Antinociceptive Activity of Intrathecally Administered Cannabinoids Alone, and in Combination with Morphine, in Mice[1]

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Accepted for publication March 6, 1992

ABSTRACT

The antinociceptive effects of various cannabinoids, alone and in combination with opiates, were evaluated in antinociceptive tests in mice. The cannabinoids tested produce marked antinociceptive effects after i.t. administration to mice. The rank order of potency for the drugs using the tail-flick test was levonantradol > CP-55,940 = CP-56,667 > 11-hydroxy-Δ^9-THC > Δ^9-THC > Δ^8-THC; dextronantradol was inactive at a dose of 25 μg/ mouse. Respective ED_{50} values in the tail-flick test were 0.4, 12.3, 4.2, 15, 45 and 72 μg/mouse. Although pretreatment with morphine somewhat enhanced the effects of Δ^9-THC, pretreatment of the mice with naloxone (1 mg/kg s.c or 1 μg/mouse i.t.) failed to block the antinociceptive effects of the cannabinoids, indicating that the cannabinoid-induced antinociception does not occur due to direct interaction with the opiate receptor. Pretreatment of mice with 3.13 μg/mouse and 6.25 μg/mouse of Δ^9-THC shifted the ED_{50} of morphine to 0.15 and 0.05 μg/mouse, respectively (a 4-and a 12-fold shift). The shifts in the dose-response curve of the morphine were parallel. Naloxone administration (1 mg/kg s.c.) completely blocked the antinociceptive effects of the combination of 6.25 μg of Δ^9-THC with morphine. The AD_{50} for naloxone blockade of the drug combination was 0.24 (0.06–0.94) mg/kg s.c. and the pA_2 was 7.7 (6.7–8.9). The pA_2 for naloxone blockade of the dimethylsulfoxide-morphine combination was 6.9 (5.7–8.1). Parallel shifts in the morphine dose-response curve were also produced by pretreatment with 11-hydroxy-Δ^9-THC (3 μg/mouse i.t.), Δ^8-THC (25 μg/mouse) and levonantradol (5 ng/mouse), but not CP-55,940 (0.01 μg/ mouse), CP-56,667 (0.1 μg/mouse) or dextronantradol (25 μg/ mouse i.t.). Thus, the antinociceptive effects of i.t.-administered morphine are enhanced by the pretreatment with the cannabinoids. The exact nature of this interaction is yet to be determined.

Cannabinoids produce diverse behavioral, physiological and pharmacological effects after systemic administration among which is the production of antinociception (for review, see Pertwee, 1988). It is evident that Δ^9-THC induces analgesia only at doses producing other behavioral side effects and is no more potent than the more commonly used opiate analgesics. However, the antinociceptive effects of the cannabinoids after injection into spinal sites have not been investigated extensively. Levonantradol, desacetyllevonantradol and nantradol have been shown to produce antinociceptive effects upon i.t. administration to rats (Yaksh, 1981) and spinal administration to the dog (Gilbert, 1981) 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. It has been shown that binding sites for the cannabinoids are present in the spinal cord in the substantia gelatinosa, an area involved with the transmission of pain signals (Her-

kenham et al., 1990). The i.t. administration of a synthetic cannabinoid, CP-55,940, has been shown to produce antinociceptive effects in rats. These effects were attenuated partially in spinalized rats. These results indicate that the cannabinoid-induced antinociceptive effects in the rat are mediated at both spinal and supraspinal sites (Lichtman and Martin, 1991). In mice, Δ^9-THC (50 μg i.t.) produced the same degree of antinociception in mice that were spinalized as compared to mice that had the spinal cord intact (Smith and Martin, 1992). These data indicate that the effects of Δ^9-THC (i.t.) in mice appear to be predominantly spinally mediated.

A better understanding of the SARs in cannabinoid pharmacological activity has developed from the synthesis of various cannabinoids which are stereoisomeric pairs (Razdan, 1986). Levonantradol and its isomer, dextronantradol, are synthetic cannabinoids. In an early clinical study on postoperative pain, levonantradol produced potent analgesic effects, but like Δ^9-THC produced marked side effects (Jain et al., 1981). However, these drugs, along with the Pfizer cannabinoids (−)CP-55,940 and its (+)-isomer CP-56,667 (Johnson et al., 1981) are valuable tools in assessing the stereoselectivity of the activity of can-

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ABBREVIATIONS: THC, tetrahydrocannabinol; i.t., intrathecal; SAR, structure-activity relationship; DMSO, dimethylsulfoxide; % MPE, percentage of the maximum possible effect.

nabinoids (Koe *et al.*, 1985; Martin *et al.*, 1987). Numerous studies have been performed to evaluate the SARs for antinociceptive effects of a large number of Δ^8- and Δ^9-THC analogs after p.o. and i.v. administration to mice and rats. These studies (see reviews by Razdan, 1986; Pertwee,1988; as well as Koe *et al.*, 1985; Martin *et al.*, 1987; Little *et al.*, 1989) indicate that a SAR and stereoselectivity exist for the production of pharmacological activity by the cannabinoids, both of which are characteristics of a receptor mediation of the effects. The identification of a specific cannabinoid receptor has been the topic of intense investigation leading to a recent report that a putative cannabinoid receptor has been cloned (Matsuda *et al.*, 1990). This study, along with work evaluating the binding of the cannabinoid, CP-55,940 (Devane *et al.*, 1988), support the hypothesis of a cannabinoid receptor linked through a G protein to the modulation of cyclic-AMP.

The interaction of the cannabinoids with the opiates is an area of research in which ambiguities exist. It has been documented that the cannabinoids produce effects which have some characteristics in common with the opiates, such as antinociception, cross-tolerance to morphine and attenuation of naloxone-precipitated withdrawal from morphine (see Martin, 1985 for a review). Most investigators have shown that naloxone fails to block the effects of various parenterally administered cannabinoids (Chesher *et al.*, 1973; Sanders *et al.*, 1979; Martin, 1985). Naloxone also fails to block the antinociception induced by a variety of i.t.-administered cannabinoids (Yaksh, 1981). *In vitro*, the effects of Δ^9-THC on adenylyl cyclase have been shown to be insensitive to naloxone blockade and additive with the decrease in adenylyl cyclase observed with morphine (Bidaut-Russell and Howlett, 1988). However, the binding of opiates has been shown to be displaced by the cannabinoids, albeit at relatively high concentrations (Bloom and Hillard, 1985; Vaysse *et al.*, 1987). The binding of CP-55,940 has been shown to be dense in the striatum and substantia gelatinosa (Herkenham *et al.*, 1990). Both are areas associated with dense binding of the opiates (Gamse *et al.*, 1979; Yaksh *et al.*, 1988) and the substantia gelatinosa is the major site of the processing of pain signals for transmission to the spinothalamic tract (Yaksh *et al.*, 1988).

