ELSEVIER

Brain Research 857 (2000) 337–342

BRAIN RESEARCH INTERACTIVE

www.elsevier.com/locate/bres

Interactive report

Dynorphin B and spinal analgesia: induction of antinociception by the cannabinoids CP55,940, Δ^9-THC and anandamide [1]

Susan J. Houser [a], Micah Eads [b], James P. Embrey [a], Sandra P. Welch [b,*]

[a] *Department of Nurse Anesthesia, Virginia Commonwealth University, Richmond, VA 23298-0613, USA*
[b] *Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298-0613, USA*

Accepted 10 January 2000

Abstract

The endogenous opioid dynorphin B was evaluated for its role in cannabinoid-induced antinociception. Previous work in our laboratory has shown that the synthetic, bicyclic cannabinoid, CP55,940, induces the release of dynorphin B whilst the naturally occurring cannabinoid, Δ^9-tetrahydrocannabinol (Δ^9-THC), releases dynorphin A. The dynorphins contribute in part to the antinociceptive effects of both cannabinoids at the level of the spinal cord. The present study compares dynorphin B released from perfused rat spinal cord in response to acute administration of anandamide (AEA), Δ^9-THC and CP55,940 at two time points, 10 min and 30 min post administration, and attempts to correlate such release with antinociceptive effects of the drugs. Dynorphin B was collected from spinal perfusates of rats pretreated with Δ^9-THC, CP55,940 or AEA. The supernatant was lyophilized and the concentrations of dynorphin B were measured via radioimmunoassay. At a peak time of antinociception (10 min), CP55,940 and Δ^9-THC induced significant two-fold increases in the release of dynorphin B. AEA did not significantly release dynorphin B. Upon a 30-min pretreatment with the drugs, no significant dynorphin B release was observed, although antinociceptive effects persisted for CP55,940 and Δ^9-THC. Previous work indicates that Δ^9-THC releases dynorphin A while AEA releases no dynorphin A. This study confirms that although all three test drugs produced significant antinociception at 10 min, the endocannabinoid, AEA, does not induce antinociception via dynorphin release. Thus, our data indicate a distinct mechanism which underlies AEA-induced antinociception.

Keywords: Cannabinoid; Dynorphin A; Dynorphin B; Antinociception; Anandamide; Rat

1. Introduction

Opioid peptide families include the endorphins, the enkephalins and the dynorphins [1]. Degradation of prodynorphin yields many dynorphins among them dynorphin A and B [13] and several sizes of dynorphin A products. These range from the dynorphin A (1–17) fragment to the smaller dynorphin A (1–8) fragment [1]. Cleavage of dynorphin A (1–17), the endogenous ligand for the kappa receptor [3], to dynorphin A (1–8) and leu-enkephalin [13,20], is well documented. Dynorphin peptides exist in high concentrations in the substantia gelatinosa of the dorsal horn in the spinal cord [32].

Dynorphin involvement in dose- and test-dependent antinociception is established [2,30,34]. Dynorphin A and B produce antinociception [11,41]. Dynorphin B, also known as rimorphin, [13] is distinct from α/β-neoendorphins and dynorphin A in amino acid sequence. It is a leucine enkephalin-containing peptide possessing potent opioid activity [11,31].

Two cannabinoid (CB) receptors have been cloned. CB1 is located in the central nervous system [18]; CB2 is located predominantly on immune cells and peripheral tissues [19]. A splice variant of CB1 called CB1A has also been identified [25]. A CB1 antagonist, SR141716A, was described by Rinaldi-Carmona et al. [24]. Δ^9-THC and CP55,940 bind CB1 and CB2 receptors. Anandamide binds the CB1 receptor with 30 times greater affinity than it binds to the CB2 receptor [19].

Intravenously (i.v.) and intrathecally administered (i.t.) cannabinoids are effective antinociceptive agents [29,39,40]. The action of intrathecally administered cannabinoids in producing antinociception is predominately at the level of the spinal cord. However, the mechanism of action of cannabinoid-induced antinociception involves spinal and supraspinal mechanisms. A recent review article clearly delineates many interactions of the

* Corresponding author. E-mail: swelch@hsc.vcu.edu
[1] Published on the World Wide Web on 18 January 2000.

