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Modulation of Cannabinoid-Induced Antinociception After Intracerebroventricular *Versus* Intrathecal Administration to Mice: Possible Mechanisms for Interaction with Morphine[1]

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ABSTRACT

Dose-effect curves were generated for the cannabinoids [intracerebroventricularly (icv.)] and compared with those previously generated after administration intrathecally (i.t.). The ED_{50} values after administration of levonantradol, CP 55,940, Δ^9-THC and Δ^8-THC i.t. *vs.* icv. did not differ significantly. CP 56,667 was significantly more potent after icv. administration than i.t. administration, and was nearly 10 times more potent than CP 55,940 (icv.). CP 55,940 and CP 56,667, which did not produce greater than additive effects in combination with morphine when the drugs were administered i.t., shifted the morphine (icv.) dose-effect curve in a parallel manner nearly 10-fold after icv. administration. The antinociceptive effects of the cannabinoids (icv.) were not blocked by ICI 174,864 (20 μg/mouse), nor-BNI (70 μg/mouse) or naloxone (20 μg/mouse or 10 mg/kg s.c.). Pertussis toxin pretreatment i.t. for 7 days totally abolished the antinociception produced by the cannabinoids (icv. and i.t.). Pretreatment of the mice with forskolin (i.t.) or Cl-cAMP (10 μg/mouse i.t.), which produced no antinociception, significantly attenuated the antinociception produced by the Δ^9-THC and CP 55,940. However, when administered icv., forskolin and Cl-cAMP produced antinociception, but did not block or produce greater than additive effects with the antinociception produced by the cannabinoids adminis icv. The i.t. administration of calcium and calcium modul failed to alter the antinociception produced by the i.t. adn tration of the cannabinoids. Conversely, calcium (icv.) blo the antinociceptive effects of the cannabinoids. The AD ues (±CL) for calcium-induced block of Δ^9-THC, Δ^8-THC CP 55,940 were 215 (94–489), 176 (122–253) and 123 (81- nmol/mouse, respectively. ω-Conotoxin (1 μg/mouse which did not alter the antinociceptive effects of Δ^9-THC nificantly reversed the calcium-induced blockade of Δ^9- Thapsigargin (icv.) blocked the antinociception produce Δ^9-THC and CP 55,940. Apamin, blocker calcium-gated p sium channels, produced a parallel rightward shift in the c effect curves of Δ^9-THC, Δ^8-THC and CP 55,940 (i.t.). How apamin (5 ng/mouse icv.) failed to block icv. administered nabinoids. Because acute administration of opiates/op have been shown to interact with $G_{i/o}$ protein-coupled re tors, decrease calcium entry to and content of neurons, re cAMP levels and produce hyperpolarization of neurons *via* ATP- and apamin-sensitive potassium channels, these intracellular systems may be common points of interaction the cannabinoids.

Recent work has shown that the cannabinoids produce potent antinociceptive effects in mice and rats through both spinal and supraspinal mechanisms (Welch and Stevens, 1992; Welch, 1993; Smith and Martin, 1992; Lichtman and Martin, 1991a). Although the spinal mechanism of cannabinoid antinociception in the rat has been proposed to involve the *alpha*-2 adrenergic receptor (Lichtman and Martin, 1991b), the mechanism by which cannabinoids produce antinociception has not been fully determined. The ident tion of a specific cannabinoid receptor has been the to intense investigation leading to the cloning of a cannab receptor (Matsuda *et al.*, 1990). In addition, an endoge ligand for the cannabinoid receptor has been identified ane *et al.*, 1992). These reports led to the next logical tion—What are the transduction mechanisms assoc with the activation of the cannabinoid receptor? Severa sible transduction mechanisms have been proposed inclu modulation of adenylate cyclase, calcium, prostaglandin opioids. The role of adenylate cyclase, calcium modul and potassium channel modulation, and to a lesser exte

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ABBREVIATIONS: i.t., intrathecally; icv, intracerebroventricularly; i.v., intravenously; s.c., subcutaneously; %MPE, percentage of the max possible effect; THC, tetrahydrocannabinol; DPDPE, [D-Pen^{2,5}]-enkephalin; nor-BNI, nor-binaltorphimine; ICI 174,864, N,N,diallyl-Tyr-Ali Leu; CL, confidence limit; AD_{50}, dose producing 50% antagonism; Cl-c-AMP, 8-(4-chlorophenylthio)-adenosine-3′:5′-monophosphate monosodium salt; 4-AP, 4-aminopyridine; TEA, tetraethylammonium; db-cAMP, dibutyryl cyclic 3′:5′-adenosine monophosphate monos salt; DMSO, dimethyl sulfoxide.

role of opioids, as possible mechanisms in the antinociceptive effects of the cannabinoids is the focus of this paper. These mechanisms will be discussed individually, although the interplay of these various systems in the transduction of the effects of the cannabinoids is clearly a possibility.

cAMP. The cloning of the cannabinoid receptor was performed in conjunction with an evaluation of cAMP modulation after receptor activation (Matsuda *et al.*, 1990). Anandamide, the endogenous cannabinoid ligand, has also been shown to inhibit cAMP production in cells (Vogel *et al.*, 1993). Numerous studies have shown the involvement of the cannabinoids in the modulation of cAMP levels in cells of various types in culture as well as in homogenates of brain regions (Kelly and Butcher, 1979; Zimmerman *et al.*, 1981; Li and Ng, 1984; Howlett and Fleming, 1984; Howlett, 1984; Howlett, 1985). The interaction of the cannabinoids with a membrane protein *via* ADP-ribosylation was shown. The ribosylated protein was identified as the G_i protein (Howlett *et al.*, 1986). The potency of numerous cannabinoids to inhibit cAMP formation in the neuroblastoma cells was found to correlate to the antinociceptive effects of the drugs *in vivo* (Howlett *et al.*, 1988). The effects of the cannabinoids on spinal cAMP accumulation have not been examined. This study utilizes modulators of adenylate cyclase or cAMP levels *in vivo* to evaluate the effects of such treatments on the antinociceptive effects of the cannabinoids in the brain and in the spinal cord.

Calcium/potassium. An alteration of intracellular calcium by the cannabinoids could be the trigger for a cascade of events leading to decreased neuronal transmission and antinociception. Previous work has indicated that Δ^9-THC decreases the release of acetylcholine presynaptically by a decrease in the influx of calcium into presynaptic nerve terminals (Kumbaraci and Nastuk, 1980). 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. Direct measurement of the effects of cannabinoids on free intracellular calcium in brain tissue (using fura-2/AM) 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). Although *in vitro* studies indicate a role for calcium in the effects of the cannabinoids, *in vivo* studies of the involvement of calcium in the antinociceptive effects of the cannabinoids have not been reported previously. In addition, potassium channel opening and the resultant hyperpolarization of the neuronal membrane could account for the antinociceptive effects of cannabinoids. The modulation of potassium channels by the cannabinoids may differ in the brain and in the spinal cord. The involvement of potassium channels in cannabinoid-induced effects has been reported *in vitro* (Childers *et al.*, 1992), although the nature of the interaction of the drugs with potassium channels has not been determined.

Opioids. The role of opioids in cannabinoid antinociception is unclear. Many investigators have shown that naloxone fails to block the effects of various parenterally administered cannabinoids (Chesher *et al.*, 1973; Chesher and Jackson, 1985; Sanders *et al.*, 1979; Martin, 1985). Naloxone (s.c., icv. or i.t.) also failed to block the antinociception induced by a variety of i.t., icv. or spinally administered cannabinoids (Yaksh, 1981; Gilbert, 1981; Welch and Stevens, 1992; Welch, 1993). It is intriguing that, despite the data suggesting that cannabinoids and opiates have distinct mechanisms of action, the effects of morphine have been found to be enhanced by crude cannabis extract (Ghosh and Bhattacharya, 1979) and by orally administered Δ^6-THC and Δ^9-THC (Mechoulam *et al.*, 1984). Intrathecal administration of several cannabinoids leads to synergism with i.t. administered morphine in the production of antinociception in mice (Welch and Stevens, 1992; Smith and Martin, 1992; Smith *et al.*, 1994). Recently, the blockade of cannabinoid antinociception by the *kappa* opioid antagonist, nor-binaltorphimine (nor-BNI) has been reported (Welch, 1993). The blockade by nor-BNI of the Δ^9-THC-induced (i.t.) antinociception, but not catalepsy, hypothermia or hypoactivity has been also reported (Smith *et al.*, 1994). Although we have evaluated the effects of opioid antagonists on the antinociceptive effects of i.t. administered cannabinoids, and the interaction of cannabinoids (i.t.) with morphine (i.t.) (Welch and Stevens, 1992; Welch, 1993), the interaction of icv. administered cannabinoids with icv. administered opioids and opioid antagonists has not been reported previously.

Thus, the research presented in this manuscript was designed to evaluate the involvement of the cannabinoids (*via* both i.t. and icv. routes of administration) on the modulation of calcium, cAMP and potassium channels *in vivo* in the production of antinociception. In addition, we wanted to compare and contrast the interaction of the cannabinoids with morphine in the brain and in the spinal cord. Finally, from our results we hoped to generate a hypothesis as to the possible points of interaction of the opiates and the cannabinoids at spinal and supraspinal sites.

Methods

Intrathecal and intraventricular injections. Intrathecal injections were performed according to the protocol of Hylden and Wilcox (1983). Unanesthetized mice were injected between the L5 and L6 area of the spinal cord with a 30-gauge, ½-inch needle. Injection volumes of 5 μl were administered. Intraventricular injections were performed according to the method of Pedigo *et al.* (1975). Mice were lightly anesthetized with ether and an incision was made in the scalp such that the bregma was exposed. Injections were performed using a 26-gauge needle with a sleeve of PE 20 tubing to control the depth of the injection. An injection volume of 5 μl at a site 2-mm rostral and 2-mm caudal to the bregma at a depth of 2 mm was administered to the mice. The cannabinoids, glyburide, thapsigargin, BAY K 8644, ryanodine and nimodipine were prepared in 100% DMSO. Nor-BNI, ICI 174,864, naloxone hydrochloride, morphine sulfate, apamin, pertussis toxin, calcium chloride, magnesium chloride, dibutyryl cAMP, Cl-cAMP, forskolin, ω-conotoxin GVIA, charybdotoxin, TEA, 4-AP and verapamil were dissolved in distilled water. The cannabinoids or DMSO vehicle (i.t.) were administered 15 min before determination of the response latency of the mice in the tail flick test. This time point represents the peak effect of the drugs as determined in previous studies in our laboratory (Welch and Stevens, 1992). DMSO vehicle produced scratching behavior in mice which lasted 2 min after injection. Other vehicles have been tested previously in our laboratory. 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 when performing i.t. injections. Preliminary time course studies of the various drugs (at the highest dose which exhibited no toxicity) or their respective controls were initially performed in combination with the ED_{80} dose of each cannabinoid. Once the time of the peak effect of those drugs which altered cannabinoid antinociception was determined, all further testing was performed at that

time point. With few exceptions, the time of the peak effects of the drugs was 5 to 10 min before the administration of the cannabinoids. The exceptions include glyburide (i.t.), which blocks both i.t. potassium channel openers and morphine (Welch and Dunlow, 1993) using a 15 min pretreatment; thapsigargin, which produced peak blockade after a 1-hr pretreatment and pertussis toxin, which was administered i.t. (0.5 μg/mouse) 7 days before testing in combination with the cannabinoids. Thapsigargin, Bay K 8644 and calcium produced antinociception when administered i.t. Forskolin, dibutyryl cAMP and Cl-cAMP and Bay K 8644 produced antinociception when administered icv. To test for greater than additive effects of the intrinsically antinociceptive drugs (i.t. or icv.) in combination with the cannabinoids, doses of the drugs were chosen which produced no significant antinociception. These doses were then tested in combination with a wide range of doses of the cannabinoids. For the studies of the enhancement of the effects of morphine (icv.) by cannabinoids (icv.), the cannabinoids were administered 10 min before morphine. The mice were tested 10 min after the administration of morphine using the tail flick test.

To study the effect of ω-conotoxin on calcium-induced blockade of the cannabinoids, the ω-conotoxin was administered icv. at 5 min before the calcium (icv.) which was administered at 10 min before the cannabinoid (icv.). The mice were tested 15 min later using the tail flick test.

The tail flick test. The tail flick procedure used was that of D'Amour and Smith (1941). Control reaction times of 2 to 4 sec and a cut-off time of 10 sec were employed. Antinociception was quantified as the %MPE as developed by Harris and Pierson (1964) using the following formula:

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

Percent MPE was calculated for each mouse using at least 12 mice per dose. Using the %MPE for each mouse, the mean effect and standard error of the mean (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 method omitting doses producing 100% or 0% MPE) and 95% confidence limits (CLs) were determined using the method of Litchfield and Wilcoxon (1949), as well as the ALLFIT program (DeLean *et al.*, 1977).

The AD_{50} for blockade the cannabinoid-induced antinociception was determined by calculation of the percent antagonism of antinociception (using a dose of the cannabinoid that resulted in at least 80% MPE) by the blockers according to the following formula:

$$\%\ \text{antagonism} = 100 \times 1 - \left[\frac{(\%\text{MPE of antagonist + cannabinoid})}{(\%\text{MPE of vehicle + cannabinoid})}\right].$$

Using at least three doses of blocker, AD_{50} values were determined by log-probit analysis (a modification of the Litchfield-Wilcoxon method omitting doses producing 100% or 0% MPE) and 95% CLs were determined using the method of Litchfield and Wilcoxon (1949). At least 12 mice per dose were used for all determinations.

Statistical analysis. Significant differences between treatment and control groups were determined using analysis of variance and the Dunnett's *t* test (Dunnett, 1955). The dose-response curves were evaluated for the parallelism of shifts using the method of Tallarida and Murray (1986), as well as the ALLFIT program (DeLean *et al.*, 1977).

Drugs. All of the cannabinoids were obtained from the National Institute on Drug Abuse with the exception of CP 55,940, CP 56,667, levonantradol and dextronantradol which were obtained from Dr. Lawrence Melvin, Pfizer Central Research. Nor-BNI, Bay K 8644, charybdotoxin and forskolin were purchased from Research Biochemicals, Inc. (Natick, MA). ICI 174,864 was purchased from Cambridge Research Biochemicals (Valley Stream, NY). Calcium chloride, magnesium chloride, verapamil, glyburide, TEA, 4-AP, db-cAMP and apamin were purchased from Sigma (St. Louis, MO). 8-(4-Chlorophenylthio)-adenosine-3′:5′-monophosphate cyclic monosodium salt was obtained from Boehringer Mannheim (Indianapolis, IN). Nimodipine was provided by Miles, Inc. Pharmaceutical Division (West Haven, CN). Thapsigargin was purchased from LC Services (Woburn, MA). ω-Conotoxin GVIA was purchased from Peninsula (Belmont, CA). Ryanodine and pertussis toxin were purchased from Calbiochem (San Diego, CA).

Results

The ED_{50} values for a series of cannabinoids are listed in table 1. The ED_{50} values of the cannabinoids after i.t. administration were taken from Welch and Stevens (1992) and were compared with the ED_{50} values generated for the cannabinoids after icv. administration. The dose-effect curves used to generate the ED_{50} values for the cannabinoids (icv.) are shown in figure 1. All of the cannabinoids were full agonists in the tail flick test (fig. 1) with the exception of Δ^8-THC which produced only an average of 68% MPE at 250 μg/mouse. We tried to administer higher doses of Δ^8-THC, but the solubility of the drug precluded the use of higher doses. The ED_{50} values after administration of levonantradol and CP 55,940 i.t. *vs.* icv. were nearly identical. The ED_{50} values for Δ^9-THC did not differ significantly after administration i.t. or icv., although a trend toward higher potency of the drug was observed after icv. administration. Conversely, the ED_{50} values for Δ^8-THC did not differ significantly after administration i.t. or icv., although a trend toward higher potency of the drug was observed after i.t. administration. CP 56,667 was significantly more potent after icv. administration than i.t. administration, and was nearly 10 times more potent than CP 55,940 (icv.). CP 55,940 and CP 56,667 are stereoisomers and CP 55,940 has been described to be the active isomer in many tests (Johnson and Melvin, 1986; Little *et al.*, 1988). We were unable to obtain enough 11-OH-Δ^9-THC to complete a dose-effect curve of the drug after icv. administration. However, 25 μg/mouse produced 44% MPE in the tail flick test. Dextronantradol was inactive at 25 μg/mouse after both i.t. and icv. administration.

Interaction with opioids. Table 2 lists the ED_{50} values for morphine (i.t.) after pretreatment with cannabinoids (i.t.) (taken from Welch and Stevens, 1992) and the ED_{50} values for morphine (icv.) after pretreatment with cannabinoids (icv.). Δ^9-THC, Δ^8-THC, 11-OH-Δ^9-THC and levonantradol produced greater than additive effects in combination with

TABLE 1

ED_{50} values (μg/mouse) and 95% confidence limits for various cannabinoids in the tail flick test in mice

The cannabinoids were administered either i.t. or icv. at 15 min before testing using the tail flick test. The ED_{50} values and 95% CLs were determined as described under "Methods."

Drug	ED_{50}	
	i.t.	icv.
Levonantradol	0.04 (0.003–0.67)	0.02 (0.007–0.06)
CP 55,940	2.28 (0.006–8.59)	2.89 (1.6–5.1)
CP 56,667	4.17 (2.19–7.94)	0.18 (0.09–0.36)
11-OH-Δ^9-THC	14.67 (7.5–28.6)	25 μg = 44%
Δ^9-THC	44.97 (22.96–88.09)	16.4 (11–24.8)
Δ^8-THC	72.07 (36.06–144)	125.8 (65–244)
Dextronantradol Inactive at 25 μg both i.t. and icv.		

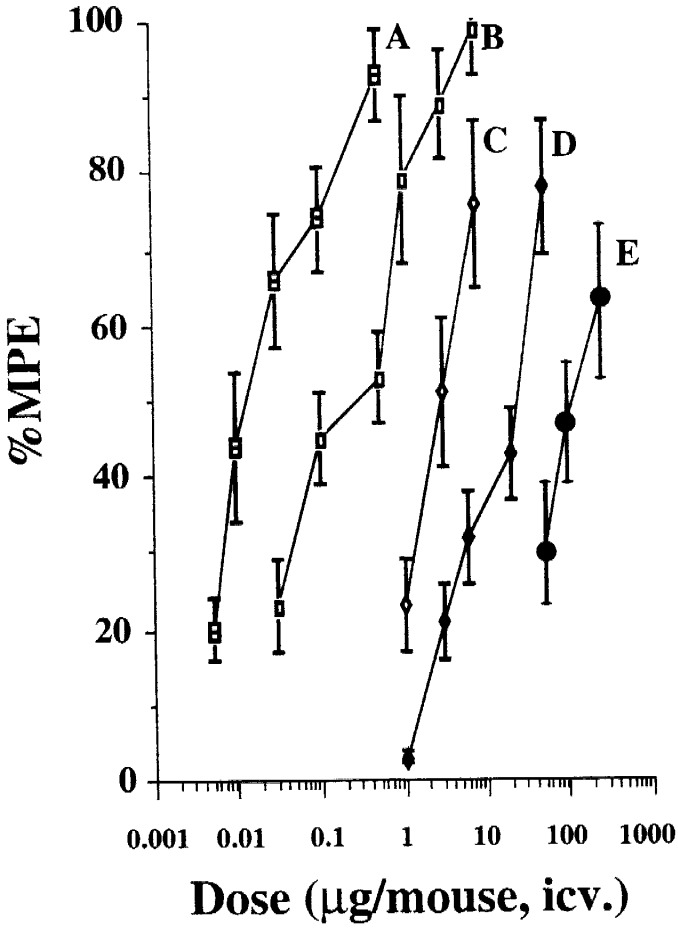

Fig. 1. Dose-effect curves for the cannabinoids after icv. administration. Cannabinoids were injected icv. and tested for the production of antinociception using the tail flick test and the ED_{50} values were determined as described under "Methods." The following cannabinoids were tested: A = levonantradol; B = CP 56,667; C = CP 55,940; D = Δ^9-THC; E = Δ^8-THC. At least 12 mice were used per dose.

morphine when the drugs were administered i.t. CP 55,940, CP 56,667 and dextronantradol (the inactive isomer of levonantradol) failed to produce greater than additive effects with morphine (i.t.). Conversely, CP 55,940 and CP 56,667 produced greater than additive effects with morphine when the drugs were administered icv. The ED_{50} values for morphine were shifted from 2.4 μg/mouse to 0.28 and 0.65 μg/mouse by CP 55,940 and CP 56,667, respectively. Δ^8-THC produced greater than additive effects with morphine when the drugs were administered either i.t. or icv. The shifts in the morphine dose-effect curve in combination with the cannabinoids were parallel.

Various opioid antagonists were administered icv. before icv. administered cannabinoids and did not alter cannabinoid-induced antinociceptive effects. These drugs are listed in Table 3 and include the *delta* opioid antagonist, ICI 174,864 (20 μg/mouse) (Mosberg *et al.*, 1983); the *kappa* opioid antagonist, nor-BNI (70 μg/mouse) (Takemori *et al.*, 1988) and naloxone (20 μg/mouse or 10 mg/kg s.c.), which at the doses used blocks *mu*, *delta* and *kappa* receptors (Heyman *et al.*, 1987).

Interaction with cAMP. Pertussis toxin pretreatment i.t. for 7 days, which ribosylates G_i and/or G_o proteins (for a review see Brown and Birnbaumer, 1990), totally abolished the antinociception produced by the cannabinoids (icv.) (fig. 2*B*), and significantly attenuated or blocked the antinociception produced by the cannabinoids (i.t.) (fig. 2*A*). Δ^9-THC administered i.t. was least affected by the pertussis toxin pretreatment. Pretreatment with pertussis toxin (i.t.) did not alter the baseline latencies for the mice in the tail flick test and did not produce any overt toxicity in the mice. However, pretreatment with pertussis toxin icv. (0.5 μg/mouse for 7 days) led to loss in body weight, and lethality in greater than 30% of the animals. Pretreatment of the mice with 5 and 25 μg/mouse forskolin (i.t.), which produced no antinociception, significantly attenuated the antinociception produced by the Δ^9-THC administered i.t. (fig. 3A). A similar effect was observed using CP 55,940. Forskolin (25 μg/mouse i.t.) pretreatment significantly reduced the antinociceptive effects of CP 55,940 (5 μg/mouse i.t.) from 83 (±6)% MPE to 16 (±5)% MPE. However, when administered icv. forskolin produced antinociception. The ED_{50} of forskolin (icv.) was 0.5 (0.18–1.6) μg/mouse. Forskolin (0.1 μg/mouse icv.), which produced no antinociceptive effects, did not block or produce greater than additive effects with the antinociception produced by the cannabinoids administered icv. Administration of dibutyryl cAMP (10 μg/mouse i.t.) failed to alter the antinociception produced by the cannabinoids administered i.t. However, Cl-cAMP (10 μg/mouse i.t.) partially, but significantly, blocked the antinociceptive effects produced by the i.t. administration of ED_{80} doses the three cannabinoids tested (fig. 3B). However, no greater blockade of the antinociception was obtained using higher doses of Cl-cAMP. Both Cl-cAMP and dibutyryl cAMP produced antinociceptive effects when administered icv. [ED_{50} values = 0.5 (0.04–2.8) and 0.3 (0.06–2.3) μg/mouse, respectively]. Inactive doses of Cl-cAMP and dibutyryl cAMP (0.02 and 0.05 μg/mouse, respectively) did not block or produce greater than additive effects with the antinociception produced by the cannabinoids administered icv.

Interaction with calcium. Various calcium modulators were tested in combination with the cannabinoids (table 3). The i.t. administration of calcium (60 nmol/mouse); thapsi-

TABLE 2

ED_{50} values (μg/mouse) for morphine in combination with various cannabinoids in the tail flick test in mice after i.t. and icv. administration

Mice were injected with cannabinoids either i.t. before i.t. morphine or icv. before icv. morphine. The dose-effect curve for morphine was determined after cannabinoid pretreatment or vehicle pretreatment. Cannabinoids were administered 10 min before morphine and the mice were tested using the tail flick test at 10 min after morphine administration. The ED_{50} values and 95% CLs were determined as described under "Methods."

Pretreatment	ED_{50} of morphine	
	i.t.	icv.
	μg/mouse	
None	1 (0.6–1.4)	2.7 (1.7–4.3)
DMSO	0.61 (0.26–1.44)	2.4 (1.7–3.3)
Δ^9-THC (3.13 μg)	0.15 (0.11–.21)	N.T.
Δ^9-THC (6.25 μg)	0.05 (0.03–0.08)	1 (0.7–1.5)
Δ^8-THC (25 μg)	0.05 (0.02–0.10)	0.18 (0.06–0.62)
Levonantradol	0.06 (0.01–0.24)	0.66 (0.29–1.5)
11-OH-Δ^9-THC (3 μg)	0.08 (0.04–0.19)	N.T.
Dextronantradol	0.51 (0.36–0.89)	N.T.
CP 55,940	0.3 (0.09–1)	0.28 (0.09–0.87)
CP 56,667	0.26 (0.08–0.82)	0.65 (0.33–1.3)

TABLE 3

Comparison of blockade of the antinociceptive effects of the cannabinoids after i.t. and icv. administration

Various drugs were administered i.t. before cannabinoids (i.t.) or icv. before cannabinoids (icv.). Antinociception was determined using the tail flick test and the time course as described under "Methods"

Drugs	Cannabinoids	
	i.t.	icv.
Opioid antagonists		
Naloxone	No block	No block
ICI 174,864	No block	No block
nor-BNI	Blocked	No block
Calcium modulators		
Calcium	No block	Blocked
Verapamil	No block	No block
ω-conotoxin	No block	No block*
Nimodipine	No block	No block
Thapsigargin	No block	Blocked
BAY-K	No block	No block
Ryanodine	No block	No block
Modulators of cAMP		
Pertussis toxin	Blocked	Blocked
db-cAMP	No block	No block
Cl-cAMP	Blocked	No block
Forskolin	Blocked	No block
Magnesium Chloride	No block	No block
Modulators of potassium channels		
4-AP	No block	No block
TEA	No block	No block
Glyburide	No block	No block
Charybdotoxin	No block	No block
Apamin	Blocked	No block

* Although ω-conotoxin itself did not alter the effects of the cannabinoids, it did significantly block calcium-induced blockade of the cannabinoids.

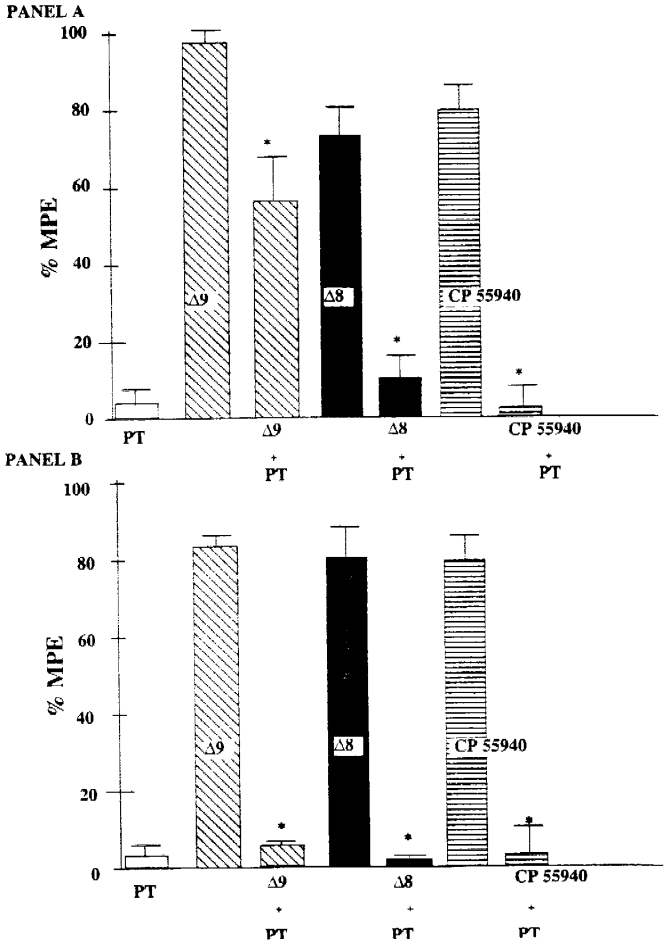

Fig. 2. The effect of pertussis toxin pretreatment (i.t.) on the antinoci ceptive effects of the cannabinoids administered either i.t. (panel A) c icv. (panel B). Pertussis toxin (PT) (0.5 μg/mouse) or distilled wate vehicle was administered i.t. 7 days before the administration of th cannabinoids (Δ^9-THC, Δ^8-THC or CP 55,940) or DMSO vehicle. Th mice were tested for antinociceptive effects after either i.t. or icv administration of the cannabinoids or DMSO. The clear bar denote "PT" represents the antinociception produced by the administration c DMSO vehicle in PT-treated mice. Not shown is the effect of DMSO (i. or icv.) in distilled water-pretreated mice (the vehicle for the PT). Th %MPE generated in those treatment groups were 5 ± 3% (i.t.) and 8 6% (icv.), respectively. Bars denoted "Δ9," "Δ8," and "CP 55,940 represent the antinociceptive effects of those cannabinoids in mic pretreated for 7 days i.t. with distilled water vehicle. The bars denote "+PT" indicate the effects of the cannabinoids in PT-pretreated mic At least 12 mice per treatment group were used. * $P < 0.01$ fro respective distilled water/cannabinoid group.

gargin (0.1 μg/mouse), which increases intracellular calcium (*via* blockade of entry of calcium into the IP_3-sensitive pools of the endoplasmic reticulum by blocking ATPase activity (Bian *et al.*, 1991; Koshiyama and Tashjian, 1991); Bay K 8644 (0.3 μg/mouse), which predominantly increases calcium entry through dihydropyridine-sensitive calcium channels; verapamil (30 μg/mouse) and nimodipine (20 μg/mouse), which block voltage-gated "L"-type calcium channels (Godfraind *et al.*, 1986; Janis *et al.*, 1987; Zernig, 1990); ω-conotoxin (1 μg/mouse) which blocks both "L-" and "N-"type calcium channels (Olivera *et al.*, 1987; Reynolds *et al.*, 1986); and ryanodine (5 μg/mouse), which alters calcium flux from the intracellular caffeine-sensitive (but not IP_3 sensitive) calcium pool (Thayer *et al.*, 1988; Palade *et al.*, 1989) failed to produce antinociception or to alter the antinociception produced by the i.t. administration of the cannabinoids. The doses listed represent the highest non-antinociceptive dose for each of the drugs. At higher doses, calcium, thapsigargin and BAY K 8644, which increase intracellular calcium, produced antinociception after i.t. administration.

Calcium (icv.) blocked the antinociceptive effects of the cannabinoids (fig. 4, panel A). The AD_{50} values (±CL) for calcium-induced block of Δ^9-THC, Δ^8-THC and CP 55,940 were 215 (94–489), 176 (122–253) and 123 (81–186) nmol/mouse, respectively. Higher doses of calcium (greater than 450 nmol/mouse icv.) produced hyperactivity in the mice. It was difficult to generate an AD_{50} for calcium *vs.* Δ^9-THC because we could not use higher doses of calcium. This difficulty is reflected in the large confidence limits for the AD_{50} of calcium *vs.* Δ^9-THC. ω-Conotoxin (1 μg/mouse icv.), which did not alter the antinociceptive effects of Δ^9-THC, significantly reversed the calcium-induced blockade of Δ^9-THC (fig. panel B).

Pretreatment of the mice icv. with thapsigargin for 1 h blocked the antinociception produced by Δ^9-THC (and C 55,940, data not shown) administered icv. (AD_{50} for thaps gargin *vs.* Δ^9-THC = 0.003 [0.001–0.012] μg/mouse) (fig. 5 Other calcium modulators were tested including: ryanodir (1 μg/mouse); omega conotoxin (5 μg/mouse); verapamil (3 μg/mouse) and nimodipine (20 μg/mouse); and magnesiu (10 nmol/mouse). None of these drugs produced antinocice tion after either i.t. or icv. administration. All of these dru were tested at the highest nontoxic doses in combination wit the cannabinoids administered either i.t. or icv. and failed alter cannabinoid-induced antinociception (table 3).

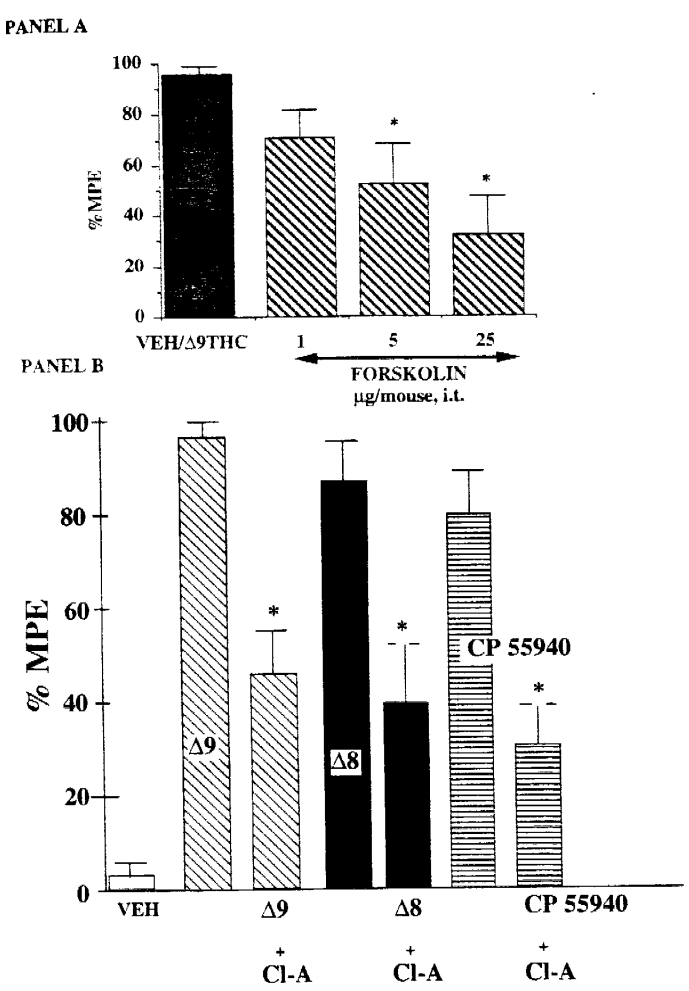

Fig. 3. The effect of forskolin pretreatment (i.t.) (panel A) or Cl-c-AMP (panel B) on the antinociceptive effects of the cannabinoids administered i.t. Panel A, Forskolin (1, 5, 25 μg/mouse) or distilled water vehicle was administered i.t. before the administration of Δ^9-THC or DMSO vehicle. The mice were tested for antinociceptive effects after i.t. administration of the cannabinoids or DMSO. The dark bar denoted "VEH/Δ9THC" represents the antinociception produced by the administration of distilled water i.t. before Δ^9-THC. Not shown is the effect of DMSO in distilled water-pretreated mice (the vehicle for the forskolin). The %MPE generated in that treatment group was 10 ± 4%. At least 12 mice were used for all treatment groups. Panel B, Bars denoted "Δ9," "Δ8," and "CP 55,940" represent the antinociceptive effects of those cannabinoids in mice pretreated i.t. with distilled water vehicle. The bars denoted "+Cl-A" indicate the effects of the cannabinoids in Cl-cAMP (10 μg/mouse, i.t.)-pretreated mice. "VEH" denotes the antinociceptive effects of distilled water administration before DMSO. At least 12 mice per treatment group were used. * $P < 0.01$.

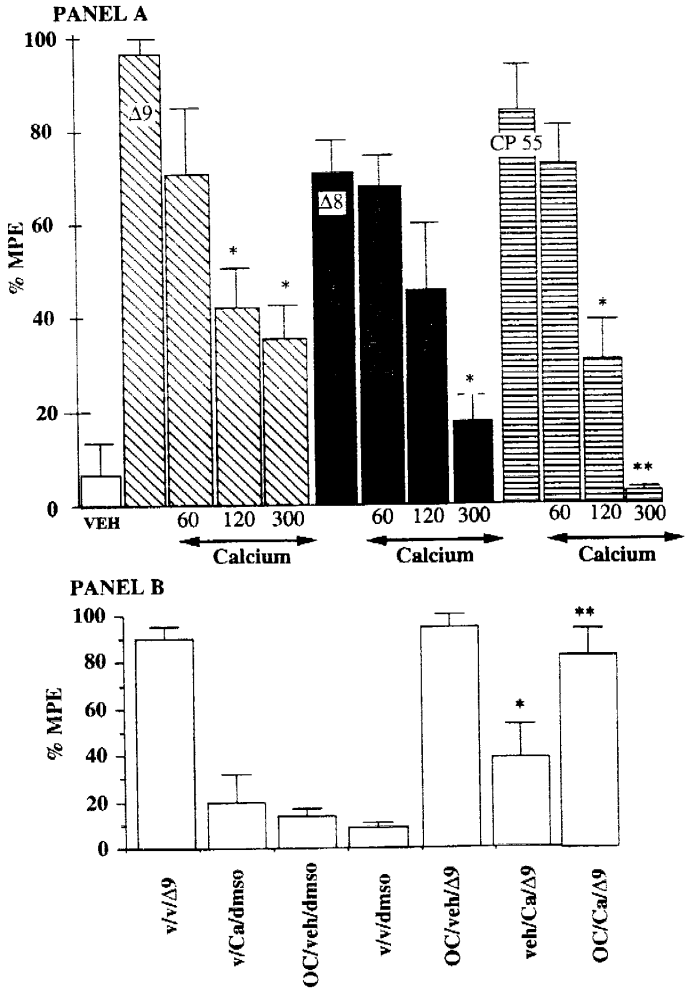

Fig. 4. The effect of calcium pretreatment (icv.) (panel A) or ω-conotoxin GVIA (OC) icv. before calcium pretreatment (panel B) on the antinociceptive effects of the cannabinoids administered icv. Panel A, Calcium (60, 120, 300 nmol/mouse) or distilled water vehicle was administered icv. before the icv. administration of Δ^9-THC, Δ^8-THC, CP 55,940 or DMSO vehicle. The mice were tested for antinociceptive effects after icv. administration of the cannabinoids or DMSO. The bar denoted "VEH" represents the antinociception produced by the administration of distilled water icv. before DMSO icv. The AD_{50} values for calcium blockade of the cannabinoids were determined as described under "Methods." At least 12 mice per treatment group were used. * $P < 0.05$; ** $P < 0.01$ from vehicle/cannabinoid. Panel B, Mice were pretreated with dual injections icv. of vehicle (v), calcium (Ca, 300 nmol/mouse), ω-conotoxin (OC, 1 μg/mouse) and/or Δ^9-THC or DMSO as described under "Methods." The mice were tested 15 min after the injection of Δ^9-THC. At least 12 mice per treatment group were used. ω-Conotoxin significantly reversed the calcium-induced blockade of Δ^9-THC-induced antinociception. ** $P < 0.01$ from veh/Ca/Δ9; * $P < 0.05$ from OC/veh/Δ9.

Interaction with potassium channels. Various potassium channel blockers were tested: TEA (1 μg/mouse) and 4-AP (0.7 μg/mouse), which block voltage-gated potassium channels (North, 1992; Aronsen, 1992); glyburide (200 μg/mouse), which blocks ATP-gated potassium channels (Fossett *et al.*, 1988; Gaines *et al.*, 1988; Panten *et al.*, 1989; and DeWeille and Lazdunski, 1990); and charybdotoxin (250 pg/mouse), which blocks the large (or fast) conductance calcium-gated potassium channels (Smith *et al.*, 1986; Gimenez-Galego *et al.*, 1988). These drugs administered either i.t. or icv. at these doses, which are the highest nontoxic doses of the drugs, failed to alter cannabinoid-induced antinociception. However, apamin, blocker of small (low) conductance calcium-gated potassium channels (Hugues *et al.*, 1982; Castle *et al.*, 1989; Strong, 1990), produced a parallel rightward shift in the dose-effect curves of Δ^9-THC, Δ^8-THC and CP 55,940 (fig. 6, panels A–C). The ED_{50} values for the cannabinoids were shifted from 6- to 33-fold by administration of apamin (5 ng/mouse i.t.). The ED_{50} of Δ^9-THC was shifted from 6 (2–19) to 82 (59–114) μg/mouse. The ED_{50} values for Δ^8-THC and CP 55,940 were shifted from 21 (9–51) and 0.6 (0.2–1.7) μg/mouse to 123 (50–306) and 20 (9–42) μg/mouse, respectively. However, apamin (5 ng/mouse icv.) failed to block icv. administered cannabinoids.

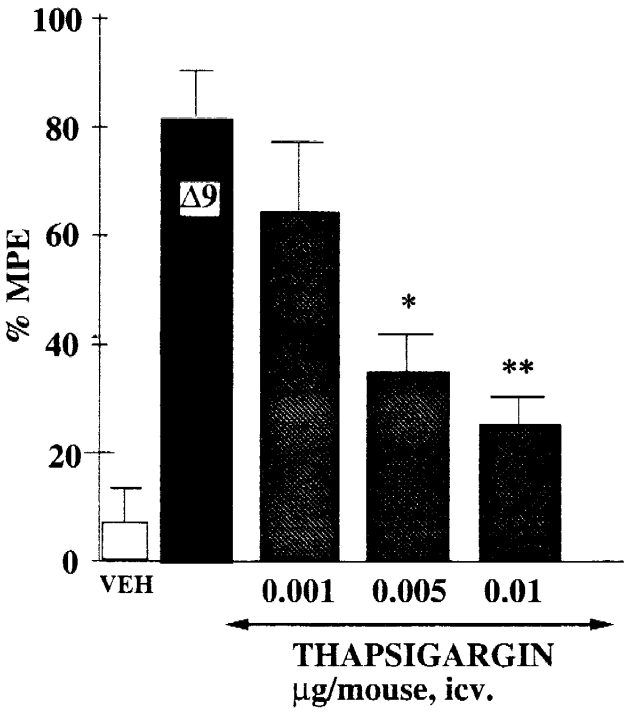

Fig. 5. Blockade of Δ^9-THC (icv.)-induced antinociception by thapsigargin (icv.). Thapsigargin, at the doses listed, or vehicle was injected at 1 hr before Δ^9-THC. The mice were tested 15 min later in the tail flick test. The clear bar denoted "VEH" represents the antinociceptive effects of DMSO icv. administered 1 hr before DMSO icv. The bar labeled "Δ9" represents DMSO vehicle icv. at 1 hr before Δ^9-THC. The remaining bars represent thapsigargin administration before Δ^9-THC. At least 12 mice were used per dose. * $P < 0.05$ from "Δ9"; ** $P < 0.01$ from "Δ9."

Discussion

Antinociceptive effects of the cannabinoids. Cannabinoids produced antinociception after both i.t. and icv. administration and are approximately equipotent at either site with the exception of CP 56,667. These results are consistent with previous studies which indicate that the cannabinoids are active as antinociceptive drugs when injected i.t. (Yaksh, 1981; Gilbert, 1981; Lichtman and Martin, 1991, a and b; Welch and Stevens, 1992). Intrathecally administered cannabinoids seem to act at predominantly spinal sites in the production of antinociception (Smith and Martin, 1992). It is not clear whether the icv. administration of the cannabinoids activates both spinal and supraspinal sites in the production of antinociception. Due to the lypophilicity of the drugs, passage to the spinal cord is very likely.

In summary, the cannabinoids seem to produce potent antinociceptive effects at both spinal and supraspinal sites. However, the mechanism by which the cannabinoids produce antinociceptive effects at these two sites seemed to differ and is the focus of the remainder of this manuscript.

Interaction with opiates. We have reported previously that the ED_{50} values for morphine were shifted from 4- to greater than 10-fold by the pretreatment with cannabinoids at doses which did not have any antinociceptive effects. When we compared the enhancement of morphine-induced antinociception by the cannabinoids after icv. administration with those data previously generated after i.t. administration, we found that some cannabinoids enhanced morphine in the

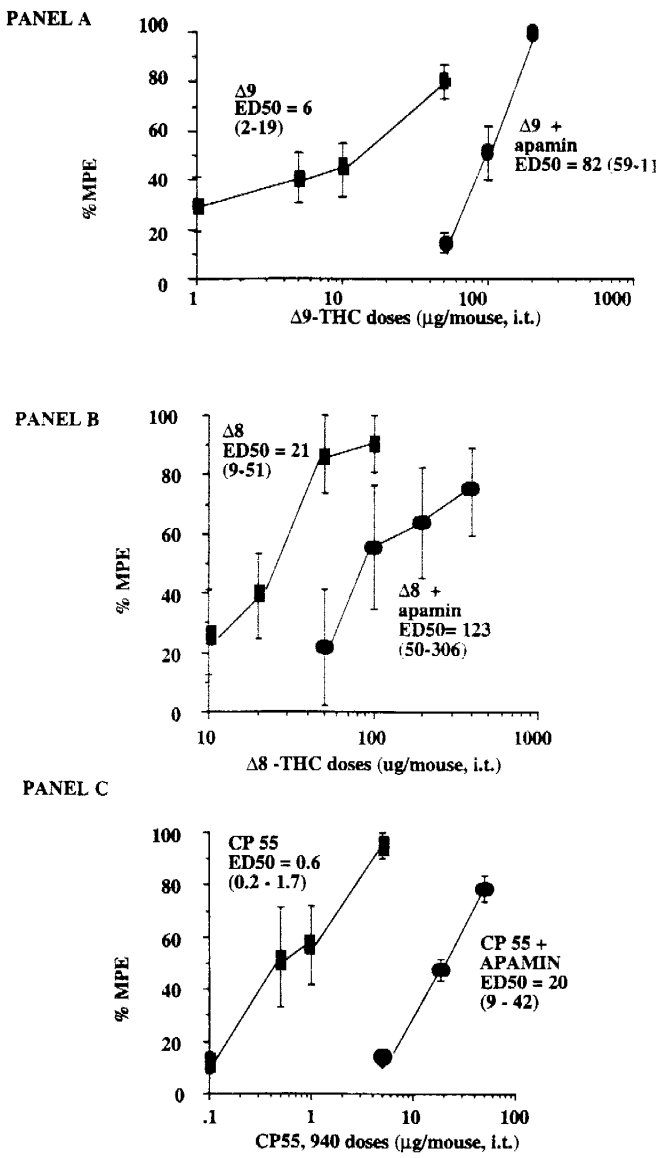

Fig. 6. Blockade of the antinociceptive effects of Δ^9-THC (panel A), Δ^8-THC (panel B) and CP 55,940 (panel C) by apamin when all drugs are administered i.t. Apamin (5 μg/mouse, i.t.) was administered at 15 min before the cannabinoids. The dose-effect curves of the cannabinoids were determined 15 min later using the tail flick test. The ED values and the parallelism of the shifts in the dose-effect curves were determined as described under "Methods" using 12 mice per dose.

brain whereas others enhanced the effects of morphine in the spinal cord. Our data are suggestive of the intriguing possibility that subtypes of cannabinoid receptors may exist in brain and spinal cord and differ in their interactions with opiate-sensitive pathways either *via* their neuroanatomic proximity to opiate receptors or differ in the convergence of second messenger systems with those of the opiates. However, in the case of one cannabinoid, CP 55,940, binding has been shown to be dense in the striatum and the substantia gelatinosa of the spinal cord (Herkenham *et al.*, 1990), areas which are associated with dense binding of the opiates (Yaksh *et al.*, 1988; Gamse *et al.*, 1979). Thus, neuroanatomical location of the receptors does not seem to account for the differences observed between the effects of CP 55,940 o

morphine-induced antinociception in the brain *vs.* in the spinal cord.

We have previously reported that the *kappa* antagonist, nor-BNI (i.t.), but not other opioid antagonists, blocks cannabinoid (i.t.)-induced antinociception (Welch, 1993). However, nor-BNI administered icv. failed to block the antinociception produced by the cannabinoids administered icv. These data support the hypothesis that the antinociceptive effects of the cannabinoids at supraspinal sites are not mediated *via* interaction with nor-BNI-sensitive receptors. Naloxone and ICI 174,864 (icv.) also failed to block the antinociceptive effects of icv. administered cannabinoids. These data suggest that the icv. administration of the cannabinoids does not result in the release of an antinociceptive endogenous opioid or the direct interaction with opiate receptors. Thus, the enhancement of opiate antinociception by cannabinoids icv. may be due to interactions with common intracellular second messenger systems. Some possible points of interaction include the modulation of calcium, cAMP and potassium flux.

Modulation of calcium and cAMP. Cannabinoids either attenuate (Martin *et al.*, 1988; Harris and Stokes, 1982), enhance or fail to alter (Okada *et al.*, 1992) calcium entry to a variety of tissues. Electrophysiological studies in neuroblastoma cells indicate cannabinoids inhibit an ω-conotoxin-sensitive, high-voltage-activated calcium channel, an effect that is blocked by the administration of pertussis toxin and independent of the formation of cAMP. Because the L-type calcium channel blocker, nitrendipine, fails to alter such an effect, the cannabinoids were hypothesized to interact with an N-type calcium channel (Mackie and Hille, 1992). A similar study produced similar results. The cannabinoids were found to inhibit I_{Ca} current in neuroblastoma cells, an effect which was not dose related, but was pertussis toxin- and ω-conotoxin-sensitive (Caulfield and Brown, 1992). All such previous studies were performed *in vitro*. Based on the results of such studies we hypothesized that the antinociception produced by the cannabinoids might be sensitive to calcium, or modulators of calcium. However, the antinociceptive effects of the cannabinoids administered i.t. were not altered by the i.t. administration of calcium chloride or a series of modulators of calcium flux across voltage-gated calcium channels (verapamil, nimodipine, Bay K 8644 or ω-conotoxin) (Godfraind *et al.*, 1986; Janis *et al.*, 1987; Zernig, 1990; Olivera *et al.*, 1987; Reynolds *et al.*, 1986) or by modulators of intracellular pools of calcium such as thapsigargin (Bian *et al.*, 1991; Koshiyama and Tashjian, 1991) or ryanodine (Thayer *et al.*, 1988; Palade *et al.*, 1989). Antinociceptive effects result from the i.t. administration of calcium to mice (Lux *et al.*, 1988; Welch *et al.*, 1992; Smith and Dewey, 1992), as well as Bay K 8644 and thapsigargin (Damaj *et al.*, 1993). Administration of non-antinociceptive doses of these drugs i.t. before the cannabinoids did not produce greater than additive effects with the cannabinoids. These data indicate that the antinociception produced by the i.t. administration of the cannabinoids is not sