

Interactive report

Synergistic interactions of endogenous opioids and cannabinoid systems¹Sandra P. Welch^{*}, Micah Eads*Department of Pharmacology and Toxicology, Box 980613, Virginia Commonwealth University, Richmond, VA 23298-0613, USA*

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Abstract

Cannabinoids and opioids are distinct drug classes historically used in combination to treat pain. Δ^9 -THC, an active constituent in marijuana, releases endogenous dynorphin A and leucine enkephalin in the production of analgesia. The endocannabinoid, anandamide (AEA), fails to release dynorphin A. The synthetic cannabinoid, CP55,940, releases dynorphin B. Neither AEA nor CP55,940 enhances morphine analgesia. The CB1 antagonist, SR141716A, differentially blocks Δ^9 -THC versus AEA. Tolerance to Δ^9 -THC, but not AEA, involves a decrease in the release of dynorphin A. Our preclinical studies indicate that Δ^9 -THC and morphine can be useful in low dose combination as an analgesic. Such is not observed with AEA or CP55,940. We hypothesize the existence of a new CB receptor differentially linked to endogenous opioid systems based upon data showing the stereoselectivity of endogenous opioid release. Such a receptor, due to the release of endogenous opioids, may have significant impact upon the clinical development of cannabinoid/opioid combinations for the treatment of a variety of types of pain in humans. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

The cannabinoid/opioid interaction differs in that cannabinoids generally fall into two categories — those that enhance the antinociceptive effects of morphine in the spinal cord (Δ^9 -THC {THC} for example) and those that do not enhance spinally administered morphine (CP55,940 {CP55} for example) [48]. The mechanisms by which the cannabinoids produce antinociception are as yet unclear. We believe that our data indicate that the mechanism by which the cannabinoids produce antinociception involves dynorphin release spinally and that the ‘greater than additive effects’ of the cannabinoids with morphine [1,36] and the delta opioid, DPDPE, are due to the initial release of dynorphin A peptides and the subsequent breakdown of the dynorphin A to leucine enkephalin [28]. We hypothesize that the functional coupling of the mu/delta and mu/kappa receptors leads to enhanced antinociceptive effects of morphine and DPDPE by the cannabinoids. We envision cannabinoid-induced release of dynorphins as an indirect process due to the disinhibition of yet unknown

neuronal processes. The localization of the cannabinoid receptors involved in dynorphin release are not known. We hypothesize that in the spinal cord, cannabinoids produce antinociceptive effects via the direct interaction of the cannabinoid receptor with Gi/o proteins resulting in a decreased c-AMP production [47], as well as hyperpolarization via interaction with specific potassium channels [3]. Thus, the cannabinoids may produce disinhibition by decreasing the release of an inhibitory neurotransmitter in dynorphinergic pathways. The net result of such an effect may be an increase in dynorphin release. The events which precede and follow the release of dynorphin remain unclear. The dynorphin most likely is a modulator of other ‘down-stream’ systems (possible substance P release or interaction with NMDA-mediated events) which culminate in antinociception upon administration of cannabinoids. What has proved intriguing is the observation that cannabinoids differ in their interactions with dynorphins (and subsequently with mu and delta opioids) [16,17].

THC appears to interact with the dynorphin A system [28,44], while CP55 appears to interact with and release dynorphin B [27], although CP55 is clearly cross-tolerant to THC [6]. THC is not cross-tolerant to dynorphin B, but is cross-tolerant to the dynorphins of the ‘A’ type [44]. In addition, as animals are rendered tolerant to THC, the

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levels of dynorphin A released are decreased. Thus, tolerance to THC involves a decrease in the release of dynorphin A [16].

The kappa antagonist, nor-binaltorphimine (nor-BNI), and dynorphin antisera block THC-induced antinociception, but do not block THC-induced catalepsy, hypothermia, or hypoactivity [28,38,43]. In addition, the discovery of the bi-directional cross tolerance of THC and CP55 to kappa agonists using the tail-flick test [38] and to dynorphin A [44], indicates that cannabinoids interact in a yet-to-be-determined manner with kappa opioids. The attenuation of the antinociceptive effects of THC by antisense oligonucleotides to the cloned kappa-1 opioid receptor further implicates the release of endogenous kappa opioids in the mechanism of action of the cannabinoids [26]. Dynorphin antibodies block THC-induced antinociception, and prevention of the metabolism of dynorphin A (1–17) to dynorphin (1–8) or to leucine enkephalin prevents the enhancement of morphine-induced antinociception by the THC [28].

The endogenous cannabinoid, AEA, appears to differ from THC and CP55 in its lack of interactions with dynorphinergic systems [38,44]. Despite similarities in the profile of action to classical cannabinoids, distinct differences between AEA and other cannabinoids in terms of behavioral effects have been reported [28,38,44,45]. AEA appears to differ from the traditional cannabinoids in that it is not active following icv. administration in several behaviors which are characteristic of cannabinoids. Other differences between anandamide and THC have been observed in tasks involving learning and memory [13], drug discrimination [49] and modulation by agonists and antagonists of classical neurotransmitter systems [45]. AEA which is neither blocked by the kappa antagonist, nor-BNI, nor cross-tolerant to any dynorphins [37,44,45] is cross-tolerant to THC and CP55 and displaces binding of the traditional cannabinoids [4,33,37,45]. Anandamide fails to enhance the activity of any opioid and does not release dynorphin A [28,44,45].

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 CB₁ receptor or activities of functional subtypes of the cannabinoid CB₁ receptor in the spinal cord. It is difficult to envision such diverse dynorphin release profiles for the drugs through actions at one receptor subtype. The mechanisms underlying the differential release of dynorphins by THC versus CP55 and AEA thus remains unknown.

Two distinct cannabinoid receptors have been cloned, the CB₁ receptor which is predominantly located in the central nervous system [18], and the CB₂ receptor which is found on immune cells and on peripheral tissues [21]. In addition, a splice variant of the CB₁ receptor termed the CB_{1A} receptor has been identified [34]. The discovery of

the cannabinoid CB₁ receptor antagonist, SR141716A [30] and the discovery of the first endogenous cannabinoid-like ligand, anandamide {AEA}, [4] greatly facilitated work with the cannabinoids and complements the discovery and cloning of the cannabinoid receptors. The newly described cannabinoid CB₂ receptor antagonist, SR144528 [31], will be of great help in elucidating cannabinoid receptor subtypes. Receptor-ligand binding studies have produced evidence suggesting the existence of cannabinoid CB₁ receptor subtypes [41]. We evaluated THC, CP55, and AEA alone and in combination with SR141716A (SR), a CB₁ antagonist, in order to better characterize potential diversity in interactions of the cannabinoids with the cannabinoid (CB₁) receptor. The effects of SR on AEA-induced antinociception were mixed. The maximum attenuation of AEA-induced antinociception (intrathecally administered, i.t.) by SR (i.t.) was only 38%. SR (administered intraperitoneally, i.p.) blockade of ANA was complete, but the AD₅₀ was nearly 15-fold higher than that required to block THC or CP55. In addition, SR (i.p. or i.t.) failed to block the hypothermic effects of AEA (i.t.), while completely reversing the hypothermic effects of THC (i.t.). Such data are suggestive of either a differential interaction of the cannabinoids at the CB₁ receptor or the existence of subtypes of the CB₁ receptor [46].

In addition to the use of the CB₁ antagonist, SR141716A, in this paper we report the stereoselectivity of opioid peptide release by the administration of levonantradol, a synthetic cannabinoid which we have shown to produce antinociception and enhance the activity of morphine [48], and dextronantradol, its inactive stereoisomer. We chose this pair of stereoisomers since the drugs had been previously evaluated in several test systems in mice and rats. Clearly, many other stereoisomeric pairs of cannabinoids have been tested in other systems (see Ref. [15] for review) but most have not been tested via the i.t. route of administration and have not been evaluated for the ability to enhance the antinociceptive effects of morphine. We hypothesized that levonantradol, which was the most potent cannabinoid that we tested [48] would release more dynorphin and produce antinociceptive effects at lower doses in the rat than THC, while dextronantradol would not release dynorphin. Such work was designed to show the stereoselectivity and thus, receptor mediation, of the dynorphin release.

2. Materials and methods

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 $22 \pm 2^\circ\text{C}$. Water and food (Harlan Rat Chow) were provided *ad libitum*.

2.2. Intrathecal administration of drugs and opioid collection

Intrathecal drug administration and opioid collection were performed using a modified version of techniques described by Mason et al. [16,17], Tseng et al. [42] and Yaksh [50]. Subjects were selected for partitioning into experimental groups at random and anesthetized via 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 subarachnoid 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, greater than 1.5 s, but less than 4 s latency, were used. Test compounds were administered in a 20 ml bolus of vehicle, via spinal catheter, at a rate of 30 ml/min. Subjects were then segregated into groups for cerebrospinal fluid sampling and tail-flick latency assessment 10 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 radioimmunoassay buffer before dynorphin A-(1–17), dynorphin B, methionine [met] enkephalin, or leucine [leu] enkephalin peptide measurement.

2.3. Measurement of opioid peptides

Measurement of opioid peptide release (pg/ml) was accomplished using a specific radioimmunoassay kits obtained from Peninsula Laboratories, Inc. The reconstituted samples were analyzed in duplicate. The manufacturer

reported cross-reactivity of dynorphin A-(1–17) antibody as 100% versus dynorphin-(1–24), a parent compound, and less than 2% versus smaller peptide fragments. We found no cross-reactivity of the antibody to dynorphin A-(1–8), dynorphin A-(1–13), dimethyl sulfoxide or Δ^9 -tetrahydrocannabinol. Similarly, the dynorphin B antibody had less than 3% cross-reactivity to dynorphin A or to the met or leu enkephalins. The met enkephalin antibody had less than 1% cross-reactivity to the leu-enkephalin antibody and vice versa. We found no cross-reactivity of the met- or leu- enkephalin antibody to dynorphin A-(1–8), dynorphin A-(1–13), dimethyl sulfoxide or Δ^9 -tetrahydrocannabinol. Only the linear portion of the radioimmunoassay standard curve, between 1 pg/ml and 64 pg/ml of the standard peptide, was used to calculate dynorphin concentration.

2.4. Assessment of tail-flick latency

Antinociceptive behavior was assessed using a modified version of that described by D'Amour and Smith [2]. 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 Harris and Pierson [9] as:

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

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

2.5. Statistical analysis

Using a randomized design, analysis of data concerning tail-flick latency or peptide concentration was done using ANOVA (analysis of variance) followed by two-tailed Dunnett's *t*-test [5].

2.6. Drugs and vehicle

For i.t. challenges, levonantradol (5 $\mu\text{g}/\text{rat}$) and dextronantradol (30 $\mu\text{g}/\text{rat}$) were administered using a 100% dimethyl sulfoxide vehicle (DMSO) at 15 min prior to collection of the CSF. At 15 min post administration, the peak antinociceptive effects of levonantradol were observed. The concentration of DMSO has been used in numerous studies and has no effect on the animals behavior (in awake, non-anesthetized animals) or on dynorphin release in our anesthetized rats [16,17]. Drugs were provided by Dr. Lawrence Melvin, Pfizer Pharmaceutical Research. The dose chosen for levonantradol was that

which produced 100% antinociceptive effect in the tail-flick test. The dose chosen for dextronantradol (30 $\mu\text{g}/\text{rat}$) was the highest dose we could run with the available drug supply.