0006-8993/00/$ - see front matter
PII: S0006-8993(00)01981-8

cannabinoids with opioid systems [15]. Nor-binaltorphimine (nor-BNI), a kappa antagonist, and dynorphin antisera block Δ^9-THC-induced (i.t.) antinociception [29,35] which suggests the release of dynorphins as a part of the mechanism of action of cannabinoids [35]. Bidirectional cross-tolerance studies of Δ^9-THC and CP55,940 to dynorphins [29] further implicate dynorphins with cannabinoids in the production of antinociception. Δ^9-THC-induced antinociception requires dynorphin A release [16,17,23,35] and such release of dynorphin A has characteristics consistent with cannabinoid (CB1) receptor mediation [38]. Like dynorphin A, Δ^9-THC significantly enhances the antinociceptive effects of morphine [4,27,40].

CP55,940 is a potent bicyclic cannabinoid analog with a pharmacological profile similar to Δ^9-THC. CP55,940-induced antinociception is blocked by nor-BNI, but not by dynorphin A antisera. Dynorphin A is not released by CP55,940 [22]. We have demonstrated that CP55,940-induced antinociception involves the release of spinal dynorphin B [22]. Like dynorphin B, CP55,940 does not enhance morphine-induced antinociception.

Anandamide is an endogenous cannabinoid and the first putative ligand isolated for the cannabinoid receptor [6]. AEA has similarities and differences with classic cannabinoids [8,14]. AEA is not blocked by nor-BNI [28,37] or dynorphin antisera suggesting AEA works via a different mechanism than CP55,940 and Δ^9-THC. Most behavioral effects of AEA, including antinociception, are similar to Δ^9-THC [10,21,28,37]. However, in arthritic rats AEA-induced antinociception is not blocked by the cannabinoid (CB1) antagonist, SR141716A [26], while the effects of Δ^9-THC are blocked by SR141716A. ANA is fully cross-tolerant to Δ^9-THC [9] and CP55,940, but not to dynorphins [36].

CP55,940 releases dynorphin B [22], but not dynorphin A. We have recently reported that levonantradol, a synthetic cannabinoid, also releases dynorphin B [38]. The release of dynorphin B by Δ^9-THC and AEA had not been previously evaluated. Thus, the goal of this study was to evaluate the release of dynorphin B by various cannabinoids and the endocannabinoid, AEA. We hypothesized that AEA would not release dynorphin B, based upon our previous work with AEA/kappa opioid interactions.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) were individually housed at a temperature of 22°C. Animals were provided free access to food and water. An acclimation period of 8–10 h was provided in the laboratory prior to testing. Adequate food and water was provided during pretesting periods. Approval for all animal experiments was received from the Institutional Animal Care and Use Committee of the Medical College of Virginia/Virginia Commonwealth University.

2.2. Drugs

Δ^9-THC was obtained from the National Institute on Drug Abuse (Rockville, MA, USA). CP55,940 was obtained from Dr. Lawrence Melvin, Pfizer Central Research. AEA was obtained from Organix, Woburn, MA, USA. Dimethyl sulfoxide (DMSO) was used as a vehicle for Δ^9-THC, CP55,940 and anandamide. Rats were exposed to spinal cord perfusion of 100 μg/rat of CP55,940 or 300 μg/rat of Δ^9-THC or 100 μg/rat of anandamide or 20 μl DMSO vehicle. Doses were chosen based upon our extensive previous work which indicated that the above doses were close to the ED80 value for each drug. Time points were chosen based upon previous time-course studies performed in our laboratory.

2.3. Intrathecal administration of drugs and quantitation of dynorphin B peptide release

Spinal perfusion and collection of dynorphin peptide is as described by Tseng [33]. Rats weighing between 320–470 g were anesthetized with sodium barbital (375 mg/kg, intraperitoneally, i.p.). A separate (i.p.) injection of methyl atropine bromide (2 mg/kg) was given immediately after sodium barbital administration. Rats were placed in a quiet location with cages tilted approximately 20° in the head down position to facilitate drainage of oral secretions during the approximate 3 h anesthetizing period. Anesthetized rats were placed in stereotaxis after shaving a posterior area from the ears to the shoulders. A 3–4 cm midline incision was made cephalad to caudad to expose the atlanto-occipital membrane. Once identified, a small incision was made to expose the cisterna magna. Positive flow of cerebral spinal fluid (CSF) confirmed the location. A 13 cm, PE-10, polyethylene catheter was inserted 8 cm caudally, through the open cisterna magna into the subarachnoid space. The tip of the catheter was located at the sacral enlargement. The PE-10 catheter was prefilled with artificial cerebral spinal fluid (ACSF). ACSF was composed of (mM): Na^+, 125; K^+, 2.6; Mg^{2+} 0.9; Ca^{2+}, 1.3; Cl, 122.7; $NaHCO_3$, 21.0; sodium phosphate buffer, 2.4; bovine serum albumin (0.5 mg/ml); bacitracin (30 μg/ml) and 0.01% Triton X-100 (to prevent dynorphin sticking to the tubing) and bubbled with 95% oxygen and 5% carbon dioxide. Anesthetized and surgically cannulated animals were placed on a warming pad (37°C). An acclimation period of approximately 30 min was provided prior to drug administration. The incision was covered with a 2×2 gauze. Tail-flick latency was determined at the end of the acclimation period immediately prior to drug administration. A peristaltic pump was used for drug administration

and ACSF perfusion. Ten and 30 min time points were selected for this study based upon previous work in this laboratory. Following acclimation and exhibition of normal tail-flick response to noxious stimulation (2–4 s baseline latency to move the tail), test compounds were administered in a 20 μl volume, via the spinal catheter and peristaltic pumping, at a rate of 30 μl /min. The 10 and 30 min time points were used and tail flick latency determined at the completion of the time period.

Immediately following tail-flick testing, rapid ACSF perfusion was initiated. As ACSF was rapidly perfused by the peristaltic pump and the spinal catheter, into the subarachnoid space; CSF (1.5 ml aliquot) was aspirated from the open cisternal space. Spinal perfusates collected were boiled for 12 min at 99°C to destroy enzymatic activity. Collected fractions were centrifuged at 10 000 rpm for 10 min. The supernatant was frozen at −70°C for later lyophilization. All samples were lyophilized and analysis of dynorphin B determined via radioimmunoassay.

2.4. Antinociception

The tail-flick test developed by D'Amour and Smith [5] with modification by Dewey et al. [7] was used to determine antinociception. The tail-flick heat lamp apparatus was calibrated to provide sufficient intensity for a control reaction time of 2–4 s and a 10-s cutoff. Antinociception was quantified as the % maximum possible effect (%MPE) according to the method described by Harris and Pierson [12]:

$$\%\text{MPE} = (\text{test} - \text{control})/(10 - \text{control}) \times 100$$

The %MPE was calculated for each rat tested. Each treatment group had a minimum of 6 rats and the average %MPE was determined (± S.E.M), standard error of the mean) for each group.

2.5. Statistical analysis

Data regarding dynorphin peptide release and %MPE was analyzed using analysis of variance (ANOVA) and Student's *t*-test, unpaired.

3. Results

Fig. 1 indicates the antinociceptive effects of the cannabinoids at 10 and 30 min prior to testing. At 10 min, animals treated acutely with DMSO, Δ^9-THC, CP55,940 and AEA had %MPE ± S.E.M. values of 8 ± 4, 64 ± 18, 100 ± 0, and 65 ± 17, respectively. At 30 min, animals treated acutely with DMSO, Δ^9-THC, CP55,940 and AEA had %MPE ± S.E.M. values of 1 ± 0.7, 49 ± 11, 100 ± 0, and 18 ± 10, respectively. demonstrated tail-flick latency of 63 ± 18%MPE) compared to DMSO (8 ± 4%MPE).

In order to determine the role of dynorphin B in production of antinociception, dynorphin B release was quantified

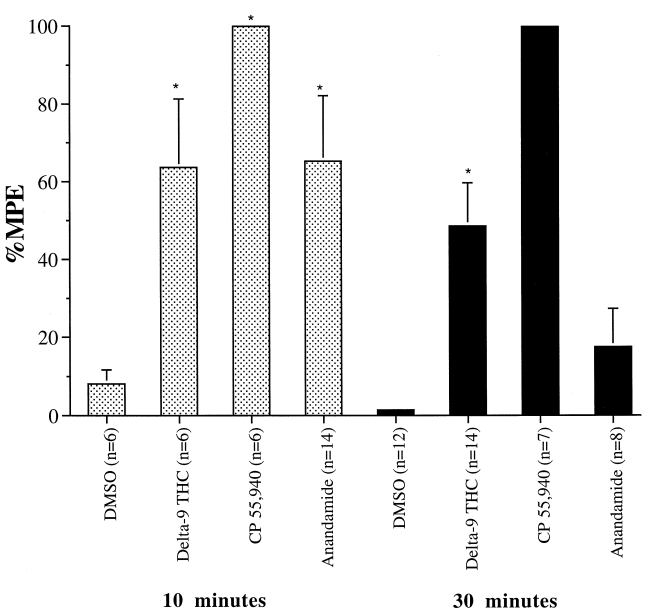

Fig. 1. Tail-flick response following the acute administration (i.t.) of the cannabinoids or DMSO. Drugs or vehicle were administered via spinal perfusion as described in the Materials and methods section. At least 6 rats were used per dose of drug or vehicle. The % MPE was quantitated for each rat and the average % MPE for a particular dosage group is presented as evaluated 10 and 30 min following drug administration. Significance levels were determined by ANOVA followed by unpaired Student's *t*-test. $^*p < 0.05$

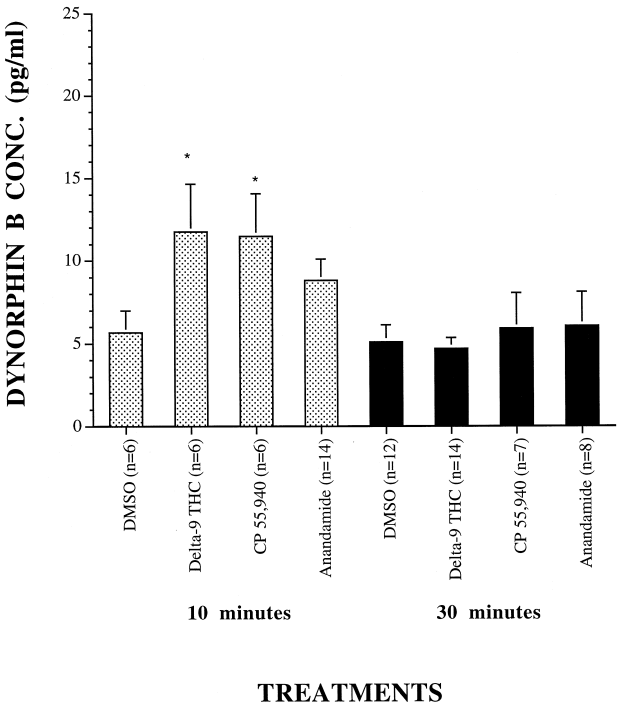

Fig. 2. Spinal dynorphin B concentrations following acute administration of intrathecal cannabinoids. Rats were exposed to spinal cord perfusion of 100 μg/rat of CP55,940 or 300 μg/rat of Δ^9-THC or 100 μg/rat of anandamide or 20 μl DMSO vehicle. Dynorphin B levels were determined at 10 and 30 min post administration of the drugs and were quantitated in pg/ml as described in the Materials and methods section. At least 6 rats were used per dose of drug or vehicle. Significance levels were determined by ANOVA followed by unpaired Student's *t*-test. $^* p < 0.05$