The purpose of this study was therefore to evaluate the antinociceptive effects of various cannabinoids after i.t. administration to mice and to determine the additivity or synergy between those cannabinoids (i.t.) and i.t.-administered morphine. In this way we hoped to gain insight into the antinociceptive effects of the cannabinoids at spinal sites and to determine interactions of the cannabinoids with the opiates in the production of antinociception.

Methods

Intrathecal injections. Intrathecal injections were performed after 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, 1/2 inch needle. Injection volumes of 5 μl were administered. The cannabinoids were prepared in 100% DMSO. Morphine sulfate was dissolved in distilled water. The cannabinoids or DMSO vehicle were administered 15 min before determination of the response latency of the mice in the tail-flick and the hot-plate tests. This time point represents the peak effect of the drugs, although significant antinociceptive effects were observed for 40 min after injection of all cannabinoids tested. Morphine or vehicle were injected 10 min before testing in the tail-flick and the hot-plate tests, the point at which maximal

antinociception occurs, although significant antinociceptive effects were observed for 45 min after the administration of the morphine. In the combination studies, the cannabinoids or vehicle were injected 15 min before morphine or vehicle and the animals were tested at 10 min later in the antinociceptive tests. The 15-min period between the injections was chosen to minimize the antinociceptive effect resulting from two i.t. injections. DMSO vehicle produced scratching behavior in mice which lasted 2 min after i.t. injection, but did not significantly effect antinociception 15 min after injection. Other vehicles were tested. Ethanol-saline (1:10) and emulphor-ethanol-saline (1:1:18) produced significant antinociceptive effects alone in the tail-flick test and were not used as the cannabinoid vehicle.

The tail-flick and hot-plate tests. The tail-flick procedure used was that of 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:

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

Percentage of MPE was calculated for each mouse using at least 12 mice per dose. By using the % MPE for each mouse, the mean effect and S.E.M. was calculated for each dose. Dose-response curves were generated using at least three doses of test drug. ED_{50} values were determined by log-probit analysis [a modification of the Litchfield Wilcoxon (1949) method omitting doses producing 100 or 0% MPE] and 95% CL were determined using the method of Litchfield and Wilcoxon (1949). The protocol for the hot-plate test was similar to that of the tail-flick test except that in the hot-plate test a copper plate was maintained at 58°C as a nociceptive stimulus. Control reaction times of 4 to 6 sec and a 20-sec cutoff time were used. The behavior quantified was licking of the hind paw or jumping by the mouse. The tail-flick test and the hot-plate test were performed as a battery on the same mice as long as no motor deficits were observed in the mice after drug administration. If motor deficits were observed, the hot-plate test was not used for testing.

In studies using naloxone blockade of the antinociceptive effects of the cannabinoids alone or in combination with morphine, naloxone was administered at 5 min before testing. Doses of naloxone tested *vs.* the cannabinoids alone were 1 mg/kg administered s.c. or 1 μg/mouse i.t. In the studies of the interaction of Δ^9-THC and morphine, the doses of naloxone tested were 0.01, 0.1, 0.3 and 1 mg/kg s.c. The blockade of the antinociceptive effects of the drugs by naloxone was calculated as follows:

$$\% \text{ blockade} = 1 - [(\%\text{MPE of drug} + \text{naloxone}/\% \text{ MPE of drug alone})] \times 100.$$

From this value the AD_{50} for naloxone blockade of antinociception was determined by using 4 doses of naloxone and the modified method of Litchfield and Wilcoxon (1949) as described above. The pA_2 values for naloxone blockade of the combination of Δ^9-THC plus morphine and DMSO vehicle plus morphine were calculated according to the method of Takemori *et al.* (1969). Confidence limits for the pA_2 were calculated using the method of Tallarida and Murray (1986).

Statistical analysis. Significant differences between treatment and control groups was determined using the Dunnett's *t* test (Dunnett, 1955). The dose-response curves were evaluated for the parallelism of shifts using the method of Colquhoun (1971).

Drugs. All of the cannabinoids were obtained from the National Institute on Drug Abuse (Rockville, MD) with the exception of CP-55,940, CP-56,667, levonantradol and dextronantradol which were obtained from Pfizer Central Research (New York, NY). All drug doses are expressed as micrograms or nanograms of the salt form of the drug used.

Results

The antinociceptive effects of Δ^9-THC were evaluated in the tail-flick and the hot-plate tests after i.t. administration to mice

(fig. 1). In the tail-flick test the peak effect of the Δ^9-THC occurred at 15 min after i.t. administration (ED_{50} = 45 μg/mouse = 148 nmol/mouse). The administration of 50 and 100 μg/mouse of Δ^9-THC produced equivalent antinociceptive effects to those observed with 25 μg/mouse. The maximal effect was elicited with 25 μg/mouse and was less than 80% MPE. The drug was prepared in DMSO vehicle (100% DMSO) which produced some scratching behavior in the mice for 2 min after the injection, but the DMSO vehicle did not produce antinociceptive effects (<18% MPE). The antinociception produced by Δ^9-THC in the hot-plate test was somewhat greater than that observed in the tail-flick test (ED_{50} = 37 μg/mouse), although these effects did not differ significantly. These results were obtained using 24 mice per dose. The effect of the opiate antagonist, naloxone (1 mg/kg s.c.), on i.t.-administered Δ^9-THC (25 μg/mouse)-induced antinociception was evaluated in the tail-flick and hot-plate tests using 12 mice per dose group. Pretreatment of the mice with naloxone failed to block the antinociceptive effects of the Δ^9-THC in either test (data not shown). In addition, the mice were injected with 1 μg/mouse of naloxone i.t. (data not shown) which failed to alter the antinociceptive activity of the Δ^9-THC. These results confirm those of Yaksh (1981) showing that the antinociceptive effects of another cannabinoid, levonantradol, are not blocked by the administration of naloxone. These results indicate that the Δ^9-THC-induced antinociception does not occur due to direct interaction with the *mu* opiate receptor.

The antinociceptive effects after i.t. administration of 11-hydroxy-Δ^9-THC, an active metabolite of Δ^9-THC, were evaluate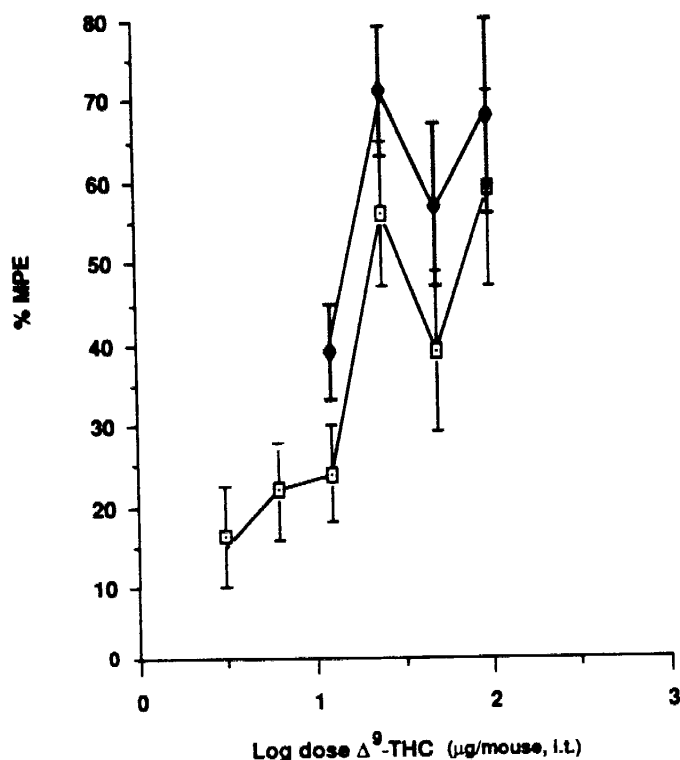d in the tail-flick and the hot-plate tests. The results are depicted in figure 2. 11-Hydroxy-Δ^9-THC produced antinociception in both tests [ED_{50} values = 15 (39 nmol) and 8 μg/mouse, respectively], although no effect greater than 85% MPE was observed. At doses higher than 50 μg/mouse the mice exhibited splaying of the hindlimbs and ataxia and were not tested in the hot-plate test. Δ^8-THC (fig. 3) also exhibited antinociceptive effects in the tail-flick (ED_{50} = 72 μg/mouse = 229 nmol/mouse) and the hot-plate (ED_{50} = 28 μg/mouse) tests. No antinociception greater than 70% MPE was observed at doses higher than 50 μg/mouse in the tail-flick test, although 100% MPE was achieved in the hot-plate test. Naloxone (1 mg/kg s.c or 1 μg i.t.) failed to block the antinociception produced by a dose of 50 μg/mouse of either drug (data not shown).

To evaluate the stereoselectivity of the antinociceptive effects of the i.t.-administered cannabinoids, the isomeric pairs levonantradol and dextronantradol and (−)CP-55,940 and its (+)-nantiomer, CP-56,667, were tested in the tail-flick and the hot-plate tests. Dextronantradol was totally inactive up to 25 μg/mouse in either test system. However, the ED_{50} calculated for levonantradol was 0.04 μg/mouse (84 pmol/mouse) (fig. 4). Thus, stereoselectivity of the response occurred with the administration of this pair of isomers. Levonantradol did not produce antinociception in the hot-plate test at the doses tested, but has been shown to produce antinociception in the hot-plate test at higher doses in rats (Yaksh, 1981). These data indicate that the levonantradol is more potent in the production of antinociception in the tail-flick test *vs.* the hot-plate test. Both

Fig. 1. Mice were injected i.t. according to the protocol described under "Methods" with Δ^9-THC in the doses listed. The drug was prepared in DMSO vehicle which produced 4% MPE in the tail-flick test and 18% MPE in the hot-plate test. Mice were tested 15 min after the i.t. injection. The % MPE was calculated as described previously using *n* = 24 mice per group. □, tail-flick test; ◆, hot-plate test.
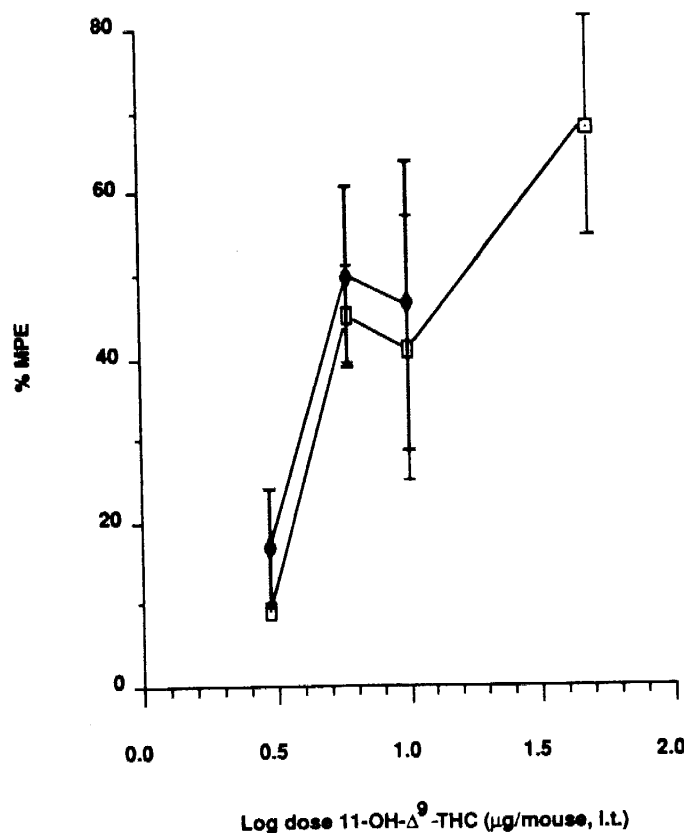

Fig. 2. Mice were injected i.t. according to the protocol described under "Methods" with 11-hydroxy-Δ^9-THC in the doses listed. The drug was prepared in DMSO vehicle which produced 4% MPE in the tail-flick test and 5% MPE in the hot-plate test. Mice were tested 15 min after the i.t. injection. The % MPE was calculated as described previously using *n* = 12 mice per group. □, tail-flick test; ◆, hot-plate test.
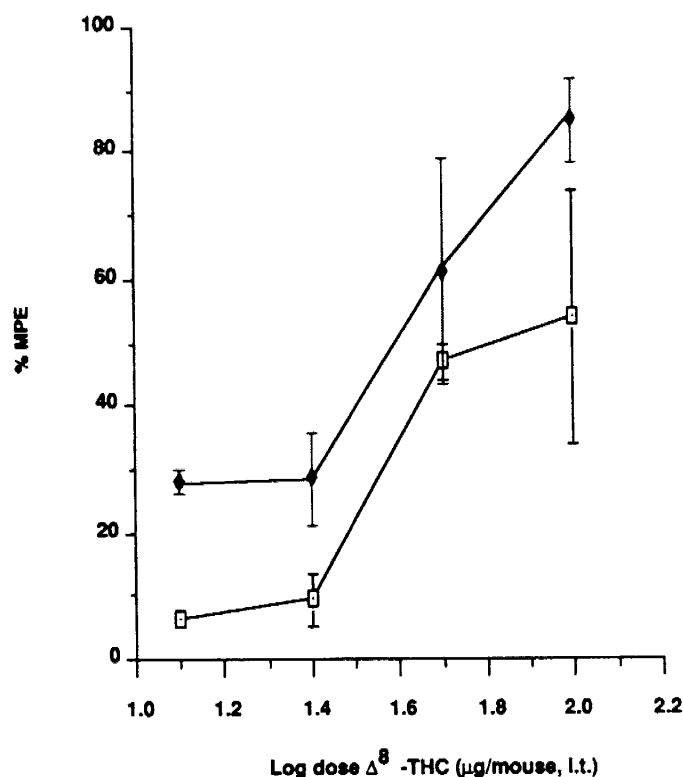
Log dose Δ^8 -THC (μg/mouse, I.t.)

Fig. 3. Mice were injected i.t. according to the protocol described under "Methods" with Δ^8-THC in the doses listed. The drug was prepared in DMSO vehicle which produced 5% MPE in the tail-flick test and 7% MPE in the hot-plate test. Mice were tested 15 min after the i.t. injection. The % MPE was calculated as described previously using $n = 24$ mice per group. □, tail-flick test; ◆, hot-plate test.

DOSE Dextronantradol   **DOSE Levonantradol**
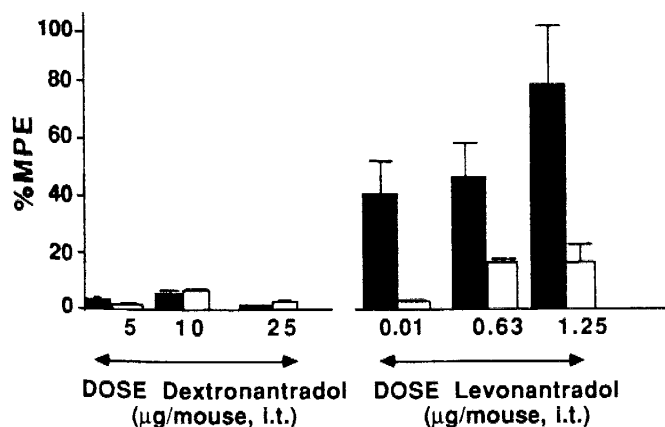   **(μg/mouse, I.t.)**   **(μg/mouse, I.t.)**

Fig. 4. Mice were injected i.t. according to the protocol described under "Methods" with dextronantradol or levonantradol in the doses listed. The drugs were prepared in DMSO vehicle which produced less than 10% MPE in the tail-flick and the hot-plate tests. Mice were tested 15 min after the i.t. injections. The % MPE was calculated as described previously using $n = 18$ mice per group. ■, tail-flick test; □, hot-plate test.

the CP-55,940 and CP-56,667 produced splaying of the hind-limbs of the mice precluding testing in the hot-plate test. CP-55,940 produced antinociception in the tail-flick test after i.t. administration.

The ED_{50} was 2.3 μg/mouse (5 nmol/mouse) (fig. 5). A maximal 100% response was achieved by using 10 μg/mouse of CP-55,940. CP-56,667 also produced antinociceptive effects in the tail-flick test ($ED_{50} = 4.2$ μg/mouse $= 11$ nmol/mouse), although higher doses (>10 μg/mouse) did not produce any

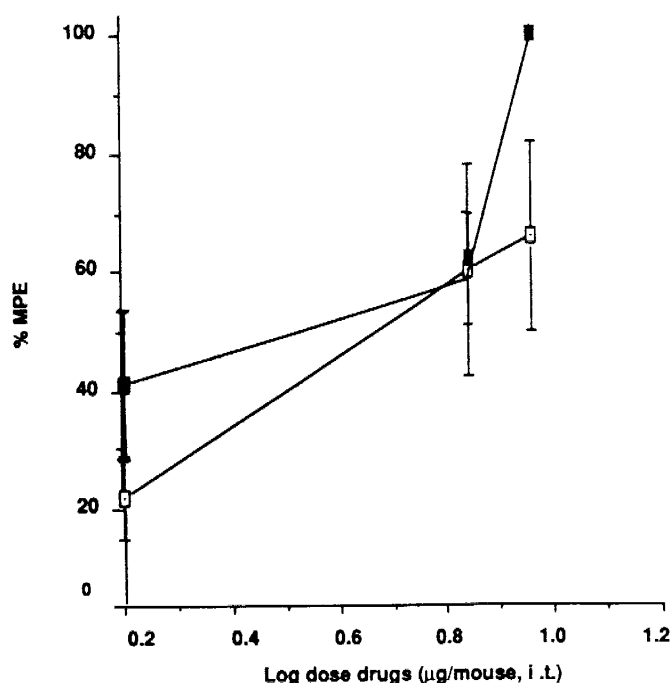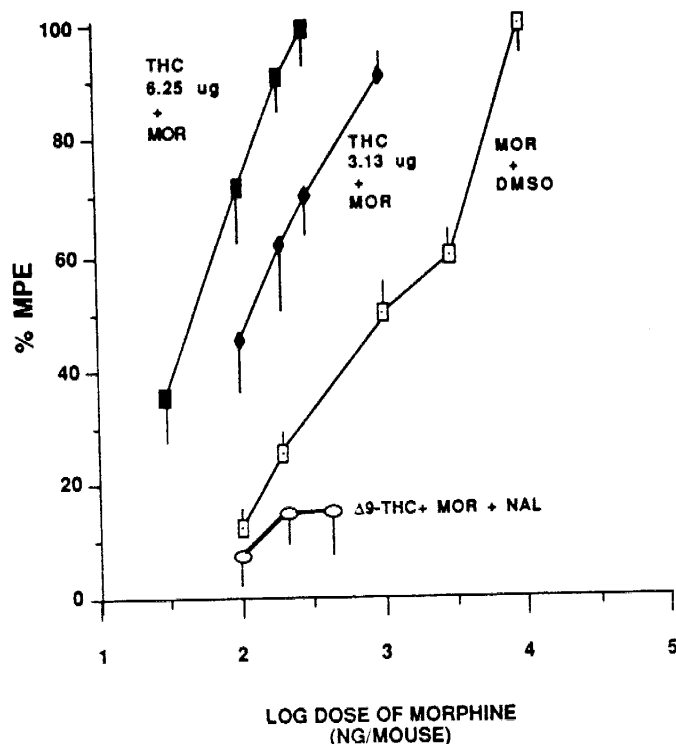**Log dose drugs (μg/mouse, i .t.)**

Fig. 5. Mice were injected i.t. according to the protocol described under "Methods" with CP-55,940 or CP-56,667 in the doses listed. The drugs were prepared in DMSO vehicle which produced less than 10% MPE in the tail-flick test. Mice were tested 15 min after the i.t. injections. The % MPE was calculated as described previously using $n = 12$ mice per group. ■, CP 55,940; □, CP 56,667.

effects greater than 60 to 70% MPE. Thus, these stereoisomers exhibited no significant stereoselectivity in the production of antinociception, but differed in efficacy. Naloxone (s.c. or i.t.) failed to alter the antinociceptive effects of maximally active doses of levonantradol, CP-55,940 or CP-56,667.
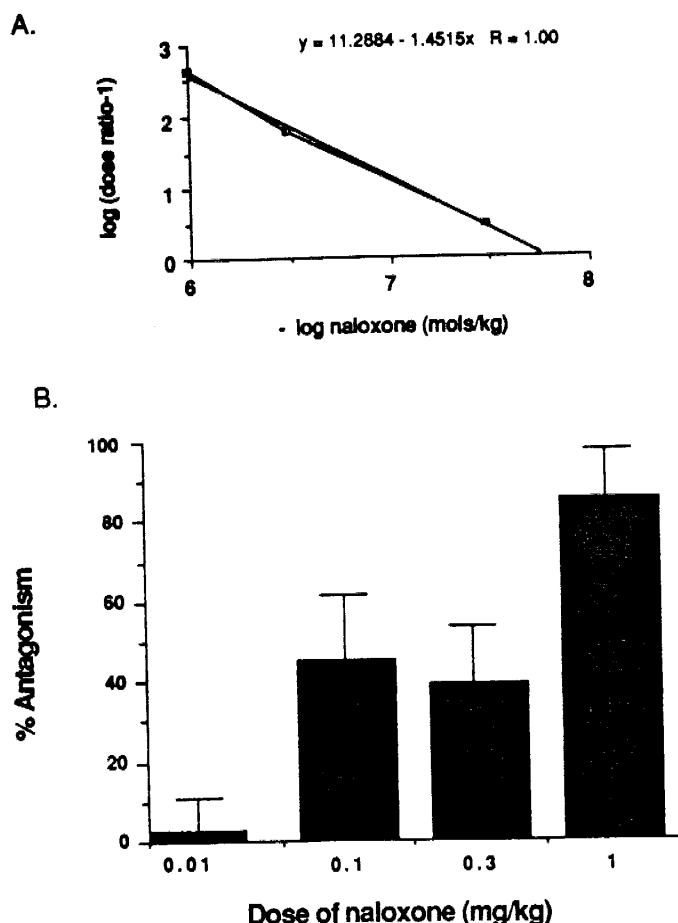
A study was performed using i.t.-administered Δ^9-THC in combination with i.t.-administered morphine in the tail-flick test. These results are shown in figure 6. Δ^9-THC at doses either devoid of antinociceptive effects or having marginal activity significantly shifted to the left the dose-response curve of i.t.-administered morphine. The ED_{50} of morphine (±95% CL) at 15 min after the DMSO vehicle was 0.61 (0.26–1.44) μg/mouse. Pretreatment with 3.13 μg/mouse of Δ^9-THC shifted the ED_{50} of morphine to 0.15(0.11–0.21) μg/mouse (a 4-fold shift). Pretreatment with 6.25 μg/mouse of Δ^9-THC shifted the dose-response curve of the morphine such that the ED_{50} for morphine was 0.05 μg/mouse (a 12-fold shift). The shifts in the dose-response curve of the morphine were parallel. These data indicate that Δ^9-THC appears to synergize with morphine in the production of antinociception when the drugs are administered i.t., although the Δ^9-THC does not appear to produce antinociception via direct interaction with the opiate receptor. However, naloxone administration (1 mg/kg s.c.) blocks completely the antinociceptive effects of the combination of 6.25 μg of Δ^9-THC with morphine. The AD_{50} for naloxone blockade of the drug combination was 0.24 (0.06–0.94) mg/kg s.c. (fig. 7B). The pA_2 determined for naloxone blockade of the Δ^9-THC/morphine interaction was 7.7 (6.7–8.9) (fig. 7A). The pA_2 determined for naloxone blockade of the DMSO-morphine interaction was 6.9 (5.7–8.1). The pA_2 for naloxone blockade of i.t. morphine in rats has been shown to be approximately 7 (Schmauss and Yaksh, 1984) and is 7.35 in mice (Roerig et al.,

THC
6.25 ug
+
MOR

THC
3.13 ug
+
MOR

MOR
+
DMSO

% MPE

Δ9-THC+ MOR + NAL

LOG DOSE OF MORPHINE
(NG/MOUSE)

Fig. 6. Mice were injected i.t. according to the protocol described under "Methods" with Δ^9-THC (3.13 or 6.25 μg/mouse) or DMSO vehicle at 15 min before morphine (MOR) in distilled water vehicle using the doses listed. The injection of DMSO vehicle i.t. 10 min before distilled water vehicle i.t. produced 15% MPE in the tail-flick test. Mice were tested 10 min after the i.t. injection of MOR. The % MPE was calculated as described previously using $n = 24$ mice per group. To evaluate naloxone (NAL) reversal of the antinociceptive effects of the drug combination, 6.25 μg of Δ-THC was inked 15 min before MOR. Ten minutes later the mice were injected with NAL (1 mg/kg s.c.). The mice were tested 5 min later in the tail-flick test.

A.

$y = 11.2884 - 1.4515x$ $R = 1.00$

log (dose ratio-1)

- log naloxone (mols/kg)

B.

% Antagonism

Dose of naloxone (mg/kg)

Fig. 7. Mice were injected i.t. according to the protocol described under "Methods" with Δ^9-Thc (25 μg/mouse) or DMSO vehicle 15 min before morphine (0.2 μg/mouse) or distilled water vehicle. Mice were injected with naloxone in the doses listed or distilled water vehicle s.c. at 10 min after the i.t. injection of the morphine or its vehicle and 5 min before testing in the tail-flick test. The percentage of blockade was calculated as described previously using $n = 12$ mice per group. The pA_2 (A) and the AD_{50} (B) for naloxone blockade of the antinociceptive effects of the drugs in combination was calculated as described under "Methods." All vehicles were devoid of significant antinociceptive activity (% MPE less than 10%). The AD_{50} for naloxone blockade of the combined effects of THC and morphine was 0.24 (0.06–0.94) mg/kg s.c. The pA_2 was 7.77.

1987), and thus DMSO pretreatment does not alter sgnificantly the affinity of naloxone for the opiate receptor. There is no significant difference in the pA_2 value for naloxone blockade of the THC-morphine interaction *vs.* the DMSO-morphine interaction.

Similar parallel shifts in the morphine dose-response curve were produced by pretreatment with 11-hydroxy-Δ^9-THC (3 μg/mouse i.t.), Δ^8-THC (25 μg/mouse) and levonantradol (5 ng/mouse). However, CP-55,940 (0.01 μg/mouse i.t., fig. 8), CP-56,667 (0.1 μg/mouse i.t.) and dextronantradol (25 μg/mouse i.t.) (both not shown) pretreatment did not significantly alter the antinociceptive activity of morphine. The effect of CP-55,940 and CP-56,667 in combination with morphine resulted in an additive interaction when doses producing significant antinociception were tested. All shifts in the morphine ED_{50} values ($\pm$ 95% CLs) with the cannabinoid pretreatments are summarized in table 1. An inactive dose of morphine (0.1 μg/ mouse) failed to significantly alter the antinociceptive effects of Δ^9-THC (fig. 9). Although a leftward shift in the dose-effect curve for the Δ^9-THC was observed in combination with morphine, no significant shift in the ED_{50} was observed due to the large variability of the CL around the ED_{50}. The variability was due to the lack of an 80% response in the mice and the flatness of the dose-effect curve. The ED_{50} for Δ^9-THC in combination with vehicle (distilled water) was 20.3 μg/mouse (6.7–61) and was shifted to 1.84 μg/mouse (0.2–18.8) in combination with

morphine. The effect of morphine (0.1 μg/mouse i.t.) in combination with DMSO vehicle i.t. was 15% MPE ($\pm$5). The effect of distilled water i.t. in combination with DMSO i.t. was 10% MPE ($\pm$5). The slopes of the dose-response curves do not deviate from parallelism.

Discussion

The cannabinoids tested produce marked antinociceptive effects after i.t. administration to mice. The rank order of potency for the drugs using the tail-flick test was levonantradol > CP-55,940 = CP-56,667 > 11-hydroxy-Δ^9-THC > Δ^9-THC >Δ^8-THC; dextronantradol was inactive at a dose of 25 μg/ mouse. This rank order is similar to that previously published data for the antinociceptive effects of i.v.-administered cannabinoids and binding data for the drugs with the exception of the effect of the CP-56,667 (Howlett *et al.*, 1990). After i.v. administration this isomer has been shown to be 6-fold less active in the tail-flick test than the CP-55,940, greater than
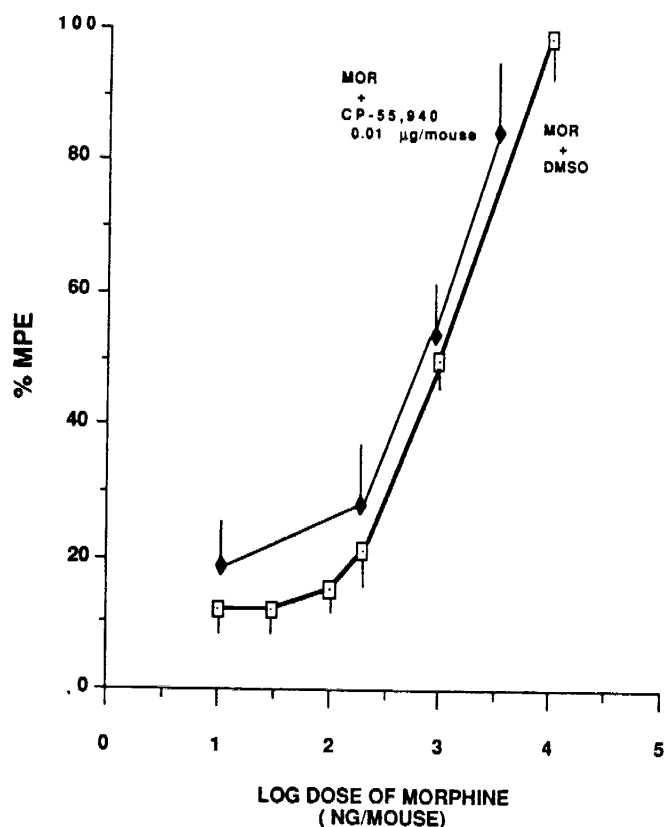
Fig. 8. Mice were injected i.t. according to the protocol described under "Methods" with CP-55,940 (0.01 μg/mouse) or DMSO vehicle 15 min before morphine (MOR; in distilled water vehicle) using the doses listed. The injection of DMSO vehicle i.t. 10 min before distilled water vehicle i.t. produced 10% MPE in the tail-flick test. Mice were tested 10 min after the i.t. injection of MOR. The % MPE was calculated as described previously using $n = 12$ mice per group.

100-fold less active in the phenylbenzylquinone writhing test (Johnson and Melvin, 1986; Little *et al.*, 1988) and to have 30- to 50-fold less affinity in binding studies (Howlett *et al.*, 1988). Although the antinociceptive potency of CP-55,940 and CP-56,667 did not differ, a difference in efficacy was observed. CP-55,940 produced full agonist effects, whereas CP-56,667 did not produce greater than 85% MPE at the doses tested. In addition, both drugs produced splaying of the hind limbs of the mice at all active doses. It is difficult to explain why stereoselectivity was not observed with these drugs. Although the mice did not appear to have any difficulty in "flicking" their tails, splayed hind limbs were observed and thus we cannot rule out the possibility that motor problems interfered with the testing and resulted in an overestimation of the antinociceptive effect of both of the drugs. This could result in the lack of the ability to measure stereoselective effects. Alternatively, after i.v. administration the CP-56,667 has been shown to be only 6-fold less potent than the CP-55,940 (Little *et al.*, 1988). This is not a high degree of stereoselectivity. After i.v. administration the drugs would likely interact with both spinal and supraspinal sites, whereas preliminary distribution studies in our laboratory indicate that CP-55,940 remains in high concentrations in the spinal cord after i.t. administration to mice (Smith and Martin, 1992). It is thus possible that the binding sites for the drugs in brain and spinal cord differ, with stereoselective differences in the potency of the drugs being observed upon interaction with brain but not spinal sites. Additional evidence to suggest that

TABLE 1

ED_{50} values (microgram per mouse) and 95% confidence levels for morphine in combination with DMSO vehicle or various cannabinoids in the tail-flick test in mice

Mice were injected i.t. according to the protocol described under "Methods" with cannabinoids or DMSO vehicle at 15 min before morphine. Mice were tested at 10 min after the i.t. injection of morphine. The average % MPE for each group was calculated as described previously. The ED_{50} for morphine alone, or in the presence of the cannabinoids or DMSO vehicle, was calculated as described under "Methods" using at least 3 doses of the morphine. This figure summarizes the shifts in the ED_{50} of morphine. All curves were tested for parallelism of the shift as described under "Methods."

Drug	ED_{50} of Morphine
	μg/mouse
None	1
	(0.6–1.4)
DMSO	0.61
	(0.26–1.44)
Δ^9-THC (3.13 μg/mouse)	0.15
	(0.11–0.21)
Δ^9-THC (6.25 μg/mouse)	0.05
	(0.03–0.08)
Δ^8-THC (25 μg/mouse)	0.05
	(0.02–0.10)
Levonantradol (0.005 μg/mouse)	0.06
	(0.01–0.24)
11-Hydroxy-Δ^9-THC (3 μg/mouse)	0.08
	(0.04–0.19)
Dextronantradol	0.51
	(0.36–0.89)
CP 55,940 (0.01 μg/mouse)	0.3
	(0.09–1)
CP 56,667 (0.5 μg/mouse)	0.26
	(0.08–0.82)

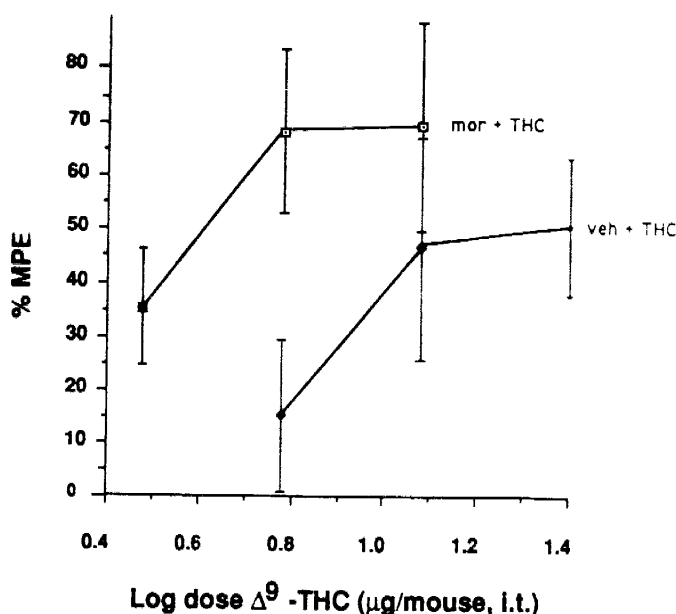**Log dose Δ^9 -THC (μg/mouse, i.t.)**

Fig. 9. Mice were injected i.t. according to the protocol described under "Methods" with morphine (mor; 0.1 μg/mouse) or vehicle (veh) in combination with Δ^9-THC or DMSO vehicle. Mice were tested 10 min after the i.t. injection of mor. The % MPE and ED_{50} values were calculated as described previously using $n = 6$ mice per group.

these drugs may differ in their spinal mechanism of action from the other cannabinoids tested comes from the lack of potentiation of morphine's antinociceptive effects by CP-55,940 and CP-56,667 (table 1). These data support the hypothesis of differences in the binding sites for the cannabinoids at the spinal level.

The cannabinoids do not appear to produce antinociception by interaction at the opiate receptor due to the lack of naloxone (s.c. or i.t.) blockade of the antinociceptive effects of the drugs. This is consistent with the reported lack of blockade of the effects of the cannabinoids by naloxone in many systems (Chesher *et al.*, 1973; Sanders *et al.*, 1979; Martin, 1985), although some investigators have found opiate antagonists effective in blocking the effects of cannabinoids (Wilson and May, 1975; Tulunay *et al.*, 1981). This hypothesis is also consistent with the lack of a significant shift of the Δ^9-THC dose-effect curve by an inactive dose of morphine (fig. 9). However, the total blockade of the antinociceptive effects of Δ^9-THC and morphine in combination by naloxone also indicates a possible interaction between these drugs at the opiate receptor. The pA_2 for naloxone blockade of the THC/morphine interaction was 7.7 and did not differ from that for DMSO/morphine (6.9) or the pA_2 for naloxone *vs.* i.t. morphine (7) reported previously in rats (Schmauss and Yaksh, 1984) or in mice (7.35) (Roerig *et al.*, 1987). Inasmuch as we are using an inactive dose of the cannabinoid in combination with morphine, it is possible that the naloxone blocks the effects of the morphine only. The cannabinoid may interact at a nonopiate site to enhance the effect of the morphine. However, we cannot eliminate the possibility that the interaction occurs at an opiate receptor.

Thus, we hypothesize that the antinociceptive effects of the cannabinoids after i.t. administration are due to interaction with a cannabinoid-specific binding site or sites which may be similar to or distinct from those sites involved in the cannabinoid/opiate interaction. The site for the interaction of the cannabinoids with morphine shows stereoselectivity to the effects of levonantradol and dextronantradol and the potency of the cannabinoids in enhancing the effects of morphine are related to their antinociceptive potency, with the notable exception of the CP-55,940 and CP-56,667. Although both of these drugs are active in antinociceptive tests, neither drug synergized with morphine in the production of antinociception. When active doses of either of these two isomers were administered i.t. before morphine, the antinociceptive effects observed were additive with those of morphine (data not shown). The lack of synergism between morphine and these two drugs may indicate the presence of different types of binding sites or receptor subtypes for the cannabinoids in the spinal cord. Because to date all of the research toward elucidation of the cannabinoid receptor has been done using brain tissue or cultured cells (Devane *et al.*, 1988; Matsuda *et al.*, 1990), the hypothesis of different types of cannabinoid receptors in the spinal cord remains untested.

Although the mechanism of the interaction of the cannabinoids with the opiates is yet to be shown, several possibilities for points of interaction exist. The parallel shift in the morphine dose-effect curve by the cannabinoids is a necessary, but insufficient, criterion for an interaction at the opiate receptor. An interaction of the cannabinoids and the opiates involving complex neuronal circuits or independent mechanisms cannot be ruled out. Although the parallel shift in the morphine dose-effect curve by the cannabinoids could be the result of chance alone, this is highly unlikely because this parallel shift was observed using several cannabinoids. The cannabinoids may act allosterically to alter the binding of the opiates. Some previous studies indicate that cannabinoids at concentrations higher than 1 μM displace the binding of *mu* and *delta* opioids in a noncompetitive manner (Bloom and Hillard, 1985; Vaysse

et al., 1987). However, in another study, the cannabinoids failed to alter *delta*-opioid binding (Devane *et al.*, 1986). All of these studies have utilized high concentrations of the cannabinoids for the displacement of the opiate binding. It is possible that at low concentrations the cannabinoids enhance rather than displace the binding of the opiates. That low concentrations the drugs may allosterically enhance the binding of the opiates in the spinal cord is one possible site for the interactions of the drugs. Investigations of this interaction are being pursued in our laboratory. Other investigators have found the cannabinoids to enhance the binding of *beta* adrenergics as well as flunitrazepam (Hillard and Bloom, 1982; Koe *et al.*, 1985), possibly by either altering the membrane fluidity or the calcium fluxes across the membrane.

The synergism between the opiates and the cannabinoids might also be due to the interplay of the opiates and cannabinoids in the modulation of neuronal second messenger systems. Opiates have been shown to decrease brain cyclic-AMP levels (Barchfeld *et al.*, 1982; Gentleman *et al.*, 1983) and thus synergistic interaction of the cannabinoids with the opiates could reflect interactions with cyclic-AMP. The cloning of the putative cannabinoid receptor was performed in conjunction with an evaluation of cyclic-AMP modulation after receptor activation (Matsuda *et al.*, 1990). Studies in NG108-15 cells have shown that cannabinoid-induced inhibition of cyclic-AMP formation is rapid and reversible (Howlett, 1985; Dill and Howlett, 1988), occurs at low concentrations of the cannabinoids (Howlett *et al.*, 1986), follows a SAR and stereoselectivity similar to that observed for psychoactivity (Howlett and Fleming, 1985; Howlett *et al.*, 1990), is not blocked by antagonists of other classical neurotransmitters (Devane *et al.*, 1986) and is mediated *via* coupling to the G_i protein (Howlett *et al.*, 1988). All of the above studies have utilized brain tissue or cells in culture. No studies using the spinal cord have been performed. The effects of Δ^9-THC on adenylyl cyclase have been shown to be insensitive to naloxone blockade and additive with the decrease in adenylyl cyclase observed with morphine (Bidaut-Russell and Howlett, 1988). However, etorphine and desacetyllevonantradol were neither additive nor synergistic in decreasing cyclic-AMP accumulation. The authors concluded that the two drugs may utilize a common second messenger for an effector system (Devane *et al.*, 1986).

Another point or potential interaction of the cannabinoids and the opiates is in the regulation of cell calcium. Previous work has indicated that Δ^9-THC decreases the release of acetylcholine presynaptically in frog nerve. This effect was proposed to occur due to a decrease in the influx of calcium into presynaptic nerve terminals (Kumbaraci and Nastuk, 1980). Similar studies have shown that cannabinoids suppress neuronal transmission (Niemi, 1979), enhance neuronal transmission (Turkanis and Karler, 1986) or produce biphasic effects on neuronal transmission (Tramposch *et al.*, 1981). Harris and Stokes (1982) found that cannabinoids decrease calcium uptake to several brain regions, an effect which did not correlate to the psychoactivity of the drugs. The only measurement of the effects of cannabinoids on free intracellular calcium (using the indicator fura-2) has shown that depolarization-induced rises in intracellular calcium are attenuated by Δ^9-THC in concentrations of 1 μM or higher (Martin *et al.*, 1988). These concentrations are similar to those required for the alteration of neuronal transmission (Kumbaraci and Nastuk, 1980), but higher than that required to block calcium uptake (Harris and

Stokes, 1982). The difference may be due to the fact that Harris and Stokes (1982) used brain regions for the study of calcium uptake. The effects of ions on the antinociceptive effects of the opiates have been investigated extensively (Harris *et al.*, 1976; Way, 1978; Guerrero-Munoz and Fearon, 1982) leading to the discovery that modulation of the antinociceptive effects of morphine in the brain by many ions, ionophores and calcium channel blockers is opposite to that observed in the spinal cord (Lux *et al.*, 1988). This finding is an important lead for the understanding of the mechanism of supraspinally and spinally mediated opiate antinociception. Because calcium modulation has been implicated in the actions of Δ^9-THC and the opiates, it may be a point for the interaction of the two drug classes.

Thus, in summary, the cannabinoids produce antinociceptive effects upon i.t.-administration to mice. These effects are not blocked by naloxone, but are somewhat enhanced by the administration of morphine i.t. and therefore are not mediated by direct action at the opiate receptor. However, the antinociceptive effects of i.t.-administered morphine are enhanced by the pretreatment with the cannabinoids. Whether the cannabinoids and the opiates act at distinct sites or a similar site and the exact nature of this interaction is yet to be determined. The interaction may involve allosteric modulation of opiate binding by the cannabinoids or interaction of the two drug classes with common neuroeffector systems in the cells.

Acknowledgments

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