by which CB1 receptor stimulation leads to antinociception. THC might act by releasing spinal norepinephrine. A study in rats revealed that THC injected intracerebroventricularly (ICV) releases norepinephrine from the spinal cord to act on alpha-2 adrenergic receptors (21). In addition, THC may release endogenous opioids based on naloxone's antagonism of antinociception (Fig. 5A). The naloxone dose was high enough to antagonize all opioid receptor subtypes, and further research is needed to identify the receptor subtype. Evidence for endogenous opioid release is supported by other data from this laboratory. Intrathecal (IT) administration of antibodies to dynorphin A (1–17) and dynorphin A (1–8) antagonizes the antinociceptive properties of IT THC (34). In addition, spinal cord perfusion of THC in anesthetized rats has been shown in this laboratory to release dynorphin A (1–17) within 10 min (50). 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 effects of THC (45,46). In addition, IT THC-induced antinociception is abolished in mice injected IT with antisense to the kappa-1 receptor (33). 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 participate in antinociception (34). The metabolism of dynorphins into Leu-enkephalin has been reported by others (12,18). Spinal cord perfusion of THC in anesthetized rats caused an increase in Leu-enkephalin CSF levels by 30 min (50). Therefore, future studies will focus on identifying the endogenous opioids and opioid receptors mediating THC's activity in the paw-pressure test.

To our knowledge, the antinociceptive effects of anandamide in the paw-pressure test of nonarthritic rats have not previously been reported. Our results indicate that 10 mg/kg of anandamide (IP) was inactive, whereas 40 mg/kg elicited an antinociception that was rapid in onset and short in duration of action. It remains to be determined whether anandamide administered by other routes is effective in the paw-pressure test. More is known about anandamide in the tail-flick test. In mice, anandamide administered IP elicits a moderate degree of antinociception (approximately 40% MPE), whereas IV and IT administration is completely efficacious (1,39,48). The only report on rats indicates that anandamide ICV is inactive in tail-flick test (22), which is consistent with the inactivity of anandamide ICV in mice (49). Further research on rats may reveal that other routes of administration are effective in the tail-flick test and other tests of nociception. Nonetheless, our results clearly indicate that anandamide was active in the paw-pressure test following IP administration.

Less is known about the underlying mechanisms of anandamide-induced antinociception. Like THC, anandamide displacement of ^{3}H-CP 55,940 in the spinal cord and brain indicates that anandamide and THC bind to a similar cannabinoid receptor (39,48). However, in the paw-pressure test, the lack of effect of SR141716A suggests that anandamide is not acting through a CB1 receptor. Others have reported a lack of effect of SR141716A in mice (B. Martin, submitted for publication). Anandamide may act through CB2 receptors, and the development of selective CB2 antagonists may allow this possibility to be tested. Other data indicate similarities and differences between THC and anandamide. Regarding antinociception, the blockade of both THC and anandamide with pertussis toxin reveals a role for G_i or G_o proteins. Yet the ability of cyclic AMP analogs to antagonize THC implicates a modulatory role for adenylyl cyclase that is not mirrored by anandamide (49). There are a number of additional similarities and differ-

ences between anandamide and THC. Bidirectional crosstolerance has been reported for anandamide and THC in mice (13,47). In the radiant-heat tail-flick test in mice, anandamide does not release endogenous opioids with activity at mu, delta, and kappa receptors. Anandamide was not antagonized by naloxone (mu), nor-binaltorphimine (kappa), and ICI 174, 864 (delta), while THC is blocked by nor-binaltorphimine (49). Thus, the naloxone-sensitive mechanisms by which anandamide blocks mechanical nociception in rats and the naloxone-insensitive mechanisms by which anandamide blocks radiant-heat nociception mice remain to be determined.

We also tested the hypothesis that Freund's adjuvant-induced arthritis would alter the antinociceptive effects of THC and anandamide. This hypothesis was based on findings that mu and kappa opioid agonist-induced antinociception is enhanced in Freund's adjuvant-treated rats compared to non-arthritic rats (41). Freund's adjuvant-treated rats have higher tissue mRNA and peptide levels of spinal prodynorphin and proenkephalin (19,32). In addition, dynorphin levels are elevated in spinal dorsal horn local circuit neurons and projection neurons in arthritic rats (29). It has been proposed that the enhancement of exogenous opioid antinociception may involve the participation of endogenous opioids. In like manner, we speculated that arthritic rats might exhibit a different antinociceptive response to THC because of the presence of higher endogenous opioid levels. Because THC releases endogenous opioids, antinociception might be enhanced by the presence of opioid peptides, or antinociception might be reduced due to prior receptor desensitization or low levels of anandamide production. Yet Freund's adjuvant-induced arthritis did not influence the antinociceptive effects of THC. Anandamide, which has not been previously shown to alter the release endogenous opioids or to be modulated by opioid antagonists (48), clearly appears to produce mechanical nociception via an opioid-related mechanism. Anandamide was equally efficacious in both nonarthritic and arthritic rats (Fig. 3). Therefore, even though Freund's adjuvant arthritis is associated with higher tissue opioid peptide levels, there appeared to be no effect on cannabinoid-induced antinociception. Finally, it is notable that both drugs were equally efficacious in arthritic rats and nonarthritic rats. Even though the baseline paw-pressure test was lower for arthritic rats (Table 1), THC and anandamide elicited the same amount of antinociception.

Finally, the hypothesis was tested that paw-pressure thresholds in arthritic rats reflect chronic cannabinoid receptor stimulation due to elevations in endogenous anandamide levels. Freund's adjuvant treatment causes chronic inflammation, edema and hyperalgesia in rats (3,7,27). Many of the mediators of inflammation in this model have been identified as metabolites of arachidonic acid. Levels of prostaglandins and leukotrienes increase 2–3 weeks after the administration of Freund's adjuvant (26). Rats administered prostaglandins and leukotrienes exhibit inflammation (6), whereas inhibitors of cyclooxygenase and lipoxygenase decrease the signs of inflammation (30,31). Furthermore, Freund's adjuvant rats treated with inhibitors of arachidonic acid metabolism exhibit a normalization of threshold to mechanical nociception (15,23). Because arachidonic acid levels are increased in arthritic rats (30,31), it is possible that levels of endogenous anandamide are also elevated, although not necessarily from the endogenous arachidonic acid release. One anandamide biosynthetic pathway involves the activation of phospholipase D (PLD) and phospholipase A_2, resulting in the liberation of free arachidonic acid and ethanolamine, and their subsequent enzymatic condensation (8,9,20). This pathway would require in the cell the presence of high levels of arachidonic acid, which are elevated in Freund's adjuvant-treated rats (30,31). Another pathway involves the calcium-dependent liberation of membrane bound N-arachidonoyl phosphatidylethanolamine, and subsequent synthesis of anandamide by PLD (11,42). The ineffectiveness of SR141716A in arthritic rats initially suggested that endogenous anandamide plays no role in chronic inflammatory nociception (Fig. 4). However, further research indicated that SR141716A, which antagonized THC, had no effect on anandamide (Fig. 5A and B). Thus, this antagonist was not able to demonstrate whether endogenous anandamide plays a role in tonic nociception in arthritic rats. SR141716A predominantly blocks CB1-mediated cannabinoid effects (2,4, 38,51). The ability of SR141716A to antagonize THC's effects indicates that it may be ineffective if anandamide were acting at a non-CB1 site in arthritic rats. These results differ from those of Richardson et al. (36), who observed hyperalgesic effects of SR141716A in the hot-plate test. Clearly, we observe differences in the effects of anandamide in the tail-flick and hot-plate tests (where we see no block by naloxone) and the paw-withdrawal test where we observe a naloxone block. The hot plate is a thermal, supraspinally mediated test with nerve fiber stimulation quite different from mechanical nociceptors. Thus, it is likely that the differences in test systems may play a role in the differences observed. In addition, the lack of block of anandamide by SR141716A indicates that in our system endogenous cannabinoid tone is not CB-1 mediated.

In summary, these results support the evidence that cannabinoids injected by various routes of administration are effective antinociceptive agents. THC elicited significant antinociception against mechanical nociception, thus indicating that different pain modalities are sensitive to cannabinoids. In addition, the endogenous cannabinoid ligand anandamide was also effective in the paw-pressure test. However, neither THC nor anandamide were more effective in arthritic vs. non-arthritic rats, and the antagonist SR141716A failed to alter paw-withdrawal thresholds, suggesting that endogenous CB-1 receptor agonists may not play a significant role in inflammatory pain.

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