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The antinociceptive effect of Delta^9-tetrahydrocannabinol in the arthritic rat

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Abstract

Our study addressed the hypothesis that spinal release of endogenous opioids underlies Delta^9-tetrahydrocannabinol (Δ^9-THC)-induced antinociception in Freund's adjuvant-induced arthritic and nonarthritic rats. The paw-pressure test was used to assess the antinociceptive effects of Δ^9-THC versus those of morphine, and opioid and cannabinoid receptor-selective antagonists were used to characterize the involved receptors. Cerebrospinal fluid was collected after Δ^9-THC injection (i.p.) for the measurement of endogenous opioid peptides. Our results indicate that morphine or Δ^9-THC is equally potent and efficacious in both nonarthritic and arthritic rats. Δ^9-THC-induced antinociception is attenuated by the κ opioid receptor antagonist, nor-binaltorphimine, in arthritic rats only. Δ^9-THC induces increased immunoreactive dynorphin A (*i*dyn A) levels in nonarthritic rats while decreasing *i*dyn A in arthritic rats. We hypothesize that the elevated *i*dyn A level in arthritic rats contributes to hyperalgesia by interaction with *N*-methyl-D-aspartate receptors, and that Δ^9-THC induces antinociception by decreasing *i*dyn A release.

Keywords: Arthritis; Δ^9-THC; Dynorphin A

1. Introduction

Cannabinoids, such as Δ^9-THC, produce a wide spectrum of pharmacological effects including antinociception in both acute and chronic pain models. Several studies have shown that cannabinoids produce antinociception in mice and rats through both supraspinal and spinal mechanisms (Lichtman and Martin, 1991a). The identification and cloning of specific cannabinoid receptors, CB1 in tissues of central nervous system origin (Matsuda et al., 1990) and CB2 in splenic macrophages (Munro et al., 1993), has led to the identification of specific antagonists for the cannabinoid receptors. The mechanism by which cannabinoids produce antinociception has not been fully determined. In the rat, cannabinoid-induced antinociception has been proposed to involve interactions with G_iG_o proteins, calcium, c-AMP, and potassium channels (Welch et al., 1995). Lichtman and Martin (1991b) suggest that cannabinoid-induced antinociception may depend in part on the release from descending neurons of noradrenaline acting on spinal α_2-adrenoreceptors.

There is also evidence that cannabinoids induce antinociception by releasing μ, δ, and κ endogenous opioids. Several lines of evidence demonstrate the role of the κ-opioid system in Δ^9-THC-induced antinociception. The κ opioid receptor antagonist, nor-binaltorphimine (nor-BNI), and dynorphin antisera block Δ^9-THC-induced antinociception (Pugh et al., 1996; Smith et al., 1994; Welch, 1993). Δ^9-THC and the nonclassical cannabinoid, CP55940, have also shown bi-directional cross-tolerance to κ agonists using the tail-flick test (Smith et al., 1994) and to dynorphin A (Welch, 1997). More directly, the spinal release of dynorphin A (1–17) has been reported following intrathecal administration of Δ^9-THC, and has been shown to be blocked by the CB1-receptor antagonist, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR141716A) (Mason et al., 1999).

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Morphine, a nonselective opioid receptor agonist, has been shown to share several pharmacological effects, including analgesia, with Δ^9-THC. Previous studies have shown that Δ^9-THC, via various routes of administration, enhances the antinociceptive effect of morphine in acute pain models (Smith et al., 1998a; Cichewicz et al., 1999). Such an enhancement of morphine by Δ^9-THC in the spinal cord has been shown to be attenuated by pretreatment with the CB1 receptor antagonist, SR141716A, enzyme inhibitors preventing the metabolism of dynorphin A (1-8) into leu-enkephalin, antisera to various dynorphins, the κ opioid receptor-selective antagonist, nor-BNI, and the opioid receptor-selective antagonist, naltrindole (Pugh et al., 1996). Taken together, these data indicate that the mechanism by which Δ^9-THC produces spinal antinociception involves dynorphin release spinally, and that synergistic interactions between Δ^9-THC and morphine (Cichewicz and McCarthy, 2003) are due to the release of dynorphin A peptides and its breakdown into leucine–enkephalin. A functional coupling of mu/kappa and mu/delta opioid receptors may lead to the enhanced antinociceptive effect of morphine by Δ^9-THC (Welch and Eads, 1999).

The interaction between Δ^9-THC and the dynorphin A system contributes to Δ^9-THC-induced antinociception in acute pain models, such as the tail-flick and hot-plate tests. Less is known about antinociceptive effects of Δ^9-THC in mechanical nociception and in chronic pain models. An early study by Sofia et al. (1973) demonstrated that Δ^9-THC (p.o.) is effective in the paw-pressure test for mechanical nociception in rats. In rats with chronic inflammatory arthritis induced by complete Freund's adjuvant, Δ^9-THC-elicited antinociceptive efficacy was no different from that in nonarthritic rats (Smith et al., 1998b).

Freund's adjuvant treatment produces chronic inflammation, edema, and hyperalgesia in rats (Millan et al., 1986a). The inflammation and hyperalgesia produced by Freund's adjuvant is associated with alterations in several neuropeptide systems, including opioids. Tissue levels of both dynorphins and enkephalins, as well as the mRNAs encoding their precursors, are up-regulated in the spinal dorsal horn of rats with chronic inflammation (Millan et al., 1986a; Pohl et al., 1997). An increased spinal release of dynorphin-like material has been reported in polyarthritic rats, and a decreased spinal release of met-enkephalin-like material has been reported in these animals (Pohl et al., 1997). The modulation of endogenous opioid systems by opioid receptor agonists and antagonists is altered in polyarthritic rats as compared to normal rats (Ballet et al., 1998, 2000). Therefore, the modulation of endogenous opioids by Δ^9-THC may likewise be modified in arthritic rats.

The goals of this study were to determine the antinociceptive potency and efficacy of both Δ^9-THC and morphine in the paw-pressure test and to further identify the receptors involved in these antinociceptive effects in nonarthritic versus arthritic rats. In addition, the hypothesis that the interaction between Δ^9-THC and endogenous opioid systems may differ in arthritic rats versus nonarthritic rats was examined by measuring the spinal release of endogenous opioids following Δ^9-THC administration.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN), which weighed 350–375 g were housed in an animal care facility maintained at 22 ± 2 °C on a 12-hr light/dark cycle with free access to food and water. All experiments were conducted according to guidelines established by the Institutional Animal Care and Use Committee of Virginia Commonwealth University, and adhered to the European Community guidelines for the use of experimental animals.

2.2. Freund's adjuvant treatment

A volume of 0.1 ml of vehicle (mineral oil) or complete Freund's adjuvant (heat-killed *Mycobacterium butyricum*; 5 mg/ml) was injected intradermally into the base of the tail. Animals remained in their cages for 12 days and were acclimated daily to paw-pressure testing until day 19, on which they were tested. Acclimation consisted of a brief pressure stimulus, less than that necessary to elicit paw withdrawal, to the hind paw of the rat using the paw-pressure apparatus. Inflammation proceeds into a generalized polyarthritis within 19 days. Paw-pressure baseline measurements on day 19 indicated that arthritic rats are more sensitive to mechanical nociception than nonarthritic rats.

2.3. 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, similar to the Randall and Sellito (1957) test of mechanical nociception. Force was applied to the hind paw that was placed under 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 withdrew its paw was defined as the paw-pressure threshold. The baseline paw-pressure was measured before injecting vehicle or drug. Nonarthritic rats that had a baseline paw-pressure greater than 100 g (average = 177 ± 6.42 g) were used in further testing. Arthritic rats that had a baseline paw-pressure less than 100 g (average = 71 ± 3.05 g) were used

in further experimentation. The upper limit of 500 g was imposed for the experiments to allow the foot to not become immobilized due to undue pressure.

2.4. Administration of Δ^9-THC and endogenous opioid collection

Nonarthritic and arthritic rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and a separate injection of atropine methyl nitrate (2 mg/kg, i.p.) was administered to decrease secretions. The anesthetized rats were placed in stereotaxis and an incision was made on the alanto–occipito membrane to expose the cisterna magna. A catheter of PE-10 polyethylene tubing was inserted through the cisternal cavity, caudally, into the subarachinoid space of the spinal cord. The catheter contained an artificial cerebrospinal fluid, composed of 125 mM $Na^{+;}$ 2.6 mM K^+; 0.9 mM Mg^{2+}; 1.3 mM Ca^{2+}; 122.7 mM Cl^-: 21.0 mM HOC^-: 2.4 mM HOP^{2-}: 0.5 mg/ml bovine serum albumin, bacitracin (30 mg/ml), 0.01% Triton X and effervesced with 95% O_2 and 5% CO_2. The catheter extended caudally 8.5 cm passing through the thoracolumbar region to an area just above the sacral enlargement. Following catheter implantation, animals were allowed to acclimate on a heating pad for approximately 30 min. Δ^9-THC or its 1:1:18 (ethanol/emulphor/saline) vehicle was administered via the i.p. route. Cerebrospinal fluid (CSF) was collected 10 or 30 min after injection of drug or vehicle. Collection entailed rapid perfusion of the spinal cavity with artificial CSF culminating in the collection of 1.5 ml of the eluting CSF from the open cisternal space. This is an open system and the sampling technique is similar to the push–pull cannula technique commonly employed in the mouse (Tseng, 1989). Collected fractions were boiled for 10–12 min and centrifuged at a rate of $10{,}000 \times g$ for 10 min. The supernatant was collected, frozen at -70 °C, and lyophilized. Samples were reconstituted in 250 μl radioimmunoassay buffer for dynorphin A (1–17) and dynorphin B peptide measurements and 500 μl for met- and leu-enkephalin measurements. One set of samples was reconstituted in 400 μl buffer for dynorphin A (1–17) and leu-enkephalin measurements. The reconstitution volumes have been previously determined to allow measurements for endogenous opioids over the linear portion of the standard curves of the radioimmunoassays (RIAs).

2.5. Measurement of spinal opioid peptides

Measurements of immunoreactive dynorphin A (1–17), dynorphin B, leu-enkephalin, and met-enkephalin (pg/100 μl) were accomplished using peptide-specific RIA kits obtained from Peninsula Laboratories. The reconstituted samples were analyzed in duplicate. The manufacturer reports cross-reactivity of dynorphin A (1–17) antibody as less than 0.43% versus smaller peptide fragments, and no cross-reactivity with leu-enkephalin. The manufacturer reports cross-reactivity of dynorphin B antiserum as 12% versus dynorphin B (1–29), and states no cross-reactivity with smaller dynorphin B fragments, dynorphin A, neoendorphins, or enkephalins. Peninsula Laboratories reports cross-reactivity of leu-enkephalin antibody as 29% versus dynorphin A, 8% versus dynorphin A (1–8), 5% versus peptide F, 3% versus met-enkephalin, and less than 0.2% versus smaller met- and leu-enkephalin fragments, adrenocorticotrophic hormone (ACTH), β-endorphin, and endothelin-1. The cross-reactivity of met-enkephalin antiserum is reported to be 3% versus leu-enkephalin, and less than 0.1% for other met-enkephalin fragments, ACTH, dynorphin A, β-endorphin, and endothelin-1. Only the linear portion of the RIA standard curves was used to calculate peptide concentrations. Before lyophilization, all samples consisted of 1.5 ml of CSF. Samples analyzed for dyn A and dyn B levels were reconstituted in 250 μl RIA buffer, resulting in a 6-fold concentration of the original CSF. Met-enk and leu-enk were analyzed from the same sample that was reconstituted in 500 μl RIA buffer, resulting in a 3-fold concentration of the CSF. The concentrations of dyn A in samples collected 10 min post-drug administration were too high to be extrapolated from the standard curve (0.1–32 pg/100 μl) when the samples were reconstituted in 250 μl. Therefore, we measured both dyn A and leu-enk levels (10 min post-drug administration) in the same samples reconstituted in 400 μl RIA buffer, resulting in a 3.75-fold concentration of the sample. This dilution allowed us to extrapolate dyn A and leu-enk concentrations from the linear portion of the standard curve. All peptide concentrations are presented as converted to pg/ml of CSF.

2.6. Drug administration protocol

For the generation of dose–response curves using the paw-pressure test of antinociception and for examination of the effect Δ^9-THC on endogenous opioid release in arthritic and nonarthritic rats, morphine was administered s.c. and Δ^9-THC was administered i.p. Morphine was prepared in distilled water and Δ^9-THC was prepared in a solution of emulphor, ethanol, and saline at a 1:1:18 ratio. Morphine (1, 2, and 4 mg/kg) or its distilled water vehicle was administered 30 min prior to antinociceptive testing. Δ^9-THC (1, 3, 4, and 5 mg/kg) or its 1:1:18 vehicle was administered 30 min prior to antinociceptive testing and 10 and 30 min prior to collection of cerebrospinal fluid. The peak times for antinociception produced by Δ^9-THC and morphine have been previously determined to be 30 min post-administration.

In the antagonism studies designed to identify the receptors involved in the antinociceptive effects of morphine and Δ^9-THC in the paw-pressure test, antagonists to each opioid receptor subtype and to the cannabinoid receptor (CB1) were administered at times previously

reported in our studies to produce maximal attenuation of antinociception. For μ-opioid receptor studies, naloxone (1 or 5 mg/kg) or saline vehicle was administered (s.c.) 5 min before the paw-pressure test. For δ-opioid receptor studies, naltrindole (2 mg/kg) or distilled water vehicle was administered (s.c.) 30 min before drug administration. The selective κ-opioid receptor antagonist, nor-BNI (2 or 10 mg/kg) (Takemori et al., 1988), or distilled water vehicle was administered (s.c.) 1 h prior to drug administration. For CB1 receptor studies, SR141716A (10 mg/kg) or 1:1:18 vehicle was administered i.p. 1 h before drug administration. All antagonists were prepared fresh each test day. Each antagonist alone was found to be inactive in the paw-pressure test. For all antagonism studies, morphine (4 mg/kg) and Δ^9-THC (5 mg/kg in nonarthritic and 4 mg/kg in arthritic rats) were administered 30 prior to testing. Doses of morphine and Δ^9-THC represent ED80 doses of the drugs and were devoid of cataleptic effects. Doses of antagonists used were those previously evaluated in proprietary studies in our laboratory versus selective κ-opioid (U50,488H) and δ-opioid (SNC-80) receptor agonists in the rat. The doses and time points for administration were selected based upon data obtained in those studies, in which both pA_2 values and AD_{50} values for naloxone, nor-BNI, and naltrindole were determined versus selective μ-, δ-, and κ-opioid receptor agonists (S.P. Welch, personal communication). In all studies, $n \geq 8$.

2.7. Statistical analysis

The average paw-pressure threshold in grams was determined before drug administration (baseline) and the selected times (test) following drug administration. The maximum possible effect (%MPE) was determined for each rat according to the following formula using a 500 g maximum pressure: %MPE=[test (g) − baseline (g)/500 g − baseline(g)] × 100. Dose–response curves were generated using three or four doses of test drug. ED_{50} values and 95% confidence limits were determined using the methods of Tallarida and Murray (1987). Data for opioid and CB1 receptor antagonists or vehicles versus morphine and Δ^9-THC, and data from opioid peptide release, were analyzed with Statview using analysis of variance (ANOVA) with comparisons using Dunnett's *t*-test. Data from endogenous peptide levels was further adjusted by the removal of outliers using a modified method of Dixon and Massey (1969).

2.8. Drugs

Complete Freund's Adjuvant was prepared in mineral oil supplied by Sigma (St. Louis, MO). Freund's complete adjuvant contains heat-killed *M. butyricum* and is supplied by Difco Laboratories (Detroit, MI). Morphine and Δ^9-THC were obtained from the National Institute on Drug Abuse (Rockville, MD). Naloxone, naltrindole, and nor-BNI were obtained from Sigma. SR141716A was supplied by Pfizer Pharmaceuticals (Groton, CT).

3. Results

3.1. Morphine is equally potent in nonarthritic and arthritic rats in the paw-pressure test

A morphine dose–response curve was generated in both nonarthritic and arthritic rats using doses of 1, 2, and 4 mg/kg morphine (s.c.) administered 30 min prior to testing. Results for these dose–response analyses, as determined by the paw-pressure test, are presented in Fig. 1A. ED_{50} values for morphine with 95% confidence limits were 2.4 mg/kg (2.2–2.8) in nonarthritic rats and 2.2 mg/kg (1.9–2.4) in arthritic rats. The ED_{50} value of morphine in the nonarthritic

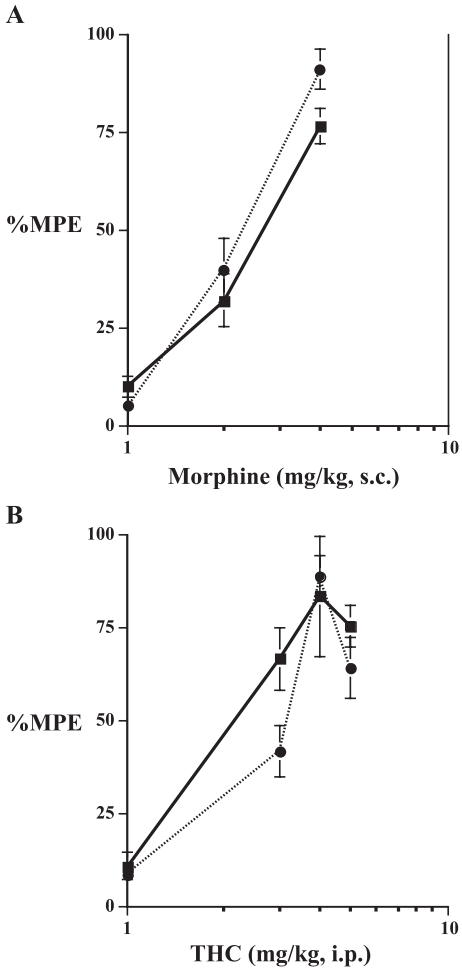

Fig. 1. (A) Dose–response curves of s.c. morphine in nonarthritic (■) and arthritic (●) rats. Animals were treated with morphine (1, 2, or 4 mg/kg, s.c.) and were tested 30 min later using the paw-pressure test. The data are presented as %MPE ± S.E.M. (B) Dose–response curves of i.p. Δ^9-THC in nonarthritic (■) and arthritic (●) rats. Animals were treated with Δ^9-THC (1, 3, 4, or 5 mg/kg, i.p.) and were tested 30 min later using the paw-pressure test. The data are presented as %MPE ± S.E.M.

rats did not differ significantly from the ED_{50} value in the arthritic rats as the confidence limits overlap.

3.2. Δ^9-THC is equally potent in nonarthritic and arthritic rats in the paw-pressure test

The Δ^9-THC dose–response curve was generated in both nonarthritic and arthritic rats using doses of 1, 3, 4, and 5 mg/kg Δ^9-THC (i.p.) administered 30 min prior to testing. Results for these dose-response analyses, as determined by the paw-pressure test, are presented in Fig. 1B. ED_{50} values for Δ^9-THC with 95% confidence limits were 2.1 mg/kg (1.8–2.5) in nonarthritic rats and 2.5 mg/kg (2.2–3.0) in arthritic rats. The ED_{50} value of Δ^9-THC in nonarthritic rats did not differ significantly from the ED_{50} value in arthritic rats as the confidence limits overlap.

3.3. The effects of μ-, δ-, κ-opioid, and CB1 receptor antagonists on morphine-induced antinociception in nonarthritic and arthritic rats

Antagonists selective for opioid receptors and the CB1 receptor were used in combination with morphine to determine the receptors involved in morphine-induced antinociception in the paw-pressure test. Results of these antagonism studies are presented in Fig. 2A and B. Naloxone (1 mg/kg), a nonspecific opioid receptor antagonist, significantly attenuated the antinociception produced by morphine (4 mg/kg) in both nonarthritic (panel A) and arthritic (panel B) rats. The δ-opioid receptor antagonist, naltrindole (2 mg/kg), and the κ-opioid receptor antagonist, nor-BNI (2 mg/kg), did not attenuate morphine-induced antinociception in nonarthritic or arthritic rats. Thus, naloxone antagonism of morphine appears to be due to activity at the mu opioid receptor. The CB1 receptor antagonist, SR141716A (10 mg/kg), did not alter morphine-induced antinociception. In summary, morphine-induced antinociception was attenuated by pretreatment with naloxone, but not by pretreatment with naltrindole, nor-BNI, or SR141716A in both nonarthritic and arthritic rats. These results indicate that morphine-induced antinociception involves the μ-, and not the δ- or κ-opioid receptors or the CB1 receptor.

3.4. The effects of μ-, δ-, κ-opioid, and CB1 receptor antagonists on Δ^9-THC-induced antinociception in nonarthritic and arthritic rats

Antagonists selective for opioid receptors and the CB1 receptor were used in combination with Δ^9-THC to determine the receptors involved in Δ^9-THC-induced antinociception in the paw-pressure test. The dose of Δ^9-THC used in these studies was 5 mg/kg in nonarthritic rats and 4 mg/kg in arthritic rats. The lower dose was chosen for arthritic rats to eliminate any possibility of catalepsy in rats that already have a reduced range of motion. Results of these

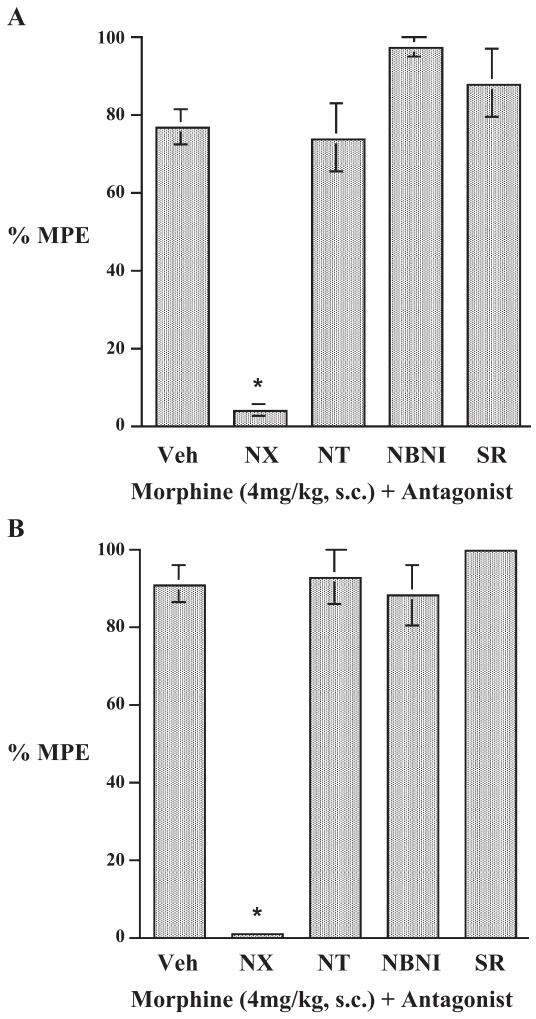

Fig. 2. (A) Evaluation of the antinociceptive effects of morphine in combination with opioid and cannabinoid receptor antagonists in the paw-pressure test in nonarthritic rats. Rats were pre-treated with vehicle, naloxone NX (1 mg/kg, s.c.; 5 min), naltrindole NT (2 mg/kg, s.c.; 30 min), nor-BNI NBNI (2 mg/kg, s.c.; 1 h), or SR141716A SR (10 mg/kg, i.p.; 1 h) before injection of morphine (4 mg/kg, s.c.). Rats were then tested for antinociception 30 min later using the paw-pressure test. $*p < 0.05$. (B) Evaluation of the antinociceptive effects of morphine in combination with opioid and cannabinoid receptor antagonists in the paw-pressure test in arthritic rats. Rats were pre-treated with vehicle, naloxone NX (1 mg/kg, s.c.; 5 min), naltrindole NT (2 mg/kg, s.c.; 30 min), nor-BNI NBNI (2 mg/kg; s.c.; 1 h), or SR141716 SR (10 mg/kg, i.p.; 1 h) before injection of morphine (4 mg/kg, s.c.). Rats were then tested for antinociception 30 min later using the paw-pressure test. $*p < 0.05$.

antagonism studies are presented in Fig. 3A and B. The CB1 receptor antagonist, SR141716A, significantly attenuated Δ^9-THC-induced antinociception in nonarthritic (panel A) and arthritic (panel B) rats. Naloxone (5 mg/kg) significantly attenuated Δ^9-THC-induced antinociception in arthritic rats. Results found previously in our laboratory by Smith et al. (1998b) indicate naloxone (5 mg/kg) attenuates Δ^9-THC-induced antinociception in nonarthritic rats in the paw-pressure test. Naloxone (1 mg/kg) attenuated Δ^9-THC-induced antinociception in nonarthritic rats, but not in

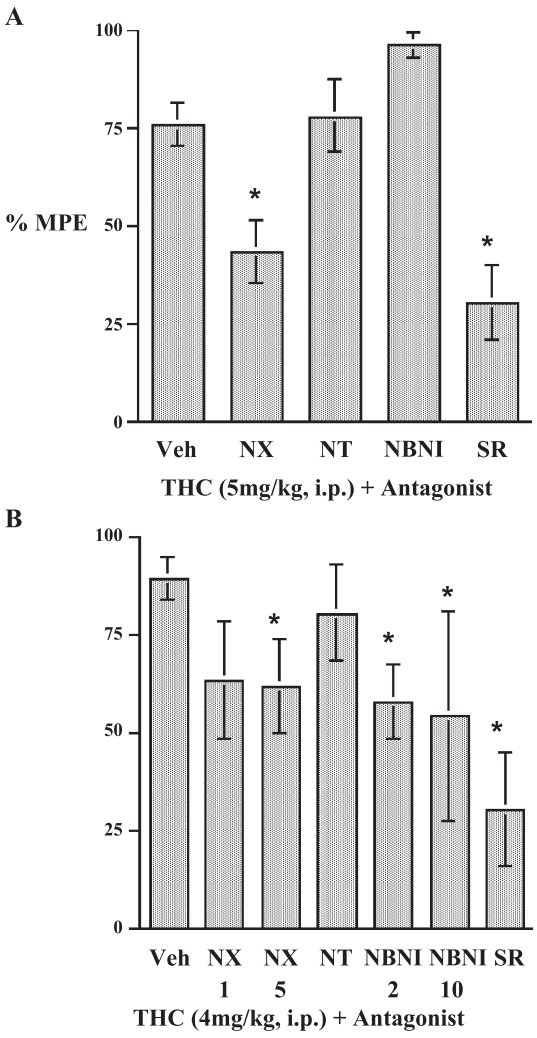

Fig. 3. (A) Evaluation of the antinociceptive effects of Δ^9-THC in combination with opioid and cannabinoid receptor antagonists in the paw-pressure test in nonarthritic rats. Rats were pre-treated with vehicle, naloxone NX (1 mg/kg, s.c.; 5 min), naltrindole NT (2 mg/kg, s.c.; 30 min), nor-BNI NBNI (2 mg/kg, s.c.; 1 h), or SR141716A SR (10 mg/kg, i.p.; 1 h) before injection of Δ^9-THC (5 mg/kg, i.p.). Rats were then tested for antinociception 30 min later using the paw-pressure test. *$p < 0.05$. (B) Evaluation of the antinociceptive effects of Δ^9-THC in combination with opioid and cannabinoid receptor antagonists in the paw-pressure test in arthritic rats. Rats were pre-treated with vehicle, naloxone NX (1 or 5 mg/kg, s.c.; 5 min), naltrindole NT (2 mg/kg, s.c.; 30 min), nor-BNI NBNI (2 or 10 mg/kg, s.c.; 1 h), or SR141716A SR (10 mg/kg, i.p.; 1 h) before injection of Δ^9-THC (4 mg/kg, i.p.). Rats were then tested for antinociception 30 min later using the paw-pressure test. *$p < 0.05$.

arthritic rats. Nor-BNI (2 mg/kg,10 mg/kg) significantly attenuated Δ^9-THC-induced antinociception in arthritic rats, but not in nonarthritic rats. Naltrindole (2 mg/kg) had no effect in arthritic or nonarthritic rats. In summary, Δ^9-THC antinociception was attenuated by pretreatment with SR141716A and naloxone in both nonarthritic and arthritic rats, but attenuated by pretreatment with nor-BNI in arthritic rats only. These results indicate that Δ^9-THC-induced antinociception involves the CB1 receptor, and the effect of Δ^9-THC on opioid systems differs between nonarthritic and arthritic rats, specifically involving a larger κ-opioid component in arthritic rats.

3.5. Release of endogenous opioids by Δ^9-THC (i.p.) in nonarthritic and arthritic rats 10 and 30 min post-administration

Samples of CSF were collected 10 or 30 min after administration of vehicle or Δ^9-THC (5 mg/kg nonarthritic; 4 mg/kg arthritic rats) to be analyzed for concentrations of immunoreactive dynorphin A (1–17) (*i*dyn A), dynorphin B (*i*dyn B), met-enkephalin (*i*met-enk), and leu-enkephalin (*i*leu-enk). Peptide-specific RIA kits were used to measure the levels of these endogenous opioids in lyophilized CSF samples from naïve, vehicle-treated, and Δ^9-THC-treated nonarthritic and arthritic rats. Data is presented in Figs. 4–6. Immunoreactive peptide concentrations are expressed as amount (pg) in 1 ml of CSF.

3.5.1. Dynorphin A

The level of immunoreactive dynorphin A (*i*dyn A) (Fig. 4) was no different between nonarthritic (11.3 pg ± 2.0) and arthritic (16.9 pg ± 2.0) naïve rats. Naïve rats were not administered drug. *i*Dyn A levels in nonarthritic vehicle-treated rats were no different at 10 min (14.7 pg ± 3.1) or 30 min (10.0 pg ± 0.7) post-administration than naïve rats. However, arthritic vehicle-treated rats 10 min post-administration (58.7 pg ± 11.0) had a significantly higher *i*dyn A level than arthritic naïve rats, and *i*dyn A remained elevated at 30 min (36.4 pg ± 6.6). Administration of Δ^9-THC to nonarthritic rats significantly elevated *i*dyn A level (40.9 pg ± 15.2) after 10 min as compared to vehicle, but after 30

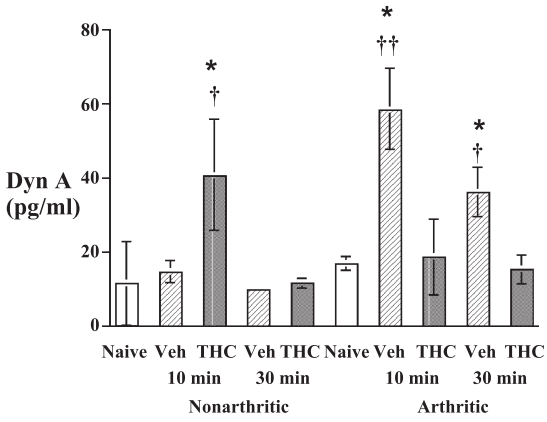

Fig. 4. Measurement of dynorphin A levels in rat CSF 10 and 30 min post-administration of Δ^9-THC. Catheters filled with artificial CSF were placed in the subarachinoid space of the spinal cord of the anesthetized rat. Δ^9-THC (5 mg/kg in nonarthritic rats and 4 mg/kg in arthritic rats; i.p.) or vehicle was injected 10 and 30 min before pumping artificial CSF through the catheter and collecting the eluting fluid. Samples were processed and later analyzed for dynorphin A levels (pg/ml) using radioimmunoassay. *$p < 0.05$, †>nonarthritic naive, and ††>arthritic naive.

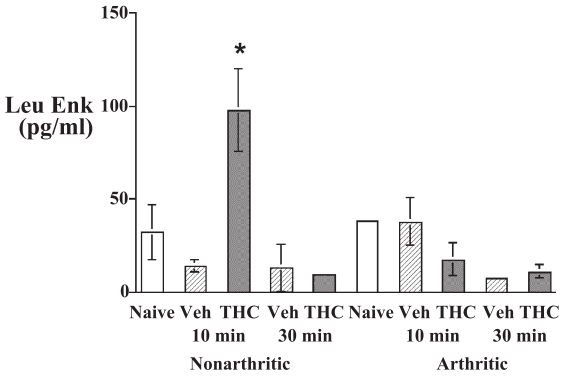

Fig. 5. Measurement of leu-enkephalin levels in rat CSF 10 and 30 min post-administration of Δ^9-THC. Catheters filled with artificial CSF were placed in the subarachinoid space of the spinal cord of the anesthetized rat. Δ^9-THC (5 mg/kg in nonarthritic rats and 4 mg/kg in arthritic rats; i.p.) or vehicle was injected 10 and 30 min before pumping artificial CSF through the catheter and collecting the eluting fluid. Samples were processed and later analyzed for leu-enkephalin levels (pg/ml) using radioimmunoassay. $*p < 0.05 >$ non-narthritic naive.

min levels returned to baseline. While *i*dyn A levels in arthritic rats were elevated after vehicle injection, Δ^9-THC elicited the opposite response. At 10 min after Δ^9-THC injection, *i*dyn A level significantly dropped (18.7 pg ± 10.3) compared to vehicle. The pattern remained the same at 30 min post-administration of Δ^9-THC when *i*dyn A level (15.3 pg ± 3.8) was lower than vehicle-treated rats at 30 min. Vehicle treatment alone raised *i*dyn A levels in the arthritic rats and Δ^9-THC reversed the effect, bringing levels back to baseline. The modulation of dyn A by Δ^9-THC is opposite in nonarthritic versus arthritic rats. Δ^9-THC increases dyn A level in nonarthritic rats but decreases it in arthritic rats.

3.5.2. Leu-enkephalin

The immunoreactive leu-enkephalin (*i*leu-enk) concentrations (Fig. 5) in naive nonarthritic (32.4 pg ± 14.5) and arthritic (38.4 pg ± 14.8) rats were no different. Vehicle treatment did not affect *i*leu-enk levels 10 (14.7 pg ± 3.2) or 30 (12.9 pg ± 5.1) min post-administration in nonarthritic rats as compared to naives. Vehicle treatment did not significantly affect *i*leu-enk levels in arthritic rats at 10 (38.1 pg ± 12.9) or 30 (7.8 pg ± 1.1) min post-administration as compared to naives. Administration of Δ^9-THC to nonarthritic rats significantly increased *i*leu-enk level (97.6 pg ± 22.3) 10 min post-administration compared to vehicle and naïve rats. Thirty minutes after Δ^9-THC injection, *i*leu-enk (10 pg ± 0.6) was not released and levels returned to baseline. Δ^9-THC did not increase or decrease release of *i*leu-enk in arthritic rats at 10 (17.8 pg ± 8.8) or 30 (11.4 pg ± 3.7) min after injection. There was a trend for Δ^9-THC to reduce the level of *i*leu-enk at 10 min, but this was not significant. No significant differences were observed in *i*leu-enk concentrations in arthritic rats. The effect of Δ^9-THC on leu-enk levels is similar to the effect of the drug on dyn A levels in the arthritic rat. In both instances, it seems that Δ^9-THC acts to reduce peptide levels toward baseline levels.

3.5.3. Met-enkephalin

The immunoreactive met-enkephalin (*i*met-enk) concentration (Fig. 6A) was significantly higher in naïve nonarthritic rats (321.1 pg ± 25.0) than naïve arthritic rats (189.4 pg ± 10.8) as well as all other nonarthritic and arthritic treatment groups. *i*Met-enk levels after vehicle treatment did not differ between nonarthritic (199.5 pg ± 23.4) and arthritic (165.0 pg ± 6.5) rats, although they were lower in naïve nonarthritic rats. Δ^9-THC-treated nonarthritic rats exhibited a significantly higher *i*met-enk level (258.0 pg ± 10.6) than all arthritic treatment groups, lower than naïve nonarthritic rats, and no different from vehicle-treated nonarthritic rats. Administration of Δ^9-THC did not alter *i*met-enk in arthritic rats.

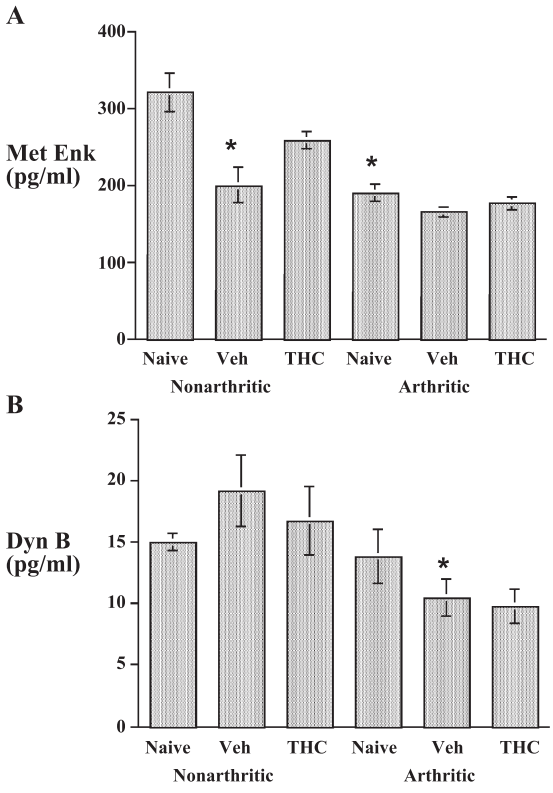

Fig. 6. (A) Measurement of met-enkephalin levels in rat CSF 30 min post-administration of Δ^9-THC. Catheters filled with artificial CSF were placed in the subarachinoid space of the spinal cord of the anesthetized rat. Δ^9-THC (5 mg/kg in nonarthritic rats and 4 mg/kg in arthritic rats; i.p.) or vehicle was injected 30 min before pumping artificial CSF through the catheter and collecting the eluting fluid. Samples were processed and later analyzed for met-enkephalin levels (pg/ml) using radioimmunoassay. $*p < 0.05 <$ non-narthritic naive. (B) Measurement of dynorphin B levels in rat CSF 30 min post-administration of Δ^9-THC. Catheters filled with artificial CSF were placed in the subarachinoid space of the spinal cord of the anesthetized rat. Δ^9-THC (5 mg/kg in nonarthritic rats and 4 mg/kg in arthritic rats; i.p.) or vehicle was injected 30 min before pumping artificial CSF through the catheter and collecting the eluting fluid. Samples were processed and later analyzed for dynorphin B levels (pg/ml) using radioimmunoassay. $*p < 0.05 <$ nonarthritic vehicle.

3.5.4. Dynorphin B

Immunoreactive dynorphin B (*i*dyn B) concentrations (Fig. 6B) did not differ between naïve nonarthritic (15 pg ± 0.7) and arthritic (13.8 pg ± 2.2) rats. The level of *i*dyn B in nonarthritic vehicle-treated rats (19.2 pg ± 3.0) was significantly higher than vehicle-treated arthritic (10.5 pg ± 1.5) and Δ^9-THC-treated arthritic rats (9.8 pg ± 1.4). Δ^9-THC did not release *i*dyn B in nonarthritic (16.7 pg ± 2.8) or arthritic (9.8 pg ± 1.4) rats.

4. Discussion

In this study, we determined if differences in efficacy and potency of morphine and Δ^9-THC exist in nonarthritic rats versus those with Freund's adjuvant-induced chronic arthritic pain. The antinociceptive effect of Δ^9-THC was further examined with regard to receptor involvement and endogenous opioid modulation. We used the Freund's adjuvant model of polyarthritis that has been frequently cited in the literature and that has been validated as an animal model of chronic pain with closely related characteristics of rheumatoid arthritis in humans (Colpaert, 1988). While the monoarthritic model, employing a within rat control, is a useful chronic pain model for behavioral testing, we chose the polyarthritic model for two reasons. (1) Our study requires a between animal design due to our endogenous opioid collection procedure and (2) the polyarthritic model is more closely related to generalized arthritic conditions in humans.

It has previously been reported that morphine is more potent in arthritic rats than normal rats (Neil et al., 1986). Our study indicates that morphine is equipotent in nonarthritic and arthritic rats in the paw-pressure test. Such differences are likely due to our methods versus those of Neil et al. The ED_{50} values for Δ^9-THC-induced antinociception in nonarthritic and arthritic rats did not differ, which is consistent with previous findings by Smith et al. (1998b).

We found that morphine-induced antinociception is naloxone-sensitive, consistent with the finding that the lack of μ-opioid receptors in μ-opioid receptor knockout mice abolishes the analgesic effect of morphine (Matthes et al., 1996). However, the attenuation of Δ^9-THC-induced antinociception requires a high dose of naloxone and nor-BNI in arthritic rats, and a low dose of naloxone in nonarthritic rats. These data indicate differences in opioid tone in arthritic and nonarthritic rats. Consistent with our data are data indicating that the inflammation and hyperalgesia produced by Freund's adjuvant is associated with alterations in several neuropeptide systems, including the endogenous opioid system, notably the κ opioid dynorphin A (Millan et al., 1985, 1986a,b). Enhanced dynorphin gene expression and peptide levels following inflammation have been implicated in the enhanced excitability and development of expanded receptive fields of spinal dorsal horn neurons observed in inflammation (Dubner, 1991; Hylden et al., 1991). Furthermore, Dubner and Ruda (1992) proposed a model in which dynorphin, substance P, and calcitonin gene-related peptide increased excitability at NMDA receptors leading to dorsal horn excitability and subsequent excessive depolarization and excitotoxicity. It is not surprising then that the alterations in dynorphinergic systems and effect of the κ-opioid receptor antagonist, nor-BNI, differ in arthritic and nonarthritic rats following Δ^9-THC administration.

A hypothetical model of the spinal nociceptive effects of Δ^9-THC was proposed by Mason et al. (1999) for acute pain models. In this model, Δ^9-THC administered spinally activates cannabinoid receptors resulting in the inhibition of tonically active inhibitory neurons which modulate dynorphinergic neurons. The disinhibition of the dynorphinergic neurons in the spinal cord leads to the release of dynorphin A. Dynorphin A (1–17) or its metabolites act via the κ- opioid receptor system, resulting in cannabinoid-induced antinociception. However, Mason et al. (1999) model of antinociception in nonarthritic rats did not fit with the data that we obtained from arthritic rats. The changes in dyn A and leu-enk levels that occurred 10 min after Δ^9-THC administration were opposite in nonarthritic versus arthritic rats. Arthritic rats have an increased level of both dyn A and leu-enk after vehicle treatment and a decreased level of both peptides after Δ^9-THC administration. To explain these discrepancies, we now propose an alternative model based on both Mason et al. (1999) model and the model proposed by Dubner and Ruda (1992) for Δ^9-THC-induced spinal antinociception in arthritic versus normal rat (Figs. 7 and 8).

We hypothesize that in normal rats, Δ^9-THC acts to inhibit an excitatory neuron that both impinges on an inhibitory interneuron, and sends a collateral that directly stimulates the dynorphinergic neuron. The inhibitory interneuron keeps the dynorphinergic neuron, on which it synapses, under tonic inhibitory control, while the excitatory collateral acts secondarily. Δ^9-THC inhibition of the excitatory input on this interneuron effectively disinhibits dynorphinergic neurons and dynorphin A is released. However, levels of dynorphin A have been shown to be higher in arthritic than nonarthritic rats. We propose that the inhibitory interneuron impinging on the dynorphinergic neuron is functionally absent, which causes a loss of tonic inhibition of dynorphinergic activity. This loss of inhibition in combination with excitatory input from the collateral causes dynorphin A to be released. Δ^9-THC restores some inhibitory control by inhibiting the excitatory input on the dynorphinergic neuron, reducing dynorphin release. This is consistent with data from Lichtman and Martin (1991a) indicating an α_2 component in Δ^9-THC-induced antinociception in normal rats. Caudle and Isaac (1988) have also shown that excessive depolarization, promoting excitotoxicity and neuronal dysfunction, could lead to a pathological state. Small local circuit

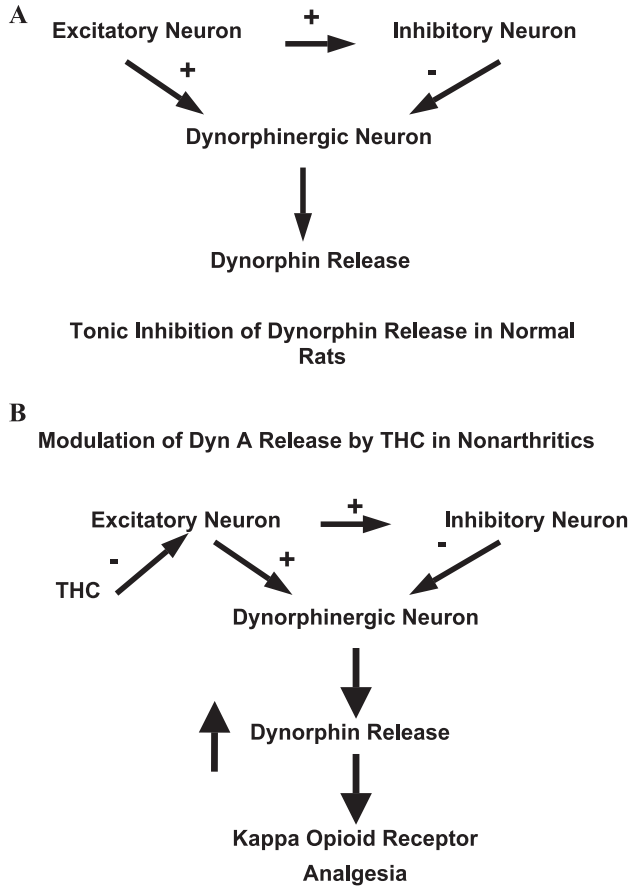

Fig. 7. Model of tonic and THC-induced dynorphin A release in nonarthritic rats.

neurons, probably inhibitory, are most sensitive to excitotoxicity and their dysfunction might contribute to a loss of inhibitory tone.

While this model fits with our peptide release data, it does not fully explain the differences between nonarthritic and arthritic rats with regard to nor-BNI attenuation of Δ^9-THC-induced antinociception. Arthritic rats demonstrated a nor-BNI attenuation of Δ^9-THC-induced antinociception although Δ^9-THC decreased dynorphin A release in these animals. This seems contradictory and might be explained by the action of dynorphin at κ-opioid receptors as well as non-kappa sites, such as NMDA receptors. Investigators have suggested that spinal dynorphin plays a role in the sensitization of nociceptive neurons, at low doses producing analgesia, while at higher doses producing hyperalgesia. Dynorphin has been shown to both excite and inhibit neurons. It has been suggested that its inhibitory effect may be due to actions at κ-opioid receptors (Caudle et al., 1994) leading to antinociception, and excitatory effects resulting from interactions with NMDA receptors (Vanderah et al., 1996) resulting in hyperalgesia. Hyperalgesia associated with Freund's adjuvant-induced inflammation may be a result of the increased release of dynorphin in the spinal cord (Laughlin et al., 2001). A study by Millan et al. (1985) demonstrated a potentiation of hyperalgesia in arthritic rats by (−)-5,9 alpha-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan (MR 2266), a potent κ-opioid receptor antagonist. This study also indicated a rise in dynorphin levels in the lumbo-sacral spinal cord. The potentiation of hyperalgesia by MR 2266 could result from the loss of inhibitory control by dynorphin at κ-opioid receptors, while dynorphin's excitatory actions at NMDA receptors are maintained.

Therefore, in arthritic rats we propose that dynorphin has excitatory actions at NMDA receptors which may predominate over its inhibitory actions at κ-opioid receptors, resulting in hyperalgesia (Laughlin et al., 2001). The decrease in release of dynorphin by Δ^9-THC is hypothesized to decrease dynorphin's actions at NMDA receptors allowing the lower levels of dynorphin to act at κ-opioid receptors to produce analgesia, consistent with nor-BNI block of κ-opioid receptors after Δ^9-THC administration.

In conclusion, we have demonstrated that Δ^9-THC differentially modulates endogenous opioids in nonarthritic versus arthritic rats depending on the basal level of dynor-

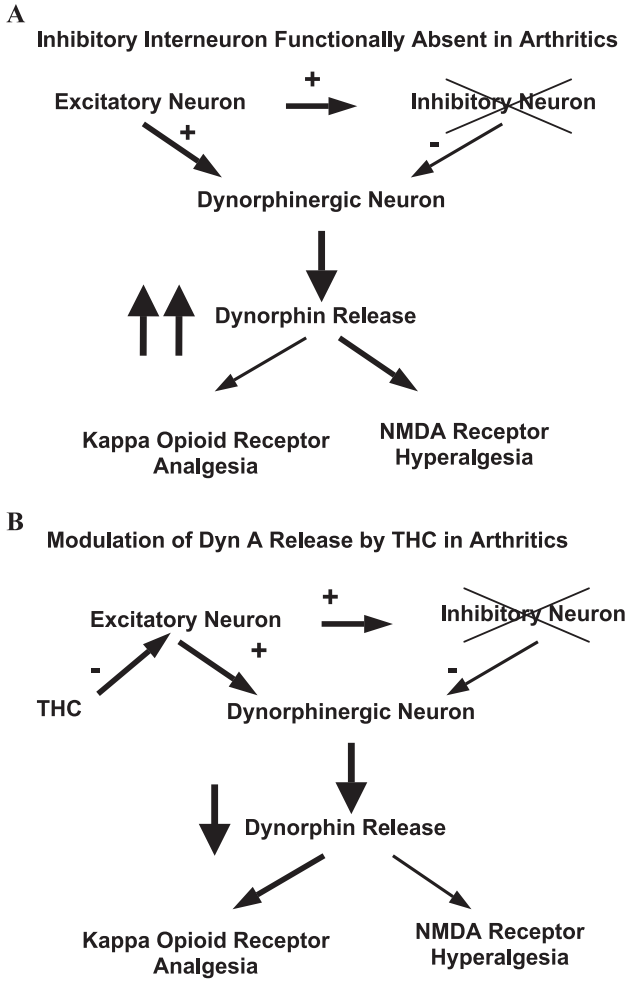

Fig. 8. Model of tonic and THC-induced dynorphin A release in arthritic rats.

phin. The cannabinoid receptor may serve as a homeostatic modulator of the tonic release of opioids in the spinal nociceptive pathways.

Acknowledgements

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