ELSEVIER

European Journal of Pharmacology 378 (1999) 237–248

ejp

www.elsevier.nl/locate/ejphar

Cannabinoid modulation of dynorphin A: correlation to cannabinoid-induced antinociception

David J. Mason Jr. [a], John Lowe [b], Sandra P. Welch [a,*]

[a] *Department of Pharmacology and Toxicology, Virginia Commonwealth University / Medical College of Virginia, Box 980613, MCV Station, Richmond, VA 23298-0613, USA*

[b] *Pfizer Central Research, Groton, CT, USA*

Received 14 April 1999; accepted 29 June 1999

Abstract

Intrathecal administration of anandamide, Δ^9-tetrahydrocannabinol (THC) and (−)-3-[2-hydroxy-4-(1,1-dimethyheptyl)ptyl)phenyl]-4-(3-hydroxypropyl)-cicloexan-1-ol (CP55,940) induced spinal antinociception accompanied by differential κ-opioid receptor involvement and dynorphin A peptide release. Antinociception using the tail-flick test was induced by the classical cannabinoid THC and was blocked totally by 17,17′-bis(cyclopropylmethyl)-6′,6,7,7′-tetrahydro-4,5,4′5′-diepoxy-6,6′-(imino)[7,7′-bimorphinan]-3,3′,14,14′-tetrol (norbinaltorphimine) indicating a significant and critical κ-opioid receptor component. The endogenous cannabinoid, anandamide and the non-classical bicyclic cannabinoid, CP55,940, induced non-nor-BNI-sensitive effects. The *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716A)-mediated attenuation of spinal antinociception imparted by the various cannabinoids indicates cannabinoid CB_1 receptor involvement. THC-induced an enhancement of immunoreactive dynorphin A release which coincided with the onset, but not duration antinociception. The release of dynorphin A was also attenuated by SR141716A suggesting it is cannabinoid CB_1 receptor-mediated. These data indicate a critical role for dynorphin A release in the initiation of the antinociceptive effects of the cannabinoids at the spinal level.

Keywords: Anandamide; Antinociception; Δ^9-Tetrahydrocannabinol; CP55,940; Dynorphin A; Opioid; SR141716A; Tail-flick latency

1. Introduction

Δ^9-Tetrahydrocannabinol (THC) is one of the 60 dibenzopyran-type compounds (known as cannabinoids) isolated from *Cannabis sativa*. In vivo studies correlate acute THC exposure with impaired motor coordination, cognitive activity, antinociception, hypothermia and decreased neurotransmitter release in various animal models (Dewey, 1986; Turkanis and Karler, 1988). Research to develop cannabinoids devoid of intoxicating side effects has lead to the synthesis of several synthetic cannabinoids such as (−)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)-cicloexan-1-ol (CP55,940) with pharmacological profiles comparable to naturally occurring cannabinoids (Compton et al., 1990, 1991; Martin et al., 1991; Melvin et al., 1993). The cannabinoid CB_1 receptor, a saturable binding site for which cannabinoids possess high affinity, has been identified primarily in tissues of central nervous system origin (Devane et al., 1988; Matsuda et al., 1990). In vitro studies reveal a distinct relationship between cannabinoid interaction with the cannabinoid CB_1 receptor and attenuation of G-protein-mediated cAMP production (Howlett, 1984; Howlett et al., 1986, 1988, 1992). The ability of cannabinoids to attenuate cAMP production correlates with their respective binding profiles and pharmacological potencies (Howlett and Flemming, 1984). A second receptor, the cannabinoid CB_2 receptor, has been described in tissues of peripheral origin (Munro et al., 1993). A splice variant of the cannabinoid CB_1 receptor, the cannabinoid CB_{1A} receptor, has also been characterized (Shire et al., 1995).

Anandamide has been described as an endogenous ligand for the cannabinoid receptor (Devane et al., 1992). The behavioral effects of anandamide are comparable to those of other psychoactive cannabinoids and cross-tolerance with other cannabinoids has been demonstrated (Fride and Mechoulam, 1993; Pertwee et al., 1993; Smith et al., 1994; Welch et al., 1995). Anandamide attenuates cAMP production in a manner akin to THC and characteristic of G-pro-

* Corresponding author. Tel.: +1-804-828-8424; fax: +1-804-828-2117; E-mail: swelch@hsc.vcu.edu

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00479-3

tein coupled functions (Felder et al., 1993; Vogel et al., 1993). A cannabinoid antagonist, *N*-piperidino-5(4-chlorophenyl)-1-(2,4-dichlorophenyl) - 4 - methyl - 3 - pyrazolecarboxamide (SR141716A), has been described (Rinaldi-Carmona et al., 1994) and appears to selectively attenuate cannabinoid CB_1 receptor-mediated activity in vivo and in vitro (Collins et al., 1995; Wiley et al., 1995a,b).

The κ-opioid receptors comprise one of three opioid receptor subtypes, including the μ- and δ-opioid receptors. They have been identified pre- and post-synaptically in the rat spinal cord (Besse et al., 1990; Yasuda et al., 1993). The dynorphins constitute a family of endogenous peptides for which the κ-opioid receptors possess great affinity (Chavkin et al., 1982; Corbett et al., 1982). Their distribution is widespread, but notably high in the spinal cord (Basbaum and Fields, 1984). Dynorphin A-(1–17) is a pharmacologically active peptide derived from a prodynorphin precursor. It may be sequentially metabolized to smaller fragments such as dynorphin A-(1–13) and dynorphin A-(1–8). Dynorphin A-(1–8) may be further metabolized to leucine–enkephalin, a δ-opioid receptor agonist (Dixon and Traynor, 1990). The pharmacological activities of the dynorphin peptides are complex. Whereas dynorphin A-(1–17) produces antianalgesic activity at low doses and antinociceptive activity at higher doses, smaller fragments such as dynorphin A-(1–13) and dynorphin A-(1–8) induce antinociception via the κ-opioid receptor, but lack the antianalgesic properties of the larger dynorphin A-(1–17) peptide (Morris and Herz, 1989; Fujimoto et al., 1990; Rady et al., 1991). Synthetic κ-opioids, such as *trans*(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide methane sulfonate (U50,488H) and (−)-[5*R*-(5α,7α,8β)]-*N*-Methyl-*N*-[7-(1-pyrrolidinyl)-1-oxa-spiro-[4.5[dec-8-yl]-4-benzofuranacetamide HCl (CI-977), demonstrate pharmacologic properties similar to those of endogenous κ-opioid receptor ligands (Hayes et al., 1990; Hunter et al., 1990).

In this study, the abilities of various cannabinoids, administered intrathecally (i.t.) to modulate spinal dynorphin A-(1–17) concentration was assessed in conjunction with tail-flick latency. Considering the previous studies conducted by Welch (1994), Pugh et al. (1996, 1997), concerning interactions between cannabinoids and κ-opioids, the following model of cannabinoid-induced antinociception was hypothesized. Following i.t. administration, cannabinoids interact with the cannabinoid receptor to increase spinal dynorphin A-(1–17) concentration. Thus, dynorphin A-(1–17) or pharmacologically active metabolites, such as dynorphin A-(1–13) and dynorphin A-(1–8), acting via the κ-opioid receptor system are responsible for the induction of cannabinoid-induced antinociception.

In testing this hypothesis, studies were performed utilizing a spinal perfusion technique which allowed for simultaneous collection of dynorphin A peptides and assessment of tail-flick latency. The mouse model, employed in previous studies, was not suitable for this work due to the small amount of cerebrospinal fluid that could be obtained. Thus, a rat model was utilized. Subsequent pharmacological studies using similar techniques were performed to determine if dynorphin A modulation was mediated via the cannabinoid receptor. Additionally, pharmacological techniques were employed to gauge the κ-opioid receptor-mediated contribution to cannabinoid-induced tail-flick latency.

2. Methods and materials

2.1. Animal husbandry

These studies were conducted using male Sprague–Dawley rats, weighing between 450 and 500 g, obtained from Harlan Laboratories. Subjects were housed individually and maintained on a fixed 12-h light cycle at a temperature of 22 ± 2°C. Water and food (Harlan Rat Chow) were provided ad libitum.

2.2. Intrathecal administration of drugs and dynorphin collection

Intrathecal drug administration and dynorphin collection were performed using a modified version of techniques described by Yaksh (1981) and Tseng (1989). Subjects were selected for partitioning into experimental groups at random and anesthetized via intraperitoneal (i.p.) injection of sodium barbital (375 mg/kg) and a separate i.p. injection of 2 mg/kg atropine methyl nitrate. The anesthetized rats were placed in stereotaxis and an incision made on the atlanto–occipito membrane to expose the cisterna magna. A catheter of PE-10 polyethylene tubing was inserted through the exposed 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 albumen, bacitracin (30 mg/ml), 0.01% Triton X and effervesced with 95% O_2 and 5% CO_2. Positioned as such, 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 approximately 30 min on a heating pad. Following acclimation, base-line tail-flick latency was assessed. Only animals exhibiting normal tail-flick response to noxious stimuli, less than 4 s latency, were used. Test compounds were administered in a 20-μl bolus of vehicle, via spinal catheter, at a rate of 30 μl/min. Subjects were then segregated into groups for cerebrospinal fluid sampling and tail-flick latency assessment at 3, 10 or 30 min post-administration of the test compound. Cerebrospinal fluid collection entailed rapid perfusion of the spinal cavity with artificial cerebrospinal fluid culminating in the collection of 1.5 ml of

the eluting artificial cerebrospinal fluid 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. Collected fractions were boiled for 12 min and centrifuged at a rate of 10,000 rpm for 10 min. The supernatant was collected, frozen at −70°C and lyophilized. Samples were reconstituted in 250 μl RIA buffer before dynorphin A-(1–17) peptide measurements.

2.3. Measurement of dynorphin A-(1–17) peptide

Measurement of dynorphin A-(1–17) (pg/ml) was accomplished using dynorphin A-(1–17) specific radioimmunoassay kits obtained from Peninsula Laboratories, The reconstituted samples were analyzed in duplicate. The manufacturer reported cross-reactivity of antibody as 100% vs. dynorphin A-(1–24), a parent compound, and less than 2% vs. smaller peptide fragments. We found no cross-reactivity of the antibody to dynorphin A-(1–8), dynorphin A-(1–13), dimethyl sulfoxide (DMSO), THC, anandamide or CP55,940. We have also recently evaluated the antibody for cross-reactivity to several opioids including morphine, metenkephalin, and leucine–enkephalin. No cross-reactivity was observed. Only the linear portion of the radioimmunoassay standard curve, between 0.1 and 64 pg/ml of the standard dynorphin peptide, was used to calculate dynorphin concentration.

2.4. Measurement of dynorphin A-(1–8) peptide

Measurement of spinal dynorphin A-(1–8) (pg/ml) was accomplished using a dynorphin A-(1–8) specific radioiummunoassay kit obtained from Peninsula Laboratories. The samples were reconstituted in 500 μl of assay buffer and analyzed in duplicate. The manufacturer reports cross-reactivity of antibody as 100% vs. dynorphin A-(1–8) and less than 0.01% vs. ''big'' dynorphin, dynorphin A-(1–17), or dynorphin A-(1–13). The manufacturer finds no cross-reactivity of the antibody to dynorphin B, or leucine–enkephalin. Only the linear portion of the radioimmunoassay standard curve, between 0.1 and 64 pg/ml of the standard dynorphin peptide, was used to calculate dynorphin concentration.

2.5. Measurement of leucine–enkephalin peptide

Measurement of spinal leucine–enkephalin (pg/ml) was accomplished using a leucine–enkephalin specific radioimmunoassay kit obtained from Peninsula Laboratories. The samples were reconstituted in 250 μl of assay buffer and analyzed in duplicate. The manufacturer reports cross-reactivity of the leucine–enkephalin antisera as 100% vs. leucine–enkephalin, 29% vs. dynorphin A-(1–17), 8% vs. dynorphin A-(1–8) and 3% vs. methionine–enkephalin. Only the linear portion of the radioimmunoassay standard curve, between 1 and 128 pg/ml of the standard leucine–enkephalin peptide, was used to calculate leucine–enkephalin concentration.

2.6. Assessment of tail-flick latency

Antinociceptive behavior was assessed using a modified version of that described by D'Amour and Smith (1941). Each animal was acclimated in the laboratory 24 h prior to experimentation. Tail-flick latency was not found to be significantly increased by sodium barbital or catheterization in comparison to unanesthetized or non-catheterized animals. Base latencies were measured as 1.5–4 s with maximal post-drug latency set at 10 s after which the noxious heat stimulus was terminated. Antinociception was measured in terms of percent maximal possible effect (% MPE) defined by Dewey et al. (1970) and Harris and Pierson (1990) as:

$$\%\,\mathrm{MPE} = \frac{[\text{test latency} - \text{control latency}]}{[10\ \text{s} - \text{control latency}]} \times 100\%$$

Each parameter (i.e., test or control tail-flick latency value) represents the mean of three recordings at 10-s intervals.

2.7. Statistical analysis

Using a randomized design, analysis of data concerning tail-flick latency or dynorphin peptide concentration was

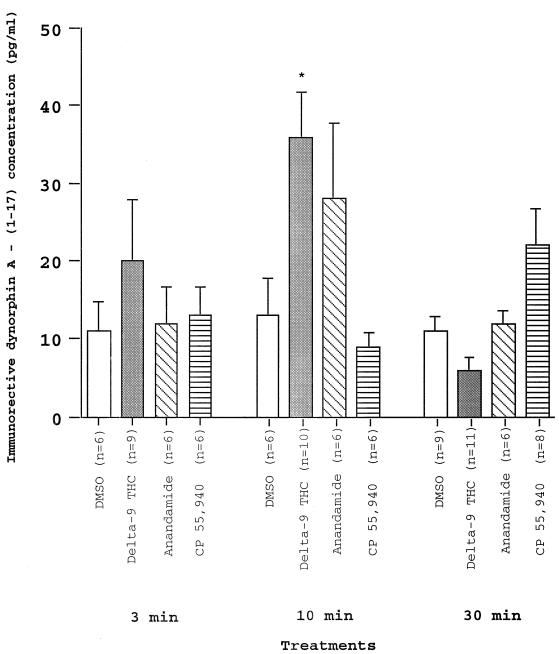

Fig. 1. Spinal dynorphin A-(1–17) concentrations following acute i.t. cannabinoid administration. Rats were exposed to 20 μl DMSO, Δ^9-THC (300 μg), CP55,940 (100 μg) or anandamide (200 μg) via catheter. Fractions of cerebrospinal fluid were collected at time periods of 3, 10 and 30 min post-administration and quantified via radioimmunoassay. Mean dynorphin A-(1–17) concentrations ± S.E. are presented. * $p < 0.05$ in comparison to control animals receiving DMSO vehicle i.t. The number of rats per treatment is shown in parentheses.

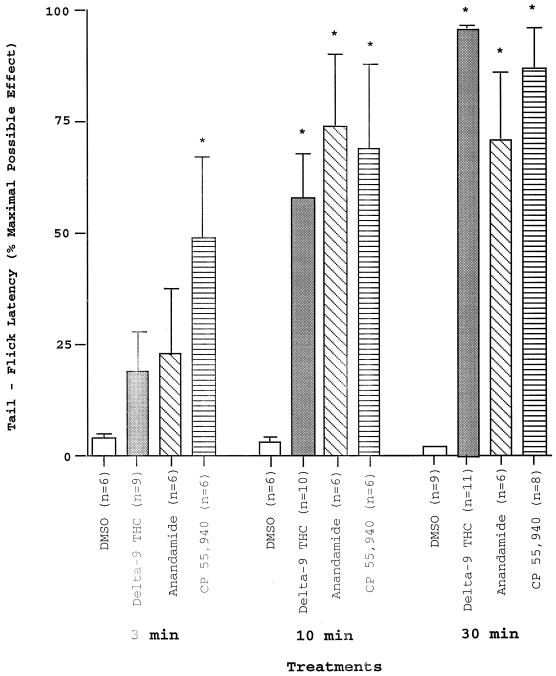

Fig. 2. Tail-flick latency following acute i.t. cannabinoid exposure. Rats were exposed to 20 μl DMSO, Δ^9-THC (300 μg), CP55,940 (100 μg) or anandamide (200 μg) via catheter and tail-flick latency assessed at time points of 3, 10 and 30 min post-administration. Mean % MPE ± S.E. are presented. $^*p < 0.05$ in comparison to control animals receiving DMSO vehicle i.t. The number of rats per treatment is shown in parentheses.

done using ANOVA (analysis of variance) followed by post-hoc Dunnett's *t*-test (Dunnett, 1955).

2.8. Drugs and vehicle

THC was obtained from the National Institute on Drug Abuse. CP55,940 was provided by Dr. Lawrence Melvin, Pfizer Pharmaceutical Research. Anandamide was obtained from Dr. Raj Razdan, Organix (Woburn, MA, USA). SR141716A was obtained from John Lowe, Pfizer Research. CI-977 was obtained from Dr. R. Martin, Parke-Davis Pharmaceutical Research Division (Ann Arbor, MI, USA). SR141716A was administered in a vehicle composed of 1 part emulphor:1 part ethanol:18 parts saline (1:1:18), CP55,940, THC and anandamide were administered using a 100% DMSO vehicle. Artificial cerebrospinal fluid was used as a vehicle for norbinaltorphimine (norBNI).

2.9. Specifics of drug administration

To assess the effect of acute cannabinoid exposure on spinal dynorphin A-(1–17) concentration, animals were treated with 20 μl DMSO vehicle, THC (300 μg), CP55,940 (100 μg) or anandamide (200 μg), via spinal catheters. Doses chosen represent the approximate doses required for equi-efficacious effects in the tail-flick latency test (Lichtman and Martin, 1991; Pugh et al., 1997).

3. Results

The mean immunoreactive dynorphin A-(1–17) concentration of fractions collected 3 min post-administration of THC was 20 ± 8 pg/ml; 13 ± 3 pg/ml following acute CP55,940 exposure and 12 ± 5 pg/ml in animals treated with anandamide (Fig. 1). These measures were not different from those of control animals receiving DMSO vehicle (11 ± 3 pg/ml).

The immunoreactive dynorphin A-(1–17) concentration 10 min after administration THC (36 ± 6 pg/ml) was greater than that of control animals treated with DMSO (12 ± 3 pg/ml) (Fig. 1). The mean immunoreactive dynorphin A-(1–17) concentration of animals treated with CP55,940 (9 ± 2 pg/ml) or anandamide (28 ± 10 pg/ml), 10 min post-administration, was not different from that of control animals receiving DMSO. Neither CP55,940- (22 ± 5 pg/ml), anandamide- (12 ± 2 pg/ml) nor THC-treated animals (6 ± 2 pg/ml) possessed immunoreactive dynorphin A-(1–17) concentrations different from that of control

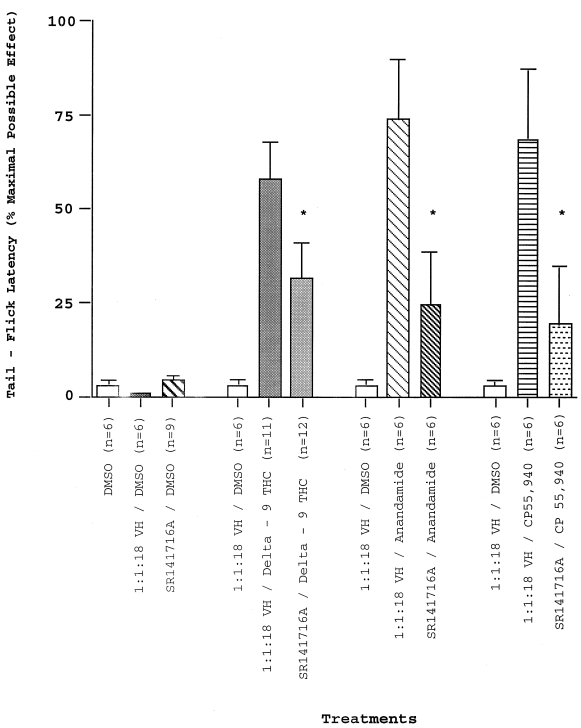

Fig. 3. Spinal dynorphin A-(1–17) concentration following acute i.t. Δ^9-THC administration and SR141716A pre-treatment. Rats were exposed to 20 μl DMSO or Δ^9-THC (300 μg) i.t. following a 60-min pre-treatment with SR141716A or 1:1:18 administered i.p. Fractions of cerebrospinal fluid were collected 10 min post-administration and quantified via radioimmunoassay. Mean dynorphin A-(1–17) concentrations ± S.E. are presented. $^*p < 0.05$ in comparison to control animals administered Δ^9-THC (i.t.) in the absence of SR141716A pre-treatment. The number of rats per treatment is shown in parentheses.

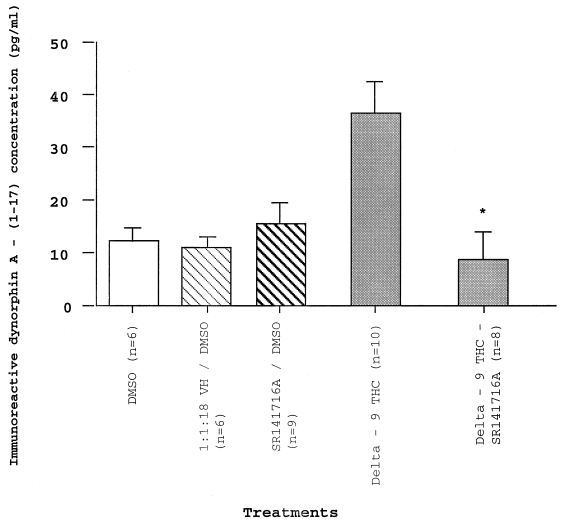

Fig. 4. Tail-flick latency following acute i.t. cannabinoid exposure and SR141716A pre-treatment. Rats were exposed to 20 μl DMSO, Δ^9-THC (300 μg), CP55,940 (100 μg) or anandamide (200 μg) via spinal catheter in either the presence or absence of a 60-min SR141716A (i.p.) pre-treatment and tail-flick latency assessed 10 min post-administration. Mean % MPE ± S.E. are presented. $^*p < 0.05$ in comparison to control animals administered either of the test cannabinoids (i.t.) in the absence of SR141716A pre-treatment. The number of rats per treatment is shown in parentheses.

animals treated with DMSO (11 ± 2 pg/ml) 30 min post-administration (Fig. 1).

Tail-flick latency was assessed in conjunction with cerebrospinal fluid collection (Fig. 2). Whereas CP55,940 increased tail-flick latency 3 min post-administration (49 ± 19% MPE) in comparison to animals treated with DMSO (4 ± 2% MPE), THC and anandamide failed to increase tail flick-latency (19 ± 10 and 23 ± 16% MPE, respectively). Each of the test cannabinoids enhanced tail-flick latency 10 min post-administration when compared to the control group (3 ± 2% MPE). THC induced 58 ± 11% MPE; anandamide 74 ± 17% MPE and CP55,940 69 ± 20% MPE, respectively (Fig. 2). While the test cannabinoids continued to significantly increase tail-flick latency 30 min post-administration (THC = 96 ± 2% MPE; anandamide = 71 ± 16% MPE; and CP55,940 = 87 ± 10% MPE), the DMSO group demonstrated 2 ± 1% MPE 30 min post-administration.

To determine if the THC-induced enhancement of spinal immunoreactive dynorphin concentration and tail-flick latency were cannabinoid receptor-mediated, animals were treated with DMSO vehicle or THC (300 μg) following a 60-min pre-treatment with 10 mg/kg SR141716A (i.p.) or 1:1:18 vehicle i.p. SR141716A pre-treatment resulted in an attenuation of the THC-induced modulation of immunoreactive dynorphin A-(1–17) 10 min post-administration (9 ± 5 pg/ml) when compared with animals receiving THC without SR141716A pre-treatment (36 ± 6 pg/ml) (Fig. 3). The acute administration of the DMSO vehicle following SR141716A pre-treatment did not increase the dynorphin A-(1–17) concentration (15 ± 5 pg/ml) significantly.

Tail-flick latencies of animals pre-treated with SR141716A 60 min prior THC administration (26 ± 10% MPE) were found to be significantly less than animals receiving a 60-min 1:1:18 vehicle pre-treatment prior to THC i.t. (55 ± 11% MPE) (Fig. 4). In a similar series of experiments animals were treated acutely with DMSO

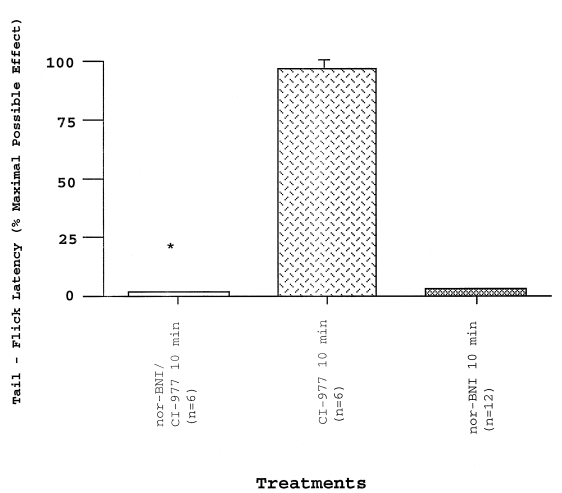

Fig. 5. Tail-flick latency following acute CI-977 exposure (i.t.) with and without nor-BNI pre-treatment. Rats were exposed to 20 μl ACSF or CI-977 (100 μg) i.t. via spinal catheter either in the presence or absence of a 10-min nor-BNI (70 μg) pre-treatment i.t. as well as nor-BNI alone and tail-flick latency assessed 10 min post-administration. Mean % MPE ± S.E. are presented. $^*p < 0.05$ in comparison to control animals administered CI-977 in the absence of nor-BNI pre-treatment. The number of rats per treatment is shown in parentheses.

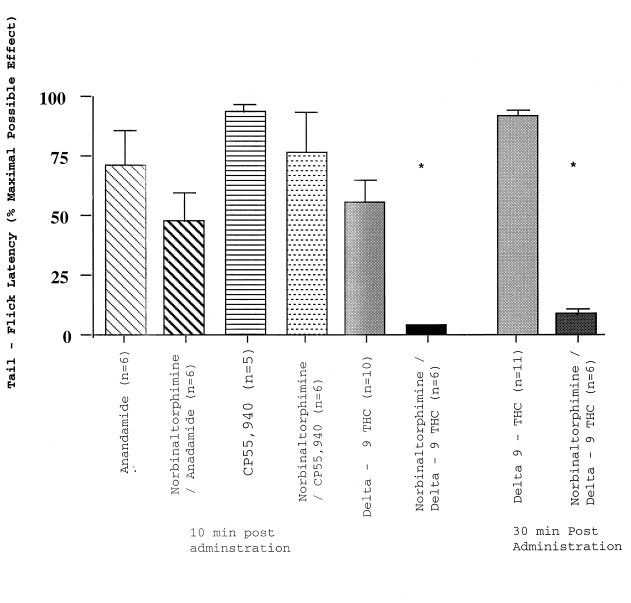

Fig. 6. Tail-flick latency following acute cannabinoid exposure (i.t.) with and without nor-BNI pre-treatment. Rats were exposed to CP55,940 (100 μg), anandamide (200 μg) or Δ^9-THC (300 μg) i.t. via spinal catheter either in the presence or absence of a 10-min nor-BNI (70 μg) pre-treatment i.t. and tail-flick latency assessed 10 and 30 min post-administration. Mean % MPE ± S.E. are presented. $^*p < 0.05$ in comparison to control animals administered with CP55,940 in the absence of nor-BNI pre-treatment. The number of rats per treatment is shown in parentheses.

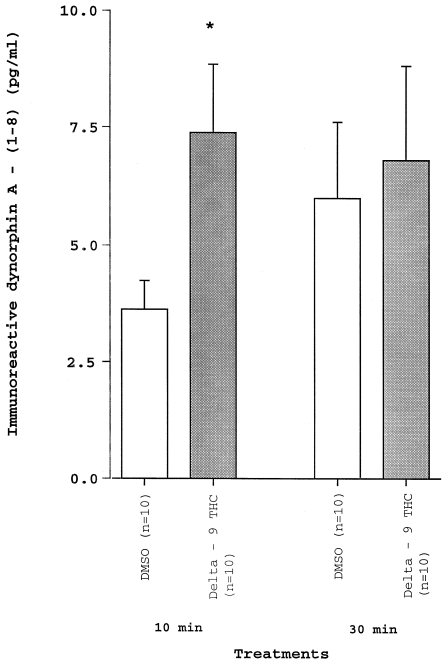

Fig. 7. Spinal dynorphin A-(1–8) concentrations following acute i.t. Δ^9-THC administration. Rats were exposed to 20 μl DMSO vehicle or Δ^9-THC (300 μg). Fractions of cerebrospinal fluid were collected at time periods of 10, 15 and 30 min post-administration and dynorphin A-(1–8) quantified via radioimmunoassay. Mean dynorphin A-(1–8) concentrations ± S.E. are presented. $^*p < 0.05$ in comparison to control animals receiving 20 μl DMSO vehicle i.t. The number of rats per treatment is shown in parentheses.

vehicle (20 μl), CP55,940 (100 μg), or anandamide (200 μg), via spinal catheter following a 60-min pre-treatment with 10 mg/kg SR141716A or 1:1:18 vehicle (i.p.) and tail-flick latency was assessed. SR141716A pre-treatment resulted in an attenuation of CP55,940 (20 ± 16% MPE), THC, and anandamide-induced antinociception (25 ± 15% MPE) to levels shown in control animals (Fig. 4).

To demonstrate the existence of a functional κ-opioid receptor system capable of mediating an antinociceptive response in the rat spinal cord, animals were treated (i.t.) with CI-977 (100 μg) or 20 μl of artificial cerebrospinal fluid vehicle in either the presence or absence of a 10-min pre-treatment with nor-BNI (70 μg, i.t.) in 20 μl of artificial cerebrospinal fluid vehicle. Nor-BNI is a highly selective κ-opioid receptor antagonist (Portoghese et al., 1988; Horan et al., 1992). Animals pre-treated for 10 min with CI-977 demonstrated significant antinociception (97 ± 3% MPE) in comparison to those receiving CI-977 following nor-BNI pre-treatment (3 ± 1% MPE) (Fig. 5). To assess the extent of any contribution to cannabinoid-induced tail-flick latency via the κ-opioid receptor system, animals were pre-treated with nor-BNI and administered either THC (300 μg), CP55,940 (100 μg) or anandamide (200 μg) and tail-flick latency was assessed 10 min post-administration of nor-BNI. Animals treated with CP55,940 (Fig. 6) following nor-BNI pre-treatment demonstrated antinociceptive activity (98 ± 2% MPE) which was not different from that seen in the absence of nor-BNI pre-treatment (80 ± 19% MPE). Animals treated with anandamide (Fig. 6) following nor-BNI pre-treatment demonstrated tail-flick latencies of 50 ± 14% MPE vs. 74 ± 17% MPE in animals treated with anandamide alone. Similar studies were conducted utilizing THC (300 μg) and nor-BNI (Fig. 6). Tail-flick latency was assessed 10 and 30 min following administration of THC. In the absence of nor-BNI pre-treatment, THC increased tail-flick latency at 10 min (58 ± 11% MPE) and 30 min (96 ± 2% MPE) post-administration. Nor-BNI pre-treatment totally blocked the ability THC to increase tail-flick latency at 10 min (4 ± 1% MPE) and 30 min (9 ± 2% MPE) post-administration.

Animals receiving appropriate single or double vehicle infusions and injections were evaluated as controls for the data presented in Figs. 5 and 6. All rats infused or injected with vehicle had % MPE < 10%.

In separate experiments assessing THC modulation of immunoreactive dynorphin A-(1–8) concentrations, fractions collected 10 min post-administration of THC contained a mean immunoreactive dynorphin A-(1–8) concentration of 7.4 ± 1.5 pg/ml which proved significantly greater than that of fractions collected 10 min post-administration of DMSO vehicle exposure (3.6 ± 0.7 pg/ml) (Fig. 7). The mean immunoreactive dynorphin A-(1–8) concentration of fractions collected 15 min (7.0 ± 1.6

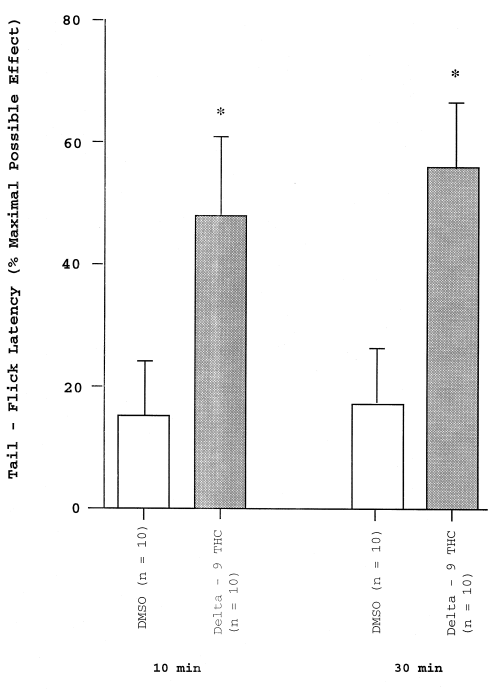

Fig. 8. Tail-flick latency following acute i.t. Δ^9-THC prior to dynorphin A-(1–8) assessment. Rats were exposed to 20 μl DMSO or Δ^9-THC (300 μg) via catheter and tail-flick latency assessed at time points of 10, 15 and 30 min post-administration prior to cerebrospinal fluid collection. Mean % MPE ± S.E. are presented. $^*p < 0.05$ in comparison to control animals receiving 20 μl DMSO vehicle i.t. The number of rats per treatment is shown in parentheses.

pg/ml) or 30 min post-administration of THC (6.8 ± 2.1 pg/ml) were not significantly increased in comparison to those of control animals treated with DMSO vehicle 15 min (5.8 ± 1.1 pg/ml) or 30 min (6.0 ± 1.7 pg/ml) post-administration.

To evaluate the antinociceptive activity associated with dynorphin modulation, tail-flick latencies were assessed in conjunction with fraction collection (Fig. 8). Ten minutes post-administration, animals treated with THC demonstrated enhanced tail-flick latency (48.0 ± 13.6% MPE) in comparison to those treated with DMSO vehicle (14.7 ± 9.6% MPE). Animals treated with THC also demonstrated increased tail-flick latency 30 min post-administration (56.1 ± 12.5% MPE) vs. 17.2 ± 9.7% MPE in those treated with DMSO.

In contrast to dynorphin A-(1–8), immunoreactive leucine–enkephalin levels were not enhanced 10 or 30 min post-administration of THC. The mean immunoreactive leucine–enkephalin concentration of artificial cerebrospinal fluid fractions collected 10 min post-administration of THC was 17.5 ± 11.2 pg/ml vs. 12.0 ± 5.0 pg/ml from those collected 10 min post-administration of DMSO vehicle (Fig. 9). Similar observations were noted 30 min post-administration. Immunoreactive leucine–enkephalin concentrations in fractions collected from subjects receiving THC (7.7 ± 4.2 pg/ml) were not found to be increased when compared to samples collected from control animals (6.8 ± 5.6 pg/ml).

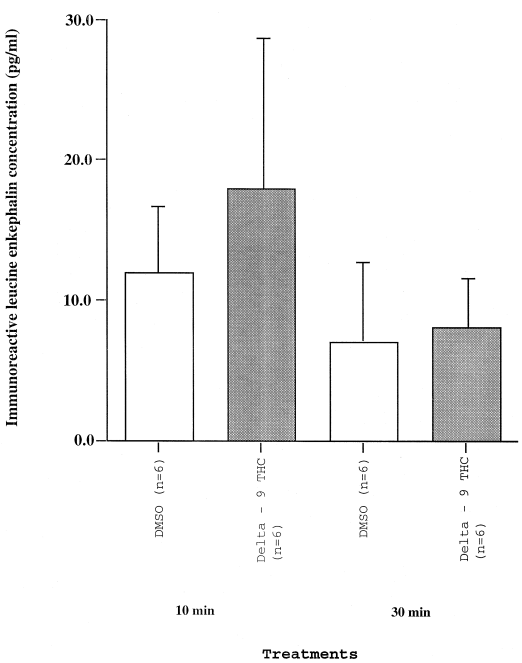

Fig. 9. Spinal leucine-enkephalin concentrations following acute i.t. Δ^9-THC administration. Rats were exposed to 20 μl DMSO vehicle or Δ^9-THC (300 μg). Fractions of cerebrospinal fluid were collected at time periods of 10, 15 and 30 min post-administration and leucine-enkephalin quantified via radioimmunoassay. Mean leucine-enkephalin concentrations ± S.E. are presented. $^*p < 0.05$ in comparison to control animals receiving 20 μl DMSO vehicle i.t. The number of rats per treatment is shown in parentheses.

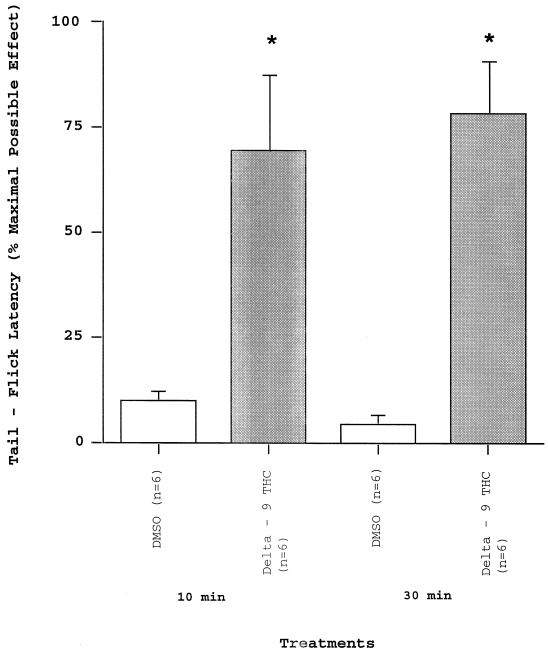

Fig. 10. Tail-flick latency following acute i.t. Δ^9-THC prior to leucine-enkephalin assessment. Rats were exposed to 20 μl DMSO or Δ^9-THC (300 μg) via catheter and tail-flick latency assessed at time points of 10 and 30 min post-administration prior ACSF collection. Mean % MPE ± S.E. are presented. $^*p < 0.05$ in comparison to control animals receiving 20 μl DMSO vehicle i.t. The number of rats per treatment is shown in parentheses.

Assessment of tail-flick latency in these animals (Fig. 10) revealed a difference between animals treated with THC (69.2 ± 19.6% MPE) and those receiving DMSO vehicle (9.7 ± 2.5% MPE) 10 min post-administration. Thirty minutes post-administration, animals receiving DMSO vehicle demonstrated little antinociceptive response whereas those treated THC demonstrated (78.2 ± 13.8% MPE).

4. Discussion

The enhanced immunoreactive dynorphin A-(1–17) concentration observed 10 min post-administration of THC, bears the correct temporal relationship to suggest dynorphin A-(1–17) involvement in the induction of antinociception. The SR141716A attenuation of THC immunoreactive dynorphin A-(1–17) enhancement, suggests the effect is cannabinoid CB_1 receptor-mediated. Furthermore, the attenuation of THC-, anandamide- and CP55,940-induced tail-flick latency by SR141716A supports the contention that each of these compounds are acting via the cannabinoid CB_1 receptor system. Thirty minutes post-administration THC, a nor-BNI-sensitive increase in tail-flick latency

was observed in spite of a decrease in immunoreactive dynorphin A-(1–17) concentration. We interpret these apparent contradictory effects as being indicative of dynorphin A-(1–17) metabolism to some non-immunoreactive, but pharmacologically active entities which act via the κ-opioid receptor. Such metabolic activity has been described in various models (Nylander et al., 1995; Chou et al., 1996; Muller et al., 1997). Two dynorphin A-(1–17) metabolites, dynorphin A-(1–13) and dynorphin A-(1–8), fit such a profile. Both are non-immunoreactive metabolic products of dynorphin A-(1–17), when the dynorphin A-(1–17) radioimmunoassay kits from Peninsula Laboratories are employed. Either peptide is capable of increasing tail-flick latency through the κ-opioid receptor system (Rady et al., 1991). Pugh et al. (1996) and Welch (1997) demonstrated that dynorphin A-(1–13) and dynorphin A-(1–8) increased tail-flick latency (i.t.) in the mouse.

The demonstration of CI-977-induced antinociception and its subsequent antagonism by nor-BNI is crucial. This experiment established the existence of a κ-opioid receptor system in the spinal cord through which dynorphin peptides might increase tail-flick latency. It also confirmed the dose of nor-BNI employed as capable of attenuating the activity of a potent and selective κ-opioid receptor agonist. The attenuation of THC-induced spinal antinociception 10- and 30 min post-administration by nor-BNI indicates that a significant portion of THC-induced tail-flick latency is derived via some κ-opioid contribution. Moreover, the attenuation of THC-induced antinociception, 30 min post-administration, supports our contention that some non-immunoreactive κ-opioid receptor agonists are present during this time period.

Anandamide-induced antinociception is mediated by a mechanism which appears to differ from THC in terms of κ-opioid receptor involvement (Smith et al., 1994; Welch, 1997). Unlike THC, anandamide does not significantly increase immunoreactive dynorphin A-(1–17) concentration nor is its ability to increase tail-flick latency nor-BNI-sensitive. However, the effect of anandamide at 10 min indicates a trend toward an increase in dynorphin A-(1–17). The role of the dynorphin released is not known, but given the lack of a nor-BNI block of anandamide-induced antinociception, it is unlikely to be a critical component in its antinociceptive effects. Similar conclusions were noted in the development of tolerance to anandamide (Welch, 1997). However, the attenuation of anandamide-induced tail-flick latency by SR141716A indicates that anandamide-induced antinociception, like that of THC and CP55,940, is mediated via the cannabinoid CB_1 receptor. Such diversity of effects, between anandamide and THC, have been reported in other models (Smith et al., 1994).

In the rat model, CP55,940-induced antinociception occurs via some mechanism independent of dynorphin A-(1–17) involvement. CP55,940 increases tail-flick latency, but fails to increase spinal dynorphin A-(1–17) concentration. Nor-BNI does not attenuate CP55,940 induced antinociception, further implicating a mechanism independent of dynorphin A-(1–17). These observations contrast with previous findings in the mouse which revealed a nor-BNI attenuation of CP55,940-induced tail-flick latency (Welch, 1994). Such divergent findings are particularly interesting as recent studies have demonstrated a CP55,940 increased immunoreactive dynorphin B peptides (Pugh et al., 1997). In light of the data concerning dynorphin B, the mechanism by which CP55,940-induced antinociception and potential dynorphin B involvement remains complex and in need of further investigation.

It is unlikely that the observed effects are the result of neurotoxicity. The antinociceptive effects of dynorphins (i.t.) have been observed at doses devoid of overt toxicity (Pugh et al., 1996; Welch, 1997). Perhaps the most convincing evidence that the observed effects are receptor-mediated vs. some form of neurotoxicity, is the attenuation of tail-latency by SR141716A and nor-BNI. The selective nature of these antagonists demonstrates that the effects are mediated by selective receptor-ligand interactions. Moreover, the i.t. administration of vehicle fails to increase tail-flick latency beyond that of naive animals. If the results represented neuronal damage or toxicity, a significant increase in tail-latency would be expected in vehicle-treated subjects. It is also unlikely that increases in tail-flick latency are the result of neuronal damage sustained during catheter implantation as only animals displaying normal basal responses post-catheterization were included in the study.

Of particular interest is the diversity of effects observed between the test cannabinoids. CP55,940 increases spinal dynorphin B concentration in rats, but not dynorphin A-(1–17), (Pugh et al., 1997) and its antinociceptive activity is not nor-BNI sensitive. Anandamide, does not appear to have significant interaction with κ-opioid systems and also fails to significantly enhance the dynorphin A-(1–17) concentration. THC enhances dynorphin A-(1–17) concentration during only the onset of antinociception, yet maintains nor-BNI-sensitive antinociceptive activity 30 min post-administration. Hence, three cannabinoids, representing three different classes, induce antinociceptive activity via the cannabinoid receptor, yet differentially modulate dynorphinergic systems. These differences may reflect differences in the interactions of cannabinoids with the cannabinoid CB_1 receptor or activities of functional subtypes of the cannabinoid CB_1 receptor in the spinal cord. Receptor–ligand binding studies have produced evidence suggesting the existence of cannabinoid CB_1 receptor subtypes (Thomas et al., 1997). The newly described cannabinoid CB_2 receptor antagonist, SR144528 (Rinaldi-Carmona et al., 1998), will be of great help in elucidating cannabinoid receptor subtypes.

From these data, it is hypothesized that THC-induced antinociception is initiated by an increase in dynorphin A-(1–17) concentration. In this model, THC, administered i.t., interacts with a cannabinoid CB_1 receptor subtype in

the outer laminae of the spinal cord, through which it demonstrates greater efficacy for inducing an enhance of spinal dynorphin A-(1–17) peptide release than CP55,940 or anandamide. These dynorphin A-(1–17) peptides initiate THC antinociception via the κ-opioid receptor. Dynorphin A-(1–17) is metabolized, by various endopeptidases present in cerebrospinal fluid, to smaller active fragments such as dynorphin A-(1–13) and dynorphin A-(1–8) as described by Dixon and Traynor (1990). These active dynorphin fragments are hypothesized to be responsible for the nor-BNI-sensitive antinociception seen in the absence of an elevated dynorphin A-(1–17) concentration 30 min post-administration.

Although the pharmacological data presented would seem support the hypothesized metabolism of dynorphin A-(1–17) to non-immunoreactive fragments, direct evidence of such was desired. Additional experiments were performed to further investigate the apparent dissociation of spinal dynorphin A-(1–17) concentration and tail-flick latency observed 30 min post-administration of THC. As dynorphin A-(1–8) is one of the metabolites of dynorphin A-(1–17), it was hypothesized that it may act during the time frame in question, 30 min post-administration. An attempt was made to assess its concentration. In addition to dynorphin A-(1–8), its metabolite, leucine–enkephalin, was also measured. The presence of leucine–enkephalin, particularly in the latter time period would support the hypothesized metabolism of dynorphin A-(1–17) peptides to smaller pharmacologically active fragments.

The temporal relationship, between increased dynorphin A-(1–8) concentration THC-induced tail flick latency, did not support our hypothesized dynorphin A-(1–8) contribution to spinal antinociception at time points beyond 10 min post-administration. The only increase of note, in spinal dynorphin A-(1–8) concentration, occurs 10 min post-administration of THC. Dynorphin A-(1–8) levels were not significantly increased, in comparison to vehicle treated animals, 30 min post-administration. In fact, dynorphin A-(1–8) concentrations remained constant throughout the 20-min period of evaluation following THC. It was the vehicle treated animals which displayed the greatest variability in immunoreactive dynorphin A-(1–8) concentration. Such a relationship suggests that dynorphin A-(1–8) is not involved in the antinociceptive activity observed beyond 10 min post-administration of THC. When these observations are considered in the context of the continued THC-induced antinociceptive activity, regardless of dynorphin A-(1–8) concentration, our hypothesized role of dynorphin A-(1–17) must be revisited.

Literature exists to support a mechanism by which the dynorphin A-(1–17) increase 10 min post-administration might be responsible for the antinociception observed throughout the time course of THC-induced antinociception. Zachariou and Goldstein (1997) demonstrated that release of substance P, a neuropeptide associated with nociceptive transmission in the spinothalamic tract (Nishiyama et al., 1995), following noxious stimuli could be attenuated by κ-opioid receptor simulation. This effect, induced via i.t. administration of dynorphin A-(1–8), continued for 20 min post-administration and was attenuated by nor-BNI (Zachariou and Goldstein, 1996). Thus, dynorphin A-(1–17) at enhanced concentrations via cannabinoid receptor activation, 10 min post-administration of THC, could elicit an antinociceptive effect by way of long-term (20 min) suppression of substance P release. The result of such dynorphinergic activity would be a THC-induced increase in tail-flick latency at time points beyond 10 min post-administration, regardless of dynorphin A concentration, yet sensitive to nor-BNI attenuation.

Alternatively, dynorphin A-(1–8) may in fact be one of several ligands acting through the κ-opioid system. Several studies, utilizing in vitro and ex vivo procedures, have identified metabolic pathways yielding varied dynorphin A metabolites, some active via the κ-opioid receptor system (Dixon and Traynor, 1990; Chou et al., 1996; Muller et al., 1997). The metabolism of dynorphin A-(1–17) into several κ-opioid receptor ligands might be expected to produce an enhancement of κ-opioid receptor stimulation akin to that expected if only one dynorphin A peptide were released in large quantity. If a mixture of kappa opioid, nor-BNI-sensitive metabolites accounts for the effects of THC at 30 min, it is unlikely that an increase in any specific metabolite could be measured. Such would explain the lack of an increase in dynorphin A-(1–8) or leucine–enkephalin concentration in the presence of a nor-BNI-sensitive antinociceptive effect of THC at 30 min.

While comparisons of peptide concentrations between collections obtained under varied conditions and time frames are valid, it is quite likely that local dynorphin concentrations vary greatly. The values presented are much like snapshots of peptide activity. Furthermore, the peptide measurements represent concentrations contained in a fraction of artificial cerebrospinal fluid collected during select time frames within the time course of THC-induced tail-flick latency. Using these techniques, it is difficult to gauge the exact peptide concentration at any specific spinal location. Thus, values for dynorphin are given as pg/ml of cerebrospinal fluid. It would be difficult, if not impossible, to determine the molarity of the spinal content of dynorphin. In addition, the total volume of spinal fluid in the rat has only been estimated. It is quite possible that dynorphin release is concentrated at local sites vs. the entire spinal cavity. Moreover, since the total volume of fluid in the subarachinoid space is clearly unknown, dynorphin A concentrations are representative of samples collected vs. actual spinal concentrations.

Our model of THC-induced spinal antinociception is as follows. Acute administration of THC into the subarachnoid cavity results in activation of cannabinoid receptors located in the substantia gelatinosa. Cannabinoid activation of these receptors results in an inhibition of tonically active inhibitory neurons responsible for modulating

dynorphinergic neurons. The resulting disinhibition of dynorphinergic neurons, located in the outer laminae of the spinal cord, leads to a release of dynorphin A peptides. We believe these peptides are responsible for the immunoreactive peptides observed in our perfusion of the spinal cavity. Dynorphin A-(1–17), its active metabolites or other κ-opioid receptor ligands interact with κ-opioid receptors located in lamina II to modulate the release of neuropeptides associated with nociceptive transmission such as substance P (Besse et al., 1990) (Fig. 11). The suppression of substance P (or that of another neuropeptide) retards amplification of the noxious transmits in the ascending pathway and diminishes the intensity of the noxious stimulus perceived. Such a mechanism would account for the attenuation of THC-induced antinociception and increased immunoreactive dynorphin A-(1–17) concentration by SR141716A, but attenuation of antinociception only by the κ-opioid antagonist nor-BNI.

This mechanism satisfies the requirements necessary for a mechanism THC-induced antinociception which includes dynorphin peptide and κ-opioid receptor system involvement. It is a model subject to attenuation by pertussis toxin as κ-opioid receptors and cannabinoid CB_1 receptor are mediated via G-protein coupled responses. The involvement of cannabinoid receptors prior to dynorphin A modulation would account for the ability of SR141716A to attenuate cannabinoid receptor-mediated activity. Similarly, the positioning of the κ-opioid receptors post-synaptically to dynorphinergic neurons would explain the ability of κ-opioid antagonists to attenuate antinociception, but not affect spinal dynorphin levels. Moreover, this model is advantageous in that it provides for fine and rapid control of the ascending limb of the pain pathway. The presence of tonically acting interneurons, allows for fine control of the nociceptive threshold in response to changing environmental conditions and stimuli. It is supported by the findings of Romero et al. (1995) who found that cannabinoid stimulation of receptors in GABAergic neurons of the striatum and substantia nigra inhibit GABA reuptake. It also provides for the attenuation of dynorphin release and THC-induced antinociception by SR141716A, but antinociception only by nor-BNI.

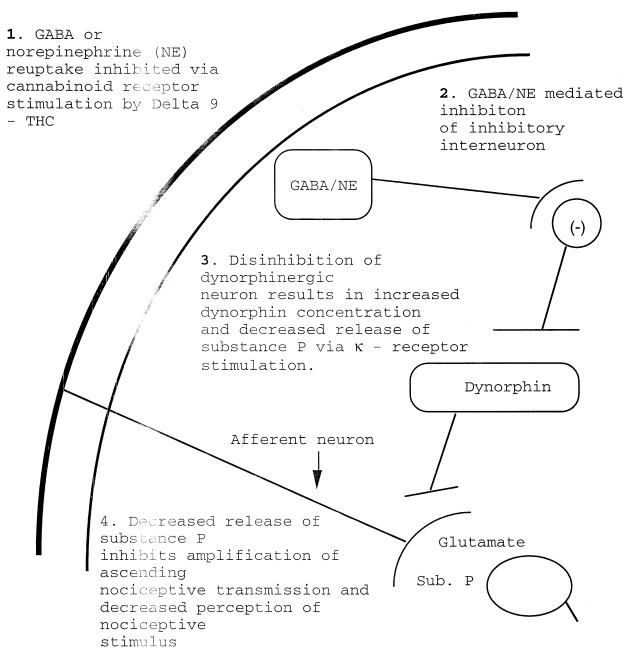

Fig. 11. Hypothesized model of Δ^9-THC-induced spinal antinociception. Δ^9-THC stimulation of cannabinoid receptors located on the synaptic terminal of a descending GABAergic neurons inhibits GABA reuptake. The increased presence of GABA results in the inhibition of a tonically active inhibitory interneuron, via GABA receptor stimulation, and disinhibition of a dynorphinergic neuron. The resulting increase in dynorphin peptides leads to a κ-opioid-receptor-mediated inhibition of substance P release. Such activity would diminish the amplification of the nociceptive stimulus and its perception. An independent noradrenergic component may also exist, in which, Δ^9-THC inhibits the reuptake or release of norepinephrine. The increased presence of norepinephrine results in stimulation of α_2-adrenergic receptors located in an inhibitory interneuron and subsequent disinhibition of the dynorphinergic neuron. The end result is a κ-receptor-mediated attenuation of substance P release.

This model depicts an entirely spinal mechanism of antinociception. Smith and Martin (1992) found that acute i.t. administration of cannabinoids results in supraspinal concentrations incapable of inducing antinociception. Thus, the antinociception induced by i.t. administration is likely a spinal system as is our rat model. Further studies of cannabinoid receptor-mediated dynorphin release and metabolism, as well as substance P release, are clearly indicated to further clarify the pathways distal to the initial dynorphin release.

The ability of THC to modulate the endogenous opioid system may prove to be a very significant discovery. The immediate benefits of elucidating the full scope of the interactions will likely come in the management of pain, particularly chronic pain. THC, in comparison to the morphine derivatives, has a greater therapeutic range. Two of the greatest problems concerning opioid use are the rapid development of tolerance and potential for overdose. These studies may lead to new techniques by which manipulation of the endogenous opioid system may be used to supplement exogenous opioid agents which would result in decreased dosage of opioids and a resulting decreased risk of toxicity and manageable tolerance development. Such would be of great benefit to persons suffering of chronic pain. Due to repeated opioid administration, tolerance development among these persons is always of concern.

Acknowledgements

This work was supported by the National Institute of Drug Abuse Grant Nos. K02 DA00186, T32DA07027, DA03672 and DA05274.

References

Basbaum, A.I., Fields, H.L., 1984. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu. Rev. Neurosci. 7, 309–388.

Besse, D., Lombard, M.C., Zajac, J.M., Roques, B.P., Besson, J.M., 1990. Pre- and postsynaptic distribution of μ, δ and κ opioid receptors in the superficial layers of the cervical dorsal horn of the spinal cord. Brain Res. 521, 15–22.

Chavkin, C., James, I., Goldstein, A., 1982. Dynorphin is a specific endogenous ligand for the kappa opioid receptor. Science 215, 413–415.

Chou, J.Z., Chait, B.T., Wang, R., Kreek, M.J., 1996. Differential biotransformation of dynorphin A (1–17) and dynorphin A (1–13) peptides in human blood, ex vivo. Peptides 17 (6), 983–990.

Collins, D.R., Pertwee, R.G., Davies, S.N., 1995. Prevention by the cannabinoid antagonist, SR 141716A, of cannabinoid mediated blockade of long term potentiation in the rat hippocampal slice. Br. J. Pharmacol. 115, 869–870.

Compton, D.R., Little, P.J., Martin, B.R., Gilma, J.W., Saha, J.K., Jorapur, V.S., Sardi, H.P., Razdan, R.K., 1990. Synthesis and pharmacological evaluation of amino, azido, and nitrogen mustard analogues of 10-cannabidiol and 11- or 12-substituted Δ^8-tetrahydrocannabinol. J. Med. Chem. 33, 1437–1443.

Compton, D.R., Johnson, M.R., Melvin, L.S., Martin, B.R., 1991. Pharmaceutical profile of a series of bicyclic cannabinoid analogs: classification as cannabimimetic agents. J. Pharmacol. Exp. Ther. 260, 201–209.

Corbett, A.D., Patterson, S.J., McKnight, A.T., Morgan, J., Kosterlitz, H.W., 1982. Dynorphin (1–8) and dynorphin (1–9) are ligands for the kappa subtype of opiate receptors. Nature 299, 79–81.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Devane, W.A., Dysarz, I., Johnson, M.R., Melvin, L.S., Howlett, A.C., 1988. Determination and characterization of a cannabinoid receptor in rat brain. Mol. Pharmacol. 34, 605–613.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Dewey, W.L., 1986. Cannabinoid pharmacology. Pharmacol. Rev. 38, 151–175.

Dewey, W.L., Peng, T., Harris, L.S., 1970. The effect of 1-*trans*-Δ^9 tetrahydrocannabinol on the hypothalamo-hypophyseal–adrenal axis of rats. Eur. J. Pharmacol. 12, 382–384.

Dixon, D.M., Traynor, J.R., 1990. Formation of [Leu^5]enkephalin from dynorphin A (1–8) by rat central nervous tissue in vitro. J. Neurochem. 54, 1379–1385.

Dunnett, C.W., 1955. A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50, 1096–1121.

Felder, C.C., Briley, E.M., Axelrod, J., Simpson, J.T., Mackie, K., Devane, W.A., 1993. Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor mediated signal transduction. Cell Biol. 90, 7656–7660.

Fride, E., Mechoulam, R., 1993. Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. Eur. J. Pharmacol. 231, 313–314.

Fujimoto, J.M., Arts, K.S., Rady, J.J., Tseng, L.F., 1990. Spinal dynorphin A (1–17). Possible mediator of antianalgesic action. Neuropharmacology 29, 609–617.

Harris, L.S., Pierson, A.K., 1990. Some narcotic antagonists in the benzomorphan series. J. Pharmacol. Exp. Ther. 143, 141–148.

Hayes, A.G., Birch, P.J., Hayward, N.J., Sheehan, M.J., Rofers, H., Tyers, M.B., Judd, D.B., Scopes, D.I.C., Naylor, A., 1990. A series of novel, highly potent and selective agonists for the κ-opioid receptor. Br. J. Pharmacol. 101, 944–948.

Horan, P., Taylor, J., Yamamura, H.I., Porreca, F., 1992. Extremely long lasting antagonistic actions of nor-binaltorphimine (nor-BNI) in the mouse tail-flick test. J. Pharmacol. Exp. Ther. 260, 1237–1243.

Howlett, A.C., 1984. Inhibition of neuroblastoma adenylate cyclase by cannabinoid and nantradol compounds. Life Sci. 35, 1803–1810.

Howlett, A.C., Flemming, R.M., 1984. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. Mol. Pharmacol. 26, 532–538.

Howlett, A.C., Qualy, J.M., Khachatrian, L.L., 1986. Involvement of G_i in the inhibition of adenylate cyclase by cannabimimetic drugs. Mol. Pharmacol. 29, 307–313.

Howlett, A.C., Johnson, M.R., Melvin, L.S., Milne, G.M., 1988. Nonclassical cannabinoid analgesics inhibit adenylate cyclase: development of a cannabinoid receptor model. Mol. Pharmacol. 33, 297–302.

Howlett, A.C., Evans, D.M., Houston, D.B., 1992. The cannabinoid receptor. In: Murphy, L., Burke, A. (Eds.), Neurobiology and Neurophysiology, pp. 35–72.

Hunter, J.C., Leighton, G.E., Meecham, K.G., Boyle, S.J., Horwell, D.C., Rees, D.C., Hughes, J., 1990. CI-977, a novel and selective agonist for the κ-opioid receptor. Br. J. Pharmacol. 101, 183–189.

Lichtman, A.H., Martin, B.R., 1991. Cannabinoid induced antinociception is mediated by spinal α_2 noradrenergic mechanism. Brain Res. 559 (2), 309–314.

Martin, B.R., Compton, D.R., Thomas, B.F., Prescott, W.R., Little, P.J., Razdan, R.K., Johnson, M.R., Melvin, L.S., Mechoulam, R., Ward, S.J., 1991. Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. Pharmacol. Biochem. Behav. 40 (3), 471–478.

Matsuda, L.A., Lolait, S.J., Bowstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

Melvin, L.S., Milne, G.M., Johnson, M.R., Subramaniam, B., Wilken, G.H., Howlett, A.C., 1993. Structure–activity relationships for cannabinoid receptor binding and analgesic activity: studies of bicyclic cannabinoid analogs. Mol. Pharmacol. 44, 1008–1015.

Morris, B.J., Herz, A., 1989. Simultaneous down-regulation of κ binding sites and up regulation of μ binding sites following chronic agonist/antagonist treatment. Adv. Biol. Sci. 75, 691–694.

Muller, S., Grand, B.L., Huchaus, G., 1997. Metabolism of dynorphin A 1–13 in human cerebrospinal fluid. Neurochem. Res. 21 (10), 1213–1219.

Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365, 61–65.

Nishiyama, K., Kwak, S., Murayama, S., Kanazawa, I., 1995. Substance P is a possible neurotransmitter in the rat spinothalamic tract. Neurosci. Res. 12, 261–266.

Nylander, I., Tan-No, K., Winter, A., Silberring, J., 1995. Processing of prodynorphin-derived peptides in striatal extracts, identification by electrospray ionization mass spectrometry linked to size-exclusion chromatography. Life Sci. 57 (2), 123–129.

Pertwee, R., Stevenson, L., Griffin, G., 1993. Cross-tolerance between Δ^9-THC and the cannabimimetic agents, CP55,940, WIN 55,212 and anandamide. Br. J. Pharmacol. 110, 1483–1490.

Portoghese, P.S., Nagase, H., Lipkowski, A.W., Larson, D.L., Takemori, A.E., 1988. Binaltorphimine-related bivalent ligands and their k opioid receptor antagonist selectivity. J. Med. Chem. 31, 836–841.

Pugh, G.J., Smith, P., Dombrowski, D., Welch, S.P., 1996. The role of endogenous opioids in enhancing the antinociception produced by the combination of Δ^9-THC and morphine in the spinal cord. J. Pharmacol. Exp. Ther. 279, 608–616.

Pugh, G.J., Mason, D.J., Combs, V., Welch, S.P., 1997. Involvement of dynorphin B in the antinociceptive effects of CP55,940, in the spinal cord. J. Pharmacol. Exp. Ther. 281, 730–737.

Rady, J.J., Fujimoto, J.M., Tseng, L.F., 1991. Dynorphins other than dynorphin lack spinal antianalgesic activity but do act on dynorphin A (1–17) receptors. J. Pharmacol. Exp. Ther. 259, 1073–1079.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B.,

Congy, C., Martinez, S., Maruani, J., Neliat, G., Caput, D., Ferrara, P., Soubrie, P., Breliere, J.C., LeFur, G., 1994. SR141716A, a potent and selective antagonist of the cannabinoid receptor. Fed. Eur. Biochem. Soc. Lett. 350, 240–244.

Rinaldi-Carmona, M., Barth, F., Millan, J., Deroq, J.M., Casellas, P., Congy, C., Oustric, D., Sarran, M., Bouaboula, M., Portier, M., Shire, D., Breliere, J.C., Le Fur, G.L., 1998. SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. J. Pharmacol. Exp. Ther. 284 (2), 644–650.

Romero, J., Garcia, L., Fernandez-Ruiz, J.J., Cebeira, M., Ramos, J.A., 1995. Changes in rat brain binding sites after acute or chronic exposure to their endogenous agonist, anandamide or Δ^9-tetrahydrocannabinol. Pharmacol. Biochem. Behav. 51, 731–737.

Shire, D., Carillon, C., Kaghad, M., Calandra, B., Rinaldi-Carmona, M., LeFur, G., Caput, D., Ferrara, P., 1995. An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. J. Biol. Chem. 270, 3726–3731.

Smith, P.B., Martin, B.R., 1992. Spinal mechanisms of Δ^9-tetrahydrocannabinol-induced analgesia. Brain Res. 578, 8.

Smith, P.B., Compton, D.R., Welch, S.P., Razdan, R.K., Mechoulam, R., Martin, B.R., 1994. The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. J. Pharmacol. Exp. Ther. 270, 219–227.

Thomas, B.F., Gilliam, A.F., Burch, D.F., Roche, M.J., Seltzman, H.H., 1997. Comparative binding analysis of cannabinoid agonists and antagonists: evidence supporting neuronal cannabinoid receptor subtypes. Int. Cann. Res. Soc. Symp. Cann.

Tseng, L.F., 1989. Intracerebroventricular administration of beta-endorphin releases immunoreactive methionine enkephalin from the spinal cord of cats, guinea pigs and mice. Neuropharmacology 28, 1333–1339.

Turkanis, S.A., Karler, R., 1988. Changes in neurotransmitter release at a neuromuscular junction of the lobster caused by cannabinoids. Neuropharmacology 22 (7), 737–742.

Vogel, Z., Barg, J., Levy, R., Saya, D., Heldman, E., Mechoulam, R., 1993. Anandamide, a brain endogenous compound, interacts specifically with cannabinoid receptors and inhibits adenylate cyclase. J. Neurochem. 61, 352–355.

Welch, S.P., 1994. Blockade of cannabinoid-induced antinociception by naloxone benzoylhydrazone (NalBZH). Pharmacol. Biochem. Behav. 49, 929–934.

Welch, S.P., 1997. Characterization of anandamide-induced tolerance: comparison to delta 9-THC-induced interactions with dynorphinergic systems. Drug Alcohol Depend. 45, 39–45.

Welch, S.P., Dunlow, L.D., Patrick, G.S., 1995. Characterization of anandamide and fluoroanandamide-induced antinociception and cross tolerance to Δ^9-THC-induced antinociception. J. Pharmacol. Exp. Ther. 273, 1235–1244.

Wiley, J.L., Barrett, R.L., Lowe, J., Balster, R.L., Martin, B.R., 1995a. Discriminative effects of CP 55,940 and structurally dissimilar cannabinoids in rats. Neuropharmacology 34, 669–676.

Wiley, J.L., Lowe, J.A., Balster, R.L., Martin, B.R., 1995b. Discriminative stimulus effect of Δ^9-tetrahydrocannabinol in rats and rhesus monkeys. J. Pharmacol. Exp. Ther. 275, 16.

Yaksh, T.L., 1981. The antinociceptive effect of intrathecally administered levonantradol and desacetyllevonantradol in the rat. J. Clin. Pharmacol. 21, 334340.

Yasuda, K., Raynor, K., Haeyoung, K., Breder, C.D., Takeda, J., Reisine, T., Bell, G.I., 1993. Cloning and functional comparison of k and d opioid receptors from mouse brain. Neurobiology 90, 67366740.

Zachariou, V., Goldstein, B.D., 1996. Kappa-opioid receptor modulation of the release of substance P in the dorsal horn. Brain Res. 706 (1), 80–88.

Zachariou, V., Goldstein, B.D., 1997. Dynorphin-(1–8) inhibits the release of substance P-like immunoreactivity in the spinal cord of rats following a noxious mechanical stimulus. Eur. J. Pharmacol. 323, 159–165.