3. Results

Fig. 1 indicates the results of antinociceptive testing on the rats subsequently utilized for the collection of CSF as described in the Materials and Methods section. The clear bars represent the average %MPE for $N = 9$ rats subsequently used to quantitate dynorphin A- (1–17) release. The striped bars represent the average %MPE for $N = 10$ rats subsequently used to quantitate the release of dynorphin B. Drugs were administered i.t. at 15 min prior to testing using the tail-flick test. Levonantradol ($N = 9$ rats; dose of 5 $\mu\text{g}/\text{rat}$ in a 20 μl volume) produced $87.6 \pm 12.3\%$ MPE versus $2.5 \pm 2.2\%$ MPE in dextronantradol-treated rats ($N = 10$; dose of 30 $\mu\text{g}/\text{rat}$ in a 20 μl volume) and $4.8 \pm 2.9\%$ MPE in DMSO-treated rats ($N = 9$; dose in a 20 μl volume) ($p < 0.0001$ from both DEXTRO and DMSO). Immediately following testing in the tail-flick test CSF was removed for testing of the endogenous opioid release from the same rats. Separate groups of

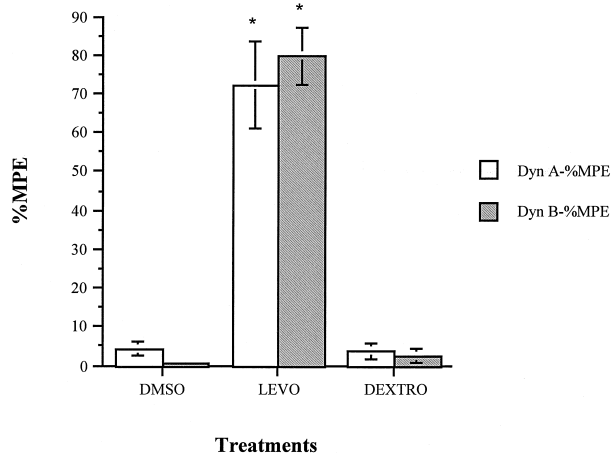


Fig. 1. Antinociceptive effects of i.t. levonantradol and dextronantradol in rats. Antinociceptive behavior was assessed using a modified version of that described by D'Amour and Smith [2] as described in the 'Materials and Methods' using the tail-flick test. %MPE was quantified for each rat. The average % MPE $\pm$ standard error of the mean is reported for separate groups of rats ($N =$ at least 8 rats per group). Clear bars represent the average %MPE for rats subsequently evaluated for the release of dynorphin A-(1–17); striped bars represent the average %MPE for rats subsequently evaluated for release of dynorphin B (data shown in Fig. 3). DMSO indicates the vehicle-treated rats. LEVO indicates rats treated with 5 $\mu\text{g}/\text{rat}$ levonantradol. DEXTRO indicates rats treated with dextronantradol (30 $\mu\text{g}/\text{rat}$). Treatments were i.t. at 15 min prior to testing. Significance was determined using ANOVA followed by the post hoc Dunnett's t -test (unpaired, two-tailed). * $p < 0.05$ from DMSO-treated rats. Similar antinociceptive effects were observed in separate groups of rats in which met- and leu-enkephalin release was quantified (Fig. 2).

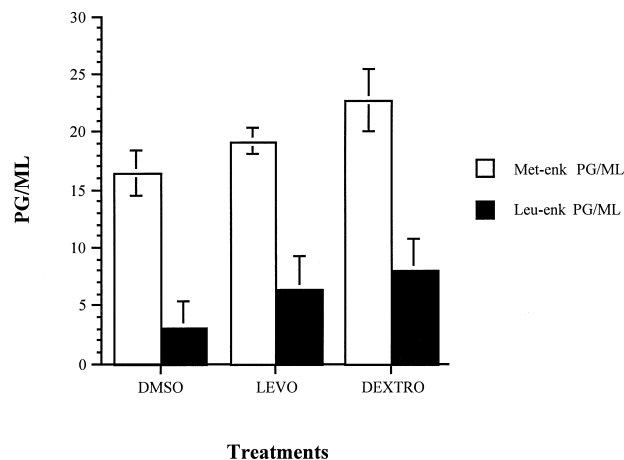


Fig. 2. Lack of the release of met- and leu-enkephalin by the i.t. administration of levonantradol and dextronantradol in rats. Antinociceptive behavior was assessed using a modified version of that described by D'Amour and Smith [2] as described in the 'Materials and Methods' using the tail-flick test. %MPE was quantified for each rat ($N =$ at least 8 rats per group). The rats were then spinally perfused and the CSF removed as described in the 'Material and Methods'. Methionine and leucine enkephalin were quantified using radioimmunoassay. Clear bars represent the average met-enkephalin release in pg/ml of CSF; filled bars represent the average leu-enkephalin release in pg/ml of CSF. DMSO indicates the vehicle-treated rats. LEVO indicates rats treated with 5 $\mu\text{g}/\text{rat}$ levonantradol. DEXTRO indicates rats treated with dextronantradol (30 $\mu\text{g}/\text{rat}$). Collection of CSF occurred at 15 min following drug or vehicle administration and concurrently with the tail-flick testing. Significance was determined using ANOVA followed by the post hoc Dunnett's t -test (unpaired, two-tailed). * $p < 0.05$ from DMSO-treated rats.

rats were administered the drugs and CSF was removed for the quantitation of met- or leu-enkephalin ($N = 10$ –12 rats per group). The average %MPE for those groups of LEVO- or DEXTRO-treated rats did not differ from the results presented in Fig. 1. (data not shown). LEVO produced significant antinociceptive effects in all groups, while DEXTRO and DMSO produced no significant effects.

Fig. 2 indicates the results of the radioimmunoassay for met-enkephalin and leu-enkephalin. DMSO-induced release of met-enkephalin was 16.4 ± 2 pg/ml. Levonantradol (LEVO) and dextronantradol (DEXTRO)- induced release was 19.2 ± 1.1 and 22.7 ± 2.7 pg/ml, respectively, and did not differ significantly from each other or DMSO control. LEVO and DEXTRO similarly did not produced a significant increase in leu-enkephalin compared to DMSO (6.3 ± 3 ; 5.8 ± 2.7 and 3.7 ± 2.3 pg/ml, respectively).

Fig. 3 indicates the results of LEVO and DEXTRO on both dynorphin A- (1–17) and dynorphin B release. The release of dynorphin A-(1–17) by DMSO was 3.6 ± 0.4 pg/ml versus 1.1 ± 0.01 pg/ml for DEXTRO and 1.8 ± 0.2 for LEVO. LEVO significantly increased dynorphin B release from 4.0 ± 0.8 pg/ml for DMSO to 9.8 ± 2.7 pg/ml ($p < 0.046$). DEXTRO did not increase dynorphin B levels (3.7 ± 0.6 pg/ml) over those observed for DMSO.

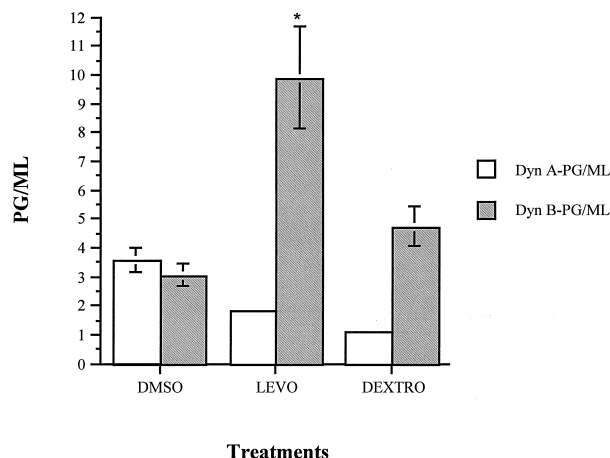


Fig. 3. Release of dynorphin B, but not dynorphin A-(1–17) by the i.t. administration of levonantradol, but not dextronantradol, in rats. Antinociceptive behavior was assessed using a modified version of that described by D'Amour and Smith [2] as described in the 'Materials and Methods' using the tail-flick test. %MPE was quantified for each rat ($N =$ at least 8 rats per group). The rats were then spinally perfused and the CSF removed as described in the 'Material and Methods'. Dynorphin A-(1–17) (clear bars) and dynorphin B (striped bars) were quantified using radioimmunoassay. DMSO indicates the vehicle-treated rats. LEVO indicates rats treated with 5 $\mu\text{g}/\text{rat}$ levonantradol. DEXTRO indicates rats treated with dextronantradol (30 $\mu\text{g}/\text{rat}$). Collection of CSF occurred at 15 min following drug or vehicle administration and concurrently with the tail-flick testing. Significance was determined using ANOVA followed by the post hoc Dunnett's t -test (unpaired, two-tailed). * $p < 0.05$ from DMSO-treated rats.

In all experiments the use of experimental animals was approved by the Institutional Care and Use Committee in accordance with all federal directives. Care was taken in all cases to minimize the numbers of animals used and the pain or discomfort of the animals.

4. Discussion

It has been documented that the cannabinoids produce effects which have much in common with the opiates, such as antinociception, hypothermia, cross tolerance to morphine, and attenuation of naloxone-precipitated withdrawal from morphine. Early experiments to evaluate the analgesic effects of the cannabinoids dealt mainly with an examination of the effects of Δ^9 -THC, the principle active ingredient in cannabis. Studies in human subjects indicated that at oral doses of 10 and 20 mg/kg Δ^9 -THC was no more effective than codeine as an analgesic, while producing a significant degree of dysphoric side effects [23]. When tested following intravenous administration to human dental patients, Δ^9 -THC produced analgesia that was accompanied by dysphoria and anxiety [29]. Thus, in these studies it was evident that Δ^9 -THC analgesia could only be elicited at doses producing other behavioral side effects. In addition, Δ^9 -THC was no more potent than the more commonly used opioid analgesics. Levonantradol has pre-

viously been shown to produce antinociceptive effects upon i.t. administration to rats [50] and spinal administration to the dog [8] at doses devoid of other behavioral side effects. These investigators concluded that a spinal site of action might be involved in the antinociceptive effects observed.

Recently the interaction of cannabinoids with opioids has been extensively revisited and reviewed [14] in terms of the interactions of the drug classes in the prevention of pain with fewer side effects of either drug class and the lack of induction of addiction. Our findings of the blockade of cannabinoids by nor-BNI, and dynorphin antibodies in spinal cord, but not the brain, implicates dynorphins in the spinal mechanism of action of the cannabinoids. This finding is exciting in that it provides a direct link between the cannabinoids and opioid systems and is the first time that the antinociceptive effects of the cannabinoids have been separated from other behavioral effects [38].

The potent, synthetic cannabinoid, CP55, was instrumental in demonstrating that cannabinoid binding sites are present in the substantia gelatinosa, an area involved with the transmission of pain signals [10]. In addition, CP55 produces many of the behavioral and physiologic effects characteristic of THC. Despite these similarities, we have found that THC, levonantradol, and CP55 differ in their interaction with morphine in the spinal cord [48]. Pretreatment of mice with CP55 (i.t.) does not enhance the antinociceptive effects of morphine (i.t.), while pretreatment with THC produces a 10-fold decrease in the morphine ED_{50} and levonantradol enhances the potency of morphine (i.t.) by greater than 10-fold [48]. Our data indicate that THC enhances the antinociception of morphine through the release of endogenous dynorphin A and its breakdown to leu-enkephalin [28]. Dynorphin B, another product of the prodynorphin precursor, also contains a copy of leucine enkephalin. However, the breakdown of dynorphin B to leucine enkephalin is less well documented [12] and dynorphin B fails to enhance the activity of morphine in antinociceptive tests [27]. It has been shown that dynorphin B produces antinociception when administered i.t. as measured by the tail-flick test [22,40]. Since it has been shown that kappa, delta, and mu ligands interact in the production of antinociceptive effects [11,20,32,39], the release of a delta or kappa opioid by the cannabinoids could also alter the antinociceptive effects of the predominantly mu opioid, morphine. Pugh et al. [28] and Welch [44] demonstrated that dynorphin A-(1–13) and dynorphin A-(1–8) increased tail-flick latency (i.t.) in the mouse and enhance the activity of morphine. Mason et al. [16,17] have shown that THC releases dynorphin A- (1–8) in the rat which would presumably enhance morphine's effects.