after administration cannabinoids. At 10 min, mean dynorphin B release in response to acute administration of DMSO, Δ^9-THC, CP55,940 and AEA was (5.7 ± 1.3 pg/ml, 11.8 ± 2.9 pg/ml, 11.5 ± 2.6 pg/ml, and 8.8 ± 1.3 pg/ml, respectively (Fig. 2). Compared to DMSO, the mean dynorphin B concentrations of Δ^9-THC and CP55,940 were significantly elevated by 2-fold. At 30 min, mean dynorphin B concentrations in response to acute administration of DMSO, Δ^9-THC, CP55,940 and AEA were (5.1 ± 1.0 pg/ml, 4.7 ± 0.6 pg/ml, 5.9 ± 2.2 pg/ml, and 6.1 ± 2.1 pg/ml, respectively (Fig. 2). None of the effects of the drugs on dynorphin at 30 min were significant. Thus, significant antinociception was evident following 30 min pretreatment with Δ^9-THC and CP55,940, but significant dynorphin B release was not observed at this time point.

4. Discussion

Findings from this study indicate that dynorphin B release is induced by Δ^9-THC and CP55-940. Our data on CP55,940 are consistent with previous data [22] regarding release of dynorphin B. However, this study provides new insight into release of dynorphin B by the cannabinoids, Δ^9-THC and AEA. Previous data indicates that Δ^9-THC stimulates the release of dynorphin A [16,17,23] which is critical for the enhancement of morphine by Δ^9-THC, as well as for Δ^9-THC-induced antinociception. Additionally, Δ^9-THC is cross-tolerant to dynorphin A and not dynorphin B [22] suggesting that antinociception induced by Δ^9-THC is not dependent upon the release of dynorphin B. However, based upon the results of this study, a dynorphin B component in Δ^9-THC-induced antinociception cannot be ruled out. Clearly, the release of dynorphin B and CP55,940- and Δ^9-THC-induced antinociception are temporally correlated at the 10 min point. It is not surprising that dynorphin B release was not observed at 30 min. Similar findings have been shown for dynorphin A release [17]. In both reports, CP55,940 and Δ^9-THC-induced antinociception was present at 30 min after administration, but significant dynorphin release was absent. These data are consistent with one of several possible scenarios. The dynorphin released could be degraded by 30 min even though inhibitors of degradation are included in the buffer. However, if this were the case, one might expect to see increased levels of leucine enkephalin at the 30 min point. Such an increase has not been observed [17]. A second and more likely possibility is that the dynorphin release is a critical mediator in a cascade of events leading to antinociception. Such a cascade of events was described by Mason et al. [16,17]. It is clear from previous data discussed in the Introduction that a kappa opioid receptor activation is critical for Δ^9-THC and CP55,940 antinociception, yet neither cannabinoid binds to kappa opioid receptors. Since CP55,940 fails to release dynorphin A, one might hypothesize that the release of dynorphin B is crucial to CP55,940-induced antinociception. Δ^9-THC releases both dynorphins A and B, but the lack of cross-tolerance of dynorphin B to Δ^9-THC [23] argues against dynorphin B being a significant component to the Δ^9-THC-induced antinociception. However, the fact that CP55,940 fails to release dynorphin A [22] is a significant finding. Such a difference between the cannabinoids may indicate that Δ^9-THC and CP55,940 have two distinct binding sites. Alternatively, it cannot be ruled out that binding of these diverse cannabinoids may produce a conformational change in the receptor resulting in the differential release of only dynorphin B by CP55,940. Two separate second messenger systems would therefore seem to have to be involved in the production of antinociception by Δ^9-THC versus CP55,940. However since Δ^9-THC and CP55,940 are cross-tolerant to each other [29], it is unlikely that these cannabinoids, which both couple to Gi proteins [8], would stimulate two diverse second messenger systems. It is our hypothesis that there exists a subtype of the CB1 receptor for which CP55,940 has higher affinity and is coupled by some yet unknown mechanism specifically to dynorphin B release. Our data indicates that another subtype of CB1 receptor may be coupled to the release of dynorphin A.

Δ^9-THC appears to have no selectivity for either subtype in that it releases both dynorphin A and dynorphin B. Another significant point in the dynorphin release process is that the release of dynorphin appears to be receptor-mediated. We have shown that dynorphin A (1–17) and dynorphin B are released by the potent synthetic cannabinoid, levonantradol, currently with the production of antinociceptive effects. The antinociceptively inactive stereoisomer of levonantradol, dextronantradol, fails to release any endogenous opioid peptide. Thus, the release of dynorphin is stereoselective [38].

Previous work has shown AEA to be uninvolved in dynorphinergic systems [36]. Additionally, AEA is fully cross-tolerant to Δ^9-THC and CP55,940, but not dynorphins [36], suggesting AEA and Δ^9-THC produce antinociception by different mechanisms. We observe a trend toward an increase in dynorphin B by AEA as shown in Fig. 1. Due to this trend, we increased the number of animals tested to an $'N' = 14$ in order to decrease the variability in the statistical measures. In spite of the increased number of rats tested, the effects of AEA on dynorphin B release were not significant. These data are similar to those observed previously for dynorphin A release following AEA administration. The CB1 receptor is activated in AEA-induced antinociception as evidenced by the attenuation by SR141716A of ANA-induced antinociception [24,36]. However, ANA-induced antinociception, unlike the other cannabinoids tested is not blocked by nor-BNI. Taken together with the dynorphin release data, it appears that AEA-induced antinociception is devoid of significant dynorphinergic components. Thus, we are presented with three cannabinoids which have diverse mechanisms of action: those such as Δ^9-THC and CP55,940 which are blocked by nor-BNI, but differ in dynorphin release and subsequent enhancement of morphine-induced antinociception, versus AEA which is not blocked by nor-BNI, and fails to enhance morphine and fails to significantly release any dynorphin. Such a finding brings up the question of a binding site for AEA. How is such a site different from that of the other cannabinoids? Such questions remain to be answered. In addition, what kappa component differentiates those cannabinoids that enhance morphine from those that fail to enhance morphine-induced antinociception? The cannabinoids may differ in the release of other kappa opioids or other non-kappa opioid peptides. Such questions remain to be answered. Clinically, the relevance of determining the underlying mechanism for the enhancement of morphine is critical to the selection of those cannabinoids which might have therapeutic usefulness in the enhancement of opioid-induced antinociception.

In summary, our findings suggest dynorphin B may play a role in cannabinoid induced antinociception. Induction of dynorphin B release by Δ^9-THC and CP55,940, but not AEA, in light of other observed differences between the drugs could implicate cannabinoid binding to multiple CB1 receptor subtypes. The complexity of dynorphin A and B interacting to enhance opioid-induced antinociception is worthy of further exploration.

Acknowledgements

This work is supported by NIDA grant numbers DA05274, DA03672, and KO2-DA00186.

References

[1] H. Akil, S.J. Watson, E. Young, M.E. Lewis, H. Khachaturian, J.M. Walker, Endogenous opioids: Biology and function, Annu. Rev. Neurosci. 7 (1984) 223–255.
[2] E.R. Butelman, C.P. France, J.H. Woods, Agonist and antagonist effects of dynorphin A (1–13) in a thermal antinociception assay in rhesus monkeys, J. Pharmacol. Exp. Ther. 275 (1995) 374–380.
[3] C. Chavkin, I. James, A. Goldstein, Dynorphin is a specific endogenous ligand for the kappa opioid receptor, Science. 215 (1982) 413–415.
[4] D.L. Cichewicz, Z. Martin, F.L. Smith, S.P. Welch, Enhancement of opioid potency with inactive oral administration of Δ9-THC: Dose-response analysis and receptor characterization, J. Pharmacol. Exp. Ther. 289 (1999) 859–867.
[5] F.E. D'Amour, D.L. Smith, A method for determining loss of pain sensation, J. Pharm. Exp. Ther. 72 (1941) 74–79.
[6] W.A. Devane, L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, Isolation and structure of a brain constituent that binds to the cannabinoid receptor, Science 258 (1992) 1946–1949.
[7] W.L. Dewey, T. Peng, L.S. Harris, The effect of 1-trans-Δ^9 tetrahydrocannabinol on the hypothalamo-hypophyseal-adrenal axis of rats, Eur. J. Pharmacol. 12 (1970) 382–384.
[8] C.C. Felder, E.M. Briley, J. Axelrod, J.T. Simpson, K. Mackie, W.A. Devane, Anandamide, and endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction, Proc. Natl. Acad. Sci. USA 90 (1993) 7656–7660.
[9] E. Fride, J. Barg, R. Levy, D. Saya, E. Heldman, R. Mechoulam, Z. Vogel, Low doses of anandamides inhibit pharmacological effects of Δ^9-THC, J. Pharmacol. Exp. Ther. 272 (1995) 699–707.
[10] E. Fride, R. Mechoulam, Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent, Eur. J. Pharmacol. 231 (1993) 313–314.
[11] J.S. Han, C.W. Xie, Dynorphin: Potent analgesic effect in spinal cord of the rat, Life Sci. 31 (1982) 1781–1784.
[12] L.S. Harris, A.K. Pierson, Some narcotic antagonists in the benzomorphan series, J. Pharm. Exp. Ther. 143 (1990) 141–148.
[13] V. Hollt, Opioid peptide processing and receptor selectivity, Annu. Rev. Pharmacol. Tox. 26 (1986) 59–77.
[14] K. Mackie, W.A. Devane, B. Hille, Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells, Mol. Pharmacol. 44 (1993) 498–503.
[15] J. Manzanares, J. Corchero, J. Romero, J.J. Fernandez-Ruiz, J.A. Ramos, J.A. Fuentes, Pharmacological and biochemical interactions between opioids and cannabinoids, Trends Pharmacol. Sci. 20 (1999) 287–294.
[16] D.L. Mason, S.P. Welch, A diminution of Δ9-tetrahydrocannabinol modulation of dynorphin A-(1–17) in conjunction with tolerance development, Eur. J. Pharmacol. (1999) in press.
[17] D.J. Mason, S.P. Welch, Cannabinoid modulation of dynorphin A:

Correlation to cannabinoid-induced antinociception, Eur. J. Pharmacol. (1999) in press.
[18] L.A. Matsuda, S.J. Lolait, M.J. Bowstein, A.C. Young, T.I. Bonner, Structure of a cannabinoid receptor and functional expression of the cloned cDNA, Nature 346 (1990) 561–564.
[19] S. Munro, K.L. Thomas, M. Abu-Shaar, Molecular characterization of a peripheral receptor for cannabinoids, Nature 365 (1993) 61–65.
[20] I. Nylander, K. Tan-No, A. Winter, J. Silsberring, Processing of prodynorphin-derived peptides in striatal extracts. Identification by electrospray ionization mass spectrometry linked to size-exclusion chromatography, Life Sci. 57 (1995) 123–129.
[21] R. Pertwee, G. Griffin, L. Hanus, R. Mechoulam, Effects of two fatty acid ethanolamides on mouse vasa deferentia, Eur. J. Pharmacol. 259 (1994) 115–120.
[22] G.J. Pugh, D.J. Mason, V. Combs, S.P. Welch, Involvement of dynorphin B in the antinociceptive effects of CP55,940, in the spinal cord, J. Pharm. Exp. Ther. 281 (1997) 730–737.
[23] G.J. Pugh, P. Smith, D. Dombrowski, S.P. Welch, The role of endogenous opioids in enhancing the antinociception produced by the combination of D^9-THC and morphine in the spinal cord, J. Pharm. Exp. Ther. 279 (1996) 608–616.
[24] M. Rinaldi-Carmona, F. Barth, M. Heaulme, D. Shire, B. Calandra, C. Congy, S. Martinez, J. Maruani, G. Neliat, D. Caput, P. Ferrara, P. Soubrie, J.C. Breliere, G. LeFur, SR141716A, a potent and selective antagonist of the cannabinoid receptor, Fed. Eur. Biochem. Soc. Lett. 350 (1994) 240–244.
[25] D. Shire, C. Carillon, M. Kaghad, B. Calandra, M. Rinaldi-Carmona, G. LeFur, D. Caput, P. Ferrara, An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing, J. Biol. Chem. 270 (1995) 3726–3731.
[26] F.L. Smith, K. Fujimori, J. Lowe, S.P. Welch, Characterization of Δ9-tetrahydrocannabinol and anandamide antinociception in non-arthritic and arthritic rats, Pharmacol. Biochem. Behav. 60 (1998) 183–191.
[27] F.L. Smith, D.L. Stevens, Z.L. Martin, S.P. Welch, The enhancement of morphine antinociception by Δ9-tetrahydrocannabinol, Pharmacol. Biochem. Behav. 60 (1998) 559–566.
[28] P.B. Smith, D.R. Compton, S.P. Welch, R.K. Razdan, R. Mechoulam, B.R. Martin, The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice, J. Pharm. Exp. Ther. 270 (1994) 219–227.
[29] P.B. Smith, S.P. Welch, B.R. Martin, Interactions between Δ^9 tetrahydrocannabinol and kappa opioids in mice J, Pharmacol. Exp. Ther. 268 (1994) 1382–1387.
[30] Z.H. Song, A.E. Takemori, Involvement of spinal kappa opioid receptors in the antagonistic effect of dynorphins on morphine antinociception, Life Sci. 48 (1991) 1447–1453.
[31] S. Spampinato, P. Romualdi, S. Candeletti, E. Cavicchini, S. Ferri, Distinguishable effects of intrathecal dynorphins, somatostatin, neurotensin, and s-calcitonin nociception and motor function in the rat, Pain 35 (1988) 94–104.
[32] C.W. Stevens, T.L. Yaksh, Dynorphin A and related peptides administered intrathecally in the rat: A search for putative kappa receptor activity, J. Pharmacol. Exp. Ther. 238 (1986) 833–838.
[33] L.F. Tseng, Intracerebroventricular administration of beta-endorphin releases immunoreactive methionine enkephalin from the spinal cord of cats, guinea pigs and mice, Neuropharmacology 28 (1989) 1333–1339.
[34] L.F. Tseng, K.A. Collins, Spinal involvement of both dynorphin A and met-enkephalin in the antinociception induced by intracerebroventricularly administered bremazocine but not morphine in the mouse, J. Pharmacol. Exp. Ther. 266 (1993) 1430–1438.
[35] S.P. Welch, Modulation of cannabinoid-induced antinociception by nor-binaltorphimine, but not ICI 174,864, in mice, J. Pharmacol. Exp. Ther. 265 (1993) 633–640.
[36] S.P. Welch, Characterization of anandamide-induced tolerance: comparison to delta 9-THC-induced interactions with dynorphinergic systems, Drug Alcohol Dep. 45 (1997) 39–45.
[37] S.P. Welch, L.D. Dunlow, G.S. Patrick, Characterization of anandamide and fluoroanandamide-induced antinociception and cross tolerance to D^9-THC-induced antinociception, J. Pharm. Exp. Ther. 273 (1995) 1235–1244.
[38] S.P. Welch, M. Eads, Synergistic interactions of endogenous opioids and cannabinoid systems, Brain Res. (1999) in press.
[39] S.P. Welch, C. Thomas, G.S. Patrick, Modulation of cannabinoid-induced antinociception following intracerebroventricular versus intrathecal administration to mice: Possible mechanisms for interaction with morphine, J. Pharmacol. Exp. Ther. 272 (1995) 310–321.
[40] S.P. Welch, D.L. Stevens, Antinociceptive activity of intrathecally-administered cannabinoids alone, and in combination with morphine, in mice, J. Pharmacol. Exp. Ther. 262 (1992) 10–18.
[41] C.W. Xie, J. Tang, J.S. Han, Clonidine-stimulated the release of dynorphin in the spinal cord of the rat: A possible mechanism for its depressor effects, Neurosci. Lett. 65 (1986) 244–248.