Involvement of Dynorphin B in the Antinociceptive Effects of the Cannabinoid CP55,940 in the Spinal Cord

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ABSTRACT
Intrathecal administration of Δ9-tetrahydrocannabinol (Δ9-THC) but not the cannabinoid agonist CP55,940 enhances the antinociception produced by morphine. In addition, CP55,940- and Δ9-THC-induced antinociception is blocked by the kappa opioid antagonist norbinaltorphimine, and both cannabinoids are cross-tolerant to kappa agonists but do not act directly at the kappa receptor. Previous work in our laboratory has implicated dynorphins in the antinociceptive effects of Δ9-THC and its enhancement of morphine-induced antinociception. The goal of the present study was to evaluate the role of dynorphins in the antinociceptive effects of CP55,940 at the spinal level. Pretreatment of mice with antisera to dynorphin A(1–17), dynorphin B or α-neoendorphin, all of which have been shown to retain specificity for blockade of their respective peptide in vivo, blocked the antinociceptive effects of Δ9-THC but not CP55,940. Dynorphin B produced antinociceptive effects on intrathecal administration to mice. Like CP55,940, dynorphin B failed to enhance the antinociceptive effects of morphine, whereas dynorphin A(1–17) and α-neoendorphin enhanced the antinociceptive effects of morphine. Using spinal catheterization of the rat, CP55,940 administration was shown to produce a significant release of dynorphin B concurrent with the production of antinociception. Our data suggest that CP55,940 induces a release of spinal dynorphin B that contributes at least in part to its antinociceptive effects in the spinal cord.

Cannabinoids are active as antinociceptive drugs when injected i.t. (Gilbert, 1981; Lichtman and Martin, 1991a, 1991b; Welch et al., 1995; Welch and Stevens, 1992; Yaksh, 1981). Intrathecally administered cannabinoids appear to act at predominantly spinal sites in the production of antinociception (Smith and Martin, 1992). The mechanisms by which the cannabinoids produce antinociception are as yet unclear. The identification of cannabinoid receptors has been the topic of intense investigation leading to the cloning of two distinct cannabinoid receptors; one is predominantly located in the central nervous system (Matsuda et al., 1990), and the other is found on immune cells and on peripheral tissues (Munro et al., 1995). In addition, a splice variant of the CB1 receptor termed the CB1A receptor has been identified (Shire et al., 1995). When the sequence for the cannabinoid receptor was published, Gérard et al. (1990) reported they had isolated the human homolog of this receptor. The discovery of the cannabinoid antagonist SR141716A (Rinaldi-Carmona et al., 1994) and the discovery of an endogenous cannabinoid-like ligand, anandamide, (Devane et al., 1992) have greatly facilitated work with the cannabinoids and complements the discovery and cloning of the cannabinoid receptors.

Our previous data indicate that the cannabinoids produce antinociception by indirect interaction with kappa opioids in the spinal cord after i.t. administration. The kappa antagonist nor-BNI and dynorphin antisera block Δ9-THC-induced i.t. antinociception but not THC-induced catalepsy, hypothermia or hypoactivity (Smith et al., 1994; Pugh et al., 1996). Such data represent the first time that the behavioral effects of the cannabinoids had been separated. In addition, the discovery of the bidirectional cross-tolerance of THC and CP55,940 to kappa agonists using the tail-flick test (Smith et al., 1994) indicates that cannabinoids interact with kappa opioids. The attenuation of the antinociceptive effects of THC by antisense to the kappa-1 receptor further implicates the release of endogenous kappa opioids in the mechanism of action of the cannabinoids (Pugh et al., 1995). Because nor-BNI-induced blockade of dynorphin-induced antinociception has been documented and the principle site of action of nor-BNI is at the kappa receptor (Clark et al., 1989), nor-BNI-induced blockade of cannabinoid antinociception is likely the result of a block of the effects of dynorphin at the kappa receptor. The blockade of cannabinoid-induced antinociception by the kappa-1 antagonist naloxone benzoylhydrazone also implicates the kappa-1 receptor in the effects of the cannabinoids (Welch, 1994). In addition, dynorphin antibodies block cannabinoid-induced antinociception, and pre-

ABBREVIATIONS: i.t., intrathecally; THC, Δ9-tetrahydrocannabinol, nor-BNI, norbinaltorphimine; MPE, maximum possible effect; CL, confidence limit; DMSO, dimethylsulfoxide; CSF, cerebrospinal fluid.
vention of the metabolism of dynorphin A(1–17) to dynorphin(1–8) or to leucine enkephalin prevents the enhancement of morphine-induced antinociception by THC (Pugh et al., 1996).

High levels of dynorphins exist in the dorsal horn of the spinal cord as well as in the brain, where they produce diverse effects on nociception (Fujimoto et al., 1990; Fujimoto and Arts, 1990; Fujimoto and Holmes, 1990; Piercey et al., 1982; Song and Takemori, 1991; Stevens and Yaksh, 1986; Tulaney et al., 1981). The dynorphins have high affinity for the kappa receptor (for a review, see Holt, 1986). Cleavage of the large precursor prodynorphin results in the release of various dynorphins, including dynorphin A(1–17), which has been proposed to be the endogenous ligand for the kappa receptor (Chavkin et al., 1982). The breakdown of dynorphin A(1–17) into dynorphin A(1–8) and subsequently into leucine enkephalin has been shown (Dixon and Traynor, 1990; Holt, 1986). Both dynorphin A(1–8) and analogs of leucine enkephalin produce antinociception when administered i.t., as tested in the tail-flick test. The antinociceptive effects of both dynorphin A(1–8) and dynorphin A(1–13) at spinal sites have been shown to result from interaction with kappa receptors (although other opioid receptor subtypes have been shown to bind these dynorphins), and both ligands have been shown to enhance the antinociceptive effects of morphine at spinal sites after i.t. administration (Jen et al., 1986; Jhamandas et al., 1986; Pugh et al., 1996). Dynorphin B and α-neoendorphin are other products of the prodynorphin precursor. It has been shown that dynorphin B produces antinociception when administered i.t., as measured by the tail-flick test (Nakazawa et al., 1991; Spampinato et al., 1988).

Despite all the data indicating involvement of kappa (and/or delta) opioids in cannabinoid-induced antinociception, mu and delta, but not kappa, receptor-selective opioids have been shown to be displaced by the cannabinoids in brain, albeit at relatively high cannabinoid concentrations (Vaysse et al., 1987). Delta opioids are not displaced by cannabinoids in neuroblastoma cells (Devane et al., 1986). In addition, binding of the cannabinoid CP55,940 in the spinal cord is not displaced by kappa agonists or the kappa antagonist nor-BNI (Welch et al., 1995). Thus, we have accumulated considerable evidence suggesting a link of the cannabinoids to the dynorphins that requires further investigation.

The potent, synthetic cannabinoid CP55,940 was instrumental in demonstrating that cannabinoid binding sites are present in the substantia gelatinosa, an area involved with the transmission of pain signals (Herkenham et al., 1990). In addition, CP55,940 produces many of the behavioral and physiological effects characteristic of THC. Despite these similarities, we found that THC and CP55,940 differ in their interaction with morphine in the spinal cord (Welch and Stevens, 1992). Pretreatment of mice with CP55,940 i.t. does not enhance the antinociceptive effects of morphine i.t., whereas pretreatment with THC produces a 10-fold decrease in the morphine ED50. Our data indicate that THC enhances the antinociception of morphine through the release of endogenous dynorphin (Pugh et al., 1996) Unfortunately, the role of endogenous opioids, particularly the dynorphins, in the antinociceptive effects of CP55,940 is unclear. In the present investigation, we examined the role of dynorphins in CP55,940-induced antinociception.

**Methods**

**Animals.** Male ICR mice (Harlan Laboratories, Indianapolis, IN) with a weight range of 23 to 27 g were housed 6 or 8 to a cage in animal care quarters maintained at 22 ± 2°C on a 12-hr light/dark cycle. Food and water were available ad libitum.

**Intrathecal injections.** Intrathecal injections were performed according to the protocol of Hylden and Wilcox (1983). Unanesthetized mice were injected between the L5 or L6 area of the spinal cord with a 30-gauge, 0.5-inch needle. Injection volumes of 5 μl were administered. THC and CP55,940 were prepared in 100% DMSO. Dynorphins and α-neoendorphin were prepared in distilled water plus Triton X-100 (0.01%). Dynorphin antisera, morphine sulfate and nor-BNI were prepared in distilled water. All drugs were kept in plastic tubes on ice and were prepared fresh daily. In studies evaluating the effects of various dynorphin antisera on the antinociceptive effects of THC and CP55,940 alone, a 45- to 60-min pretreatment time of the antisera was used before testing the animals in the tail-flick test. This time course was consistent with our previous studies (Pugh et al., 1996) and those of others that indicate that peak blockade of antinociception occurs when the antibodies are injected 60 min before testing (Fujimoto et al., 1990). α-Neoendorphin (75 μg/mouse i.t.) was administered at 10 min before testing after a 45-min vehicle (distilled water i.t.) or antisera (10 μg/mouse i.t.) pretreatment and tested for antinociception using the tail-flick test. Dynorphin B (85 μg/mouse i.t.) was tested similarly in combination with vehicle, IgG or dynorphin B antisera. Other doses of antisera (≤100 μg/mouse) and time points of pretreatment of ≤2 hr were evaluated. For studies of the combination of morphine with dynorphins, the highest inactive dose of the respective dynorphins was administered 10 min before morphine. Inactive doses of the dynorphins (μg/mouse) were as follows: dynorphin A(1–8), 10; dynorphin A(1–17), 1; dynorphin B, 10; and α-neoendorphin, 10. At 10 min after the morphine administration, the mice were tested using the tail-flick test.

The dynorphins produce splaying of the hindlimbs at doses that produce >80% MPE. Clearly, the motor effects at such doses could contribute to the antinociceptive effects of the drugs. However, we can block the antinociceptive effects of the dynorphins at high doses with nor-BNI (kappa antagonist), which implies that the antinociception produced at such high doses may not be related to nonspecific toxic effects. At the low, inactive doses used in combination with morphine, there are no toxic effects of the dynorphins observed. The dynorphin antisera were devoid of any observable side effects. At the high, toxic doses used in combination with morphine, the motor effects at such doses could contribute to the antinociceptive effects of the drugs.

**Tail-flick test.** The tail-flick procedure was performed according to D’Amour and Smith (1941). Control reaction times of 2 to 4 sec and a cutoff time of 10 sec were used. Antinociception was quantified as the % MPE as developed by Harris and Pierson (1964) using the following formula: % MPE = 100 × [(test – control)/10 – control]. Percentage of MPE was calculated for each mouse using at least 6 mice per dose. By using the % MPE for each mouse, the mean effect and S.E.M. were calculated for each dose. Dose-response curves were generated using at least three doses of test drug. ED50 values were determined by log-probit analysis, and 95% CLs were determined using the method of Litchfield and Wilcoxon (1949).

**Tolerance to THC.** Mice were rendered tolerant to the effects of Δ9-THC by repetitive administration of 15 mg/kg s.c. Δ9-THC over a 7-day period according to the method of Tsou et al. (1995). The animals received two subcutaneously administered injections per day at 8:00 a.m. and 6:00 p.m. for the first 6 days and a single injection at 8:00 a.m. on day 7. Testing was performed at 8:00 a.m. on day 8. Control groups receiving appropriate vehicle administration were also tested. Dynorphin B was administered i.t. to THC-tolerant and nontolerant mice, and the antinociceptive effects were evaluated 10 min later.

**Spinal cord perfusion and quantification of dynorphin B release.** Spinal dynorphin release in rat has been documented in superfused isolated spinal cords (Song and Takemori, 1992) and...
spinal cord slices (Przewlocka et al., 1990), and it has been directly released from rat spinal cord in response to clonidine (Xie et al., 1986) at levels consistent with sensitivity of our radioimmunoassays. Using the methods of Tseng (1989), rats were injected with sodium barbital (300 mg/kg i.p.) and methylatropine bromide (2.0 mg/kg i.p.) and placed on a 37°C heating pad. Administration of CP55,940 (100 μg/rat i.t.) or DMSO vehicle to the rat was performed according to the method of Yaksh (1981) by the insertion of an indwelling intrathecal cannula via incision on the basal occipital membrane and insertion of PE-10 polyethylene tubing caudally into the subarachnoid space. (The dose of CP55,940 was the ED80 dose in the rat as previously determined by Lichtman and Martin, 1991a). The catheter was designed to be 8.5 cm in length and extend into the lumbar enlargement and was prefilled with artificial CSF. A peristaltic pump perfused artificial CSF or drugs at a rate of 30 μl/min. Drug and DMSO vehicle were administered in a 30-μl volume. The artificial CSF was composed of 125 mM Na+, 2.6 mM K+, 0.9 mM Mg2+, 1.3 mM Ca2+, 122.7 mM Cl-, 21.0 mM NaHCO3, 2.4 mM sodium phosphate buffer, 120 μg/ml bovine serum albumin, 30 μg/ml bacitracin and 0.01% Triton X-100 to prevent sticking of the dynorphin to the tubing, and bubbled with 95% O2/5% CO2 immediately before use. Outflow for CSF occurred by making an midline skull incision to expose the bregma and cisterna. The cisternal membrane was opened and PE-50 tubing was placed in the open cisternal space. The outflow cannula rapidly collected perfusate (one 1.5-ml aliquot in 1 min) into polypropylene tubes on ice. The antinociceptive effects of CP55,940 are significant for %MPE in the tail-flick test (fig. 2). Dynorphin B i.t. produced antinociception with dynorphins, we would expect the dynorphins to induce antinociception. Our previous work indicates that dynorphins A(1–8), A(1–13) and A(1–17) produce antinociception (Pugh et al., 1996). We evaluated the antinociceptive effects of two additional dynorphins, α-neoendorphin and dynorphin B (Figure 1) and blockade of those effects by the respective antisera to the dynorphins (Figure 2). Our results indicated that α-neoendorphin (75 μg/mouse i.t.) produced a 78% MPE in the tail-flick test at 10 min before testing (time point of maximal antinociception) and after a 45-min vehicle (distilled water i.t.) pretreatment. The ED50 value for α-neoendorphin was 38 μg/mouse (95% CLs, 20–71; fig. 1). Pretreatment of mice with the kappa antagonist nor-BNI (3 μg/mouse i.t.) or distilled water vehicle at 5 min before α-neoendorphin (75 μg/mouse i.t.) significantly attenuated the antinociception produced by this endogenous opioid peptide (MPE = 12 ± 3%; fig. 2). The effects of a 5-min distilled water vehicle pretreatment (not shown in fig. 2) did not differ from a 45-min vehicle pretreatment (data shown in fig. 2). Pretreatment of mice with α-neoendorphin antisera (10 μg/mouse i.t.) 45 min before α-neoendorphin injection significantly decreased the antinociception from 78 ± 7% MPE to 23 ± 8% MPE in the tail-flick test (fig. 2). Dynorphin B i.t. produced

\[ \text{MPE} = \text{100} \times \frac{\text{C0} - \text{Ct}}{\text{C0}} \]

\[ \text{ED50} = 38 \mu\text{g/mouse} (95\% \text{ CLs, 20–71; fig. 1}). \]

\[ \text{Pretreatment of mice with the kappa antagonist nor-BNI (3 μg/mouse i.t.) or distilled water vehicle at 5 min before α-neoendorphin (75 μg/mouse i.t.) significantly attenuated the antinociception produced by this endogenous opioid peptide (MPE = 12 ± 3%; fig. 2). The effects of a 5-min distilled water vehicle pretreatment (not shown in fig. 2) did not differ from a 45-min vehicle pretreatment (data shown in fig. 2). Pretreatment of mice with α-neoendorphin antisera (10 μg/mouse i.t.) 45 min before α-neoendorphin injection significantly decreased the antinociception from 78 ± 7% MPE to 23 ± 8% MPE in the tail-flick test (fig. 2). Dynorphin B i.t. produced}
Characterization of the selectivity of dynorphin antisera administered i.t. to mice

Mice were injected i.t. with dynorphin antisera or IgG at either 60 min (dynorphin A(1–8) or A(1–17) antisera or IgG) or 45 min (α-neoendorphin or dynorphin B antisera) before various dynorphins. At 10 min later, the mice were tested using the tail-flick test. All the dynorphins tested represented the ED₈₀ dose range for the peptides. Because antisera to dynorphin B failed to attenuate the antinociceptive effects of dynorphin B, no further testing was performed with antisera to dynorphin B.

<table>
<thead>
<tr>
<th>Antisera to:</th>
<th>Dynorphin A(1–8) (125 µg/mouse)</th>
<th>Dynorphin A(1–17) (50 µg/mouse)</th>
<th>α-Neoendorphin (75 µg/mouse)</th>
<th>Dynorphin B (85 µg/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (distilled H₂O) pretreatment</td>
<td>97 ± 8%</td>
<td>88 ± 6%</td>
<td>78 ± 7%</td>
<td>75 ± 10%</td>
</tr>
<tr>
<td>Dynorphin A(1–8) (30 µg/mouse)</td>
<td>15 ± 5%&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>90 ± 3%</td>
<td>83 ± 8%</td>
<td>80 ± 9%</td>
</tr>
<tr>
<td>Dynorphin A(1–17) (30 µg/mouse)</td>
<td>99 ± 2%</td>
<td>30 ± 5%&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>81 ± 9%</td>
<td>71 ± 5%</td>
</tr>
<tr>
<td>α-Neoendorphin (10 µg/mouse)</td>
<td>95 ± 5%</td>
<td>82 ± 7%</td>
<td>23 ± 8%&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>76 ± 3%</td>
</tr>
<tr>
<td>Dynorphin B (10 µg/mouse)</td>
<td>94 ± 4%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89 ± 4%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78 ± 2%</td>
<td>79 ± 9%</td>
</tr>
<tr>
<td>IgG (10 µg/mouse)</td>
<td>89 ± 4%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78 ± 2%</td>
<td>79 ± 9%</td>
<td>79 ± 9%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from Pugh et al. (1996).

<sup>b</sup> Significant (P < 0.05) decrease from vehicle or IgG control pretreatments.

Characterization of the blockade of THC- and CP55-induced antinociception by dynorphin antisera administered i.t. to mice

Mice were injected i.t. with dynorphin antisera or IgG at either 60 min (dynorphin A(1–8) or A(1–17) antisera or IgG) or 45 min (α-neoendorphin antisera) before the cannabinoids. At 10 min later, the mice were tested using the tail-flick test. All the cannabinoids were tested using the ED₈₀ dose range for each (50 µg/mouse for THC and 2.5 µg/mouse for CP55). Because antisera to dynorphin B failed to attenuate the antinociceptive effects of dynorphin B, no further testing was performed with antisera to dynorphin B.

<table>
<thead>
<tr>
<th>Cannabinoid</th>
<th>THC (50 µg/mouse)</th>
<th>CP55,940 (2.5 µg/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (distilled H₂O) pretreatment</td>
<td>97 ± 8%</td>
<td>88 ± 6%</td>
</tr>
<tr>
<td>Antisera to:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dynorphin A(1–8) (30 µg/mouse)</td>
<td>19 ± 4%&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>73 ± 10%</td>
</tr>
<tr>
<td>Dynorphin A(1–17) (30 µg/mouse)</td>
<td>23 ± 8%&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>75 ± 8%</td>
</tr>
<tr>
<td>α-Neoendorphin (10 µg/mouse)</td>
<td>14 ± 4%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80 ± 4%</td>
</tr>
<tr>
<td>IgG (10 µg/mouse)</td>
<td>79 ± 5%</td>
<td>86 ± 6%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from Pugh et al. (1996).

<sup>b</sup> Significant (P < .05) decrease from vehicle or IgG control pretreatments.
A(1–8), A(1–13), A(1–17) and α-neoendorphin enhanced the antinociceptive potency of morphine as observed by a decrease in the ED$_{50}$ of morphine (table 3). The effects observed with dynorphins A(1–8), (1–13), and (1–17) were significant. The effect with α-neoendorphin approached significance (nearly a 5-fold shift in the morphine ED$_{50}$ value) but due to variability and wider 95% CLs was not a significant effect. However, using the highest inactive dose of dynorphin B (10 µg/mouse), no enhancement of the antinociceptive potency of morphine was observed.

We also performed a limited number of experiments to evaluate whether the enhancement of CP55,940- or dynorphin A(1–17)- or dynorphin B-induced antinociception could be enhanced by a low dose of morphine i.t. We have previously shown that an inactive dose of morphine i.t. shifts the dose–effect curve of THC to the left but that the shift produces a flattening of the slope of the THC curve and results in a nonsignificant (wide intervals) shift in the ED$_{50}$ value for THC (Welch and Stevens, 1992). Using a 10-min pretreatment with an inactive dose of morphine (0.1 µg/mouse) before dynorphin A(1–17), the ED$_{50}$ value was shifted from 20 (11–36) to 5 (2–10) µg/mouse. Unlike our previous results with THC, the effect was significant. Thus, dynorphin A appeared to somewhat mimic the effects of THC in terms of enhancement by morphine. The ED$_{50}$ value for dynorphin B was not shifted significantly [41 (26–54) µg/mouse] by morphine pretreatment. The ED$_{50}$ value for CP55,940 was also not altered by morphine pretreatment [1.5 (0.5–2.8) in the presence of morphine vs. 1.3 (0.2–2) in the presence of vehicle]. Thus, the enhancement of morphine-induced antinociception by dynorphin A(1–17) was bidirectional, whereas CP55,940 and dynorphin B were unaffected by pretreatment with morphine.

To further characterize the lack of interaction of dynorphin B with THC, we evaluated the cross-tolerance of dynorphin B to THC (fig. 3). Animals were rendered tolerant to THC as described in “Methods.” The ED$_{50}$ value (µg/mouse i.t.) for THC was significantly shifted by 6.7-fold in THC-tolerant mice [ED$_{50}$ = 11.5 (5.8–22.9) µg/mouse i.t. vs. 77.7 (45.6–132.5)]. Dynorphin B showed no cross-tolerance to THC. The ED$_{50}$ values (µg/mouse i.t.) for dynorphin B in the nontolerant vs. the THC-tolerant mice were 40 (21.7–75.8) and 49 (28.1–86), respectively. We have previously demonstrated that dynorphin A(1–17) is cross-tolerant to THC (Welch, in press).

table 3
Enhancement of morphine-induced antinociception by dynorphin A(1–8), dynorphin A(1–17) and α-neoendorphin but not by dynorphin B after i.t. administration to mice

<table>
<thead>
<tr>
<th>Pretreatment (i.t.)</th>
<th>Morphine (i.t.) ED$_{50}$ values (µg/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.62 (0.13–2.82)</td>
</tr>
<tr>
<td>Dynorphin A(1–8)*</td>
<td>0.04 (0.02–0.09)</td>
</tr>
<tr>
<td>Dynorphin A(1–17)</td>
<td>0.02 (0.01–0.10)</td>
</tr>
<tr>
<td>α-Neoendorphin</td>
<td>0.11 (0.09–1.17)</td>
</tr>
<tr>
<td>Dynorphin B</td>
<td>0.50 (0.12–1.76)</td>
</tr>
</tbody>
</table>

* Data from Pugh et al. (1996).

Cross-tolerance to CP55,940 was not evaluated due to the lack of adequate drug supplies of CP55,940 for such studies. Because dynorphin B, like CP55,940, failed to enhance morphine-induced antinociception, we hypothesized that CP55,940 might release dynorphin B (because the dynorphin A antisera studies appeared to rule out dynorphin A release by CP55,940). For these studies, the rat was used to obtain adequate dynorphin B for testing. Spinal cord perfusion with a 100 µg/rat dose of CP55,940 (ED$_{50}$ = CP55,940 in the rat; Lichtman and Martin, 1991a) resulted in an antinociceptive effect of 79 ± 2% MPE (n = 5 rats) vs. 16 ± 8% MPE (n = 7 rats) for the 100% DMSO vehicle. Dynorphin B levels were increased significantly from 5.4 ± 0.4 µg/ml in the DMSO-treated rats to 14.0 ± 2.8 pg/ml in the CP55,940-pretreated rats (fig. 4).

**Discussion**

Several attempts have been made to understand how the cannabinoids produce their pharmacological effects, particularly antinociception. Intrathecal administration of the cannabinoids in spinally transected rats has shown that both spinal and supraspinal mechanisms are involved in cannabinoid-induced antinociception (Lichtman and Martin, 1991a). In addition, it has been shown that cannabinoid and opiate receptors are co-localized in areas involved with the transmission of pain signals (Herkenham et al., 1990). Based on these studies, it is not unlikely that an interaction would occur between the cannabinoids and opiates in the production of antinociception. Additional evidence that indicates the
The existence of a cannabinoid/opiate functional interaction is the observation that THC ameliorates naloxone-precipitated withdrawal (Bhargava, 1976). Vaysee et al. (1987) have shown that high concentrations of THC inhibit agonist binding at mu and delta receptors but not kappa receptors. The kappa antagonist nor-BNI does not displace cannabinoid binding in brain or spinal cord (Welch, 1993); however, the kappa receptor seems to be important in mediating cannabinoid-induced antinociception. It was observed that the kappa receptor antagonist nor-BNI specifically blocked the antinociceptive effects of THC without altering its hypothermic, hypopactive or cataleptic effects (Smith et al., 1984). Subsequent studies designed to determine the nature of the THC/kappa receptor interaction indicate that THC interacts indirectly with the kappa receptor through endogenous opioid release (Pugh et al., 1996).

The endogenous opioid peptides are derived from three different gene families; each has a distinct anatomical distribution (Akil et al., 1984). Prodynorphin produces three main [Leu5]enkephalin-containing peptides: α/β-neoendorphin, dynorphin A and dynorphin B. High levels of dynorphins are found in the brain as well as the dorsal horn of the spinal cord (Lewis et al., 1982; Slater and Patel, 1983; Weber et al., 1982), show a high affinity for the kappa receptor and have been suggested as the endogenous ligands for the kappa receptor (Chavkin et al., 1982; Chavkin and Goldstein, 1981). In addition, the dynorphin A fragments, as well as dynorphin B, and α/β-neoendorphins have been shown to produce antinociception when administered i.t. (Han and Xie, 1982; Piercey et al., 1982). The release of kappa opioids by THC, in combination with the activation of mu receptors by morphine, has been attributed to the greater-than-additive antinociceptive effect produced by the THC/morphine combination.

The synthetic cannabinoid CP55,940 is more potent than THC in both in vivo and in vitro assays and has been useful in determining the site and mechanism of action of the cannabinoids (Welch, 1993; Welch et al., 1995; Welch and Stevens, 1992). The block of CP55,940-induced antinociception with nor-BNI and the lack of a greater-than-additive effect between CP55,940 and morphine in antinociceptive tests was hypothesized to be due to the release of a pool of endogenous kappa opioids that do not enhance morphine-induced antinociception. We concluded that dynorphin A fragments are not involved in mediating the antinociceptive effects of CP55,940 on the basis of antiserum studies. Furthermore, on the basis of data from previous experiments, we would not have expected CP55,940-induced antinociception to be mediated by such dynorphins because all of these dynorphin peptides administered i.t. enhance the antinociceptive potency of morphine in the spinal cord. In subsequent experiments, we examined the role of a different prodynorphin product, α-neoendorphin, on CP55,940-induced antinociception. We were able to demonstrate that α-neoendorphin does enhance the antinociceptive effects of morphine in the spinal cord and that antiserum to this peptide fail to alter CP55,940-induced antinociception. Thus, we concluded that CP55,940 does not modulate the activity of α-neoendorphin in the spinal cord.

Xie et al. (1986) have shown that dynorphin B produces antinociception in the spinal cord. Our studies replicate those of Xie et al. We observed that dynorphin B, unlike any of the other dynorphins we tested in combination with morphine, does not increase the antinociceptive potency of morphine. Similarly, morphine fails to enhance the antinociceptive effects of dynorphin B, an effect also observed with CP55,940. Thus, the effects of dynorphin B are similar to those of CP55,940 with respect to modulation by morphine. Dynorphin B is not cross-tolerant to THC, even though CP55,940 is cross-tolerant to THC and THC displaces CP55,940 binding (Smith et al., 1994). Dynorphin A is cross-tolerant to THC (Welch, 1996). Thus, THC appears linked in some unknown way to the modulation of dynorphin A, whereas CP55,940 appears to be linked to modulation of dynorphin B. Clearly, an interesting but technically difficult study would be to evaluate the cross-tolerances of dynorphins A and B to each other. If such a cross-tolerance were to be observed, it might enhance our understanding of the cross-tolerance of CP55,940 and THC.

Fujimoto et al. (1990) have also shown that dynorphin B does not enhance the antinociception of morphine in the spinal cord. We hypothesized that CP55,940-induced release of dynorphin B could account for the observed nor-BNI blockade of CP55,940, as well as for the lack of enhancement produced by the combination of CP55,940 and morphine in combination. Direct measurement of dynorphin B release by CP55,940 in animals that also showed antinociceptive effects of the peptide appears to confirm a role for dynorphin B in the action of CP55,940.

The existence of multiple cannabinoid receptor subtypes may underlie the differences seen between THC and CP55,940 in the spinal cord. We hypothesize that with the existence of multiple cannabinoid receptor subtypes, different pools of endogenous opioids may be altered by THC and CP55,940. Thus, the antinociceptive potency of morphine could be modulated differently depending on whether CP55,940 or THC pretreatment is evaluated.

We envision release of dynorphins as an indirect process due to the disinhibition of yet unknown neuronal processes. The localization of the cannabinoid receptors involved in

Fig. 4. Release of dynorphin B by CP55,940 (CP55) in rat spinal cord. Rats were used to obtain adequate dynorphin B for testing. The rat spinal cord was perfused with a 100 μg/rat dose of CP55,940 or DMSO vehicle in 30-μl volumes. CSF was perfused and collected (1.5 ml/min) for analysis of dynorphin B at 10 min later. Concurrent with the drawing of the CSF, the rats were evaluated in the tail-flick test for antinociceptive effects. Spinal cord perfusion with a 100 μg/rat dose of CP55,940 resulted in an antinociceptive effect of 79 ± 2% MPE (n = 5 rats) vs. 16 ± 8% MPE (n = 7 rats) for the 100% DMSO vehicle. Dynorphin B levels in pg/ml were determined as described in the text using 10 rats for CP55,940 and 7 rats for DMSO administration. *P < 0.05 from DMSO pretreatment.

**Table 1.** Summary of Dynorphin B Release

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dynorphin B Release (pg/ml)</th>
</tr>
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<tbody>
<tr>
<td>DMSO</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>CP55</td>
<td>12.0 ± 0.5</td>
</tr>
</tbody>
</table>

**Note:** DMSO = dimethyl sulfoxide.
dynorphin release is not known. We hypothesize that in the spinal cord, cannabinoids produce antinociceptive effects via the direct interaction of cannabinoids with G protein proteins, resulting in a decreased cAMP production (Welch et al., 1995), as well as hyperpolarization via interaction with specific potassium channels (Deadwyler et al., 1993). Thus, the cannabinoids produce disinhibition by decreasing the release of an inhibitory neurotransmitter in dynorphinergic pathways. The net result of such an effect is an increase in dynorphin release. The events that follow the release of dynorphin also remain unclear. The dynorphin most likely is a modulator of other "downstream" systems (possible substance P release or interaction with N-methyl-d-aspartate-mediated events) that culminate in antinociception on administration of cannabinoids.

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References


Welch, S. P.: Blockade of cannabinoid-induced antinociception by norbinaltor-

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