Dextronantradol, which produced no antinociceptive effects and did not enhance the effects of morphine, was hypothesized to not release opioids. Our hypothesis proved correct. However, we were not expecting levonantradol to be inactive in release of dynorphin A. Since levonantradol

potently enhanced the effects of morphine, we expected it to potently release dynorphin A. Such was not the case. Levonantradol released only dynorphin B which was temporally correlated to its antinociceptive effect. Since levonantradol-induced antinociception is blocked totally by nor-BNI [43], the antinociceptive effects of levonantradol appear to be related in part to dynorphin B release and subsequent interaction at the kappa-opioid receptor. CP55 releases only dynorphin B [27]. Like levonantradol, it does not release met- or leu-enkephalin or dynorphin A- (1–8) (data not shown). Since dynorphin B does not enhance morphine-induced antinociception, the enhancement of morphine by levonantradol appears to not be related to dynorphin B release. Since levonantradol does not release dynorphin A, which does enhance morphine's effects, the mechanism by which levonantradol acts to enhance morphine is obviously unique from that of THC. However, the effects of levonantradol on dynorphin B release are stereoselective and blocked by SR141716A and are thus, receptor-mediated.

AEA-induced antinociception is mediated by a mechanism which appears to differ from THC, CP55 and levonantradol in terms of kappa-opioid receptor involvement [16,17,38,44,45]. Unlike THC, AEA does not significantly increase immunoreactive dynorphin A-(1–17) concentration, nor is its ability to increase tail-flick latency nor-BNI-sensitive. Given the lack of a nor-BNI block of AEA-induced antinociception, it is unlikely to be a critical component in its antinociceptive effects [17]. Similar conclusions were noted in the development of tolerance to AEA [44]. AEA does not release any opioid peptide tested (data not shown). However, the attenuation of AEA-induced tail-flick latency by SR141716A indicates that AEA-induced antinociception, like that of THC and CP55, is mediated via the cannabinoid CB₁ receptor. Such diversity of effects, between AEA and THC, have been reported in other models [35,37]. Such data are suggestive of either a differential interaction of AEA versus the classical cannabinoids at the CB₁ receptor or the existence of subtypes of the CB₁ receptor.

Thus, the mechanism of action of anandamide, its activity in modulating pain, and its lack of interaction with opioids remains unclear and appears to differ from that of the THC, CP55, and levonantradol. Anandamide is but one of a family of arachadonic acid derivatives which have cannabinoid-like effects [7,19,24,25]. The elucidation of distinct mechanisms by which the body produces and utilizes endogenous cannabinoid substances in the modulation of nociception could have a potentially important impact on the design of new analgesics for clinical use. The immediate benefits of elucidating the full scope of the interactions of cannabinoids (exogenous or endogenous) with opioids will likely come in the management of pain, particularly chronic pain [35]. THC, in comparison to the morphine derivatives, has a greater therapeutic range. Opioid use can lead to development of tolerance, undesirable

side effects, and tolerance. Studies such as ours and those of others [1,14,16,17,36] may lead to new techniques by which manipulation of the endogenous opioid system by cannabinoids can be used to supplement exogenous opioid agents resulting in decreased dosage of opioids and a resulting decreased risk of toxicity, as well as manageable tolerance development. Alternatively, the elucidation of the mechanisms underlying the differences in the interaction of distinct cannabinoids with distinct endogenous opioid systems may lead to the discovery of novel CB receptors as potential targets for development of either opioid adjuncts or non-opioid analgesics.

In summary, we have shown that THC and levonantradol enhance the antinociceptive effects of morphine. THC releases dynorphin A, but levonantradol releases dynorphin B. CP55 and AEA do not enhance the effects of morphine spinally. CP55 releases dynorphin B. AEA releases no opioid peptides. Antinociceptive effects of THC, levonantradol, CP55, and AEA are all blocked by the CB₁ antagonist, SR141716A. Thus, four distinct cannabinoids exert four distinct patterns of interaction or lack of interaction with endogenous opioid systems. We hypothesize that such a diversity of interactions may be indicative of CB₁ receptor subtypes in the spinal cord. Such subtypes may be clinically important in the development or use of cannabinoids in the treatment of pain.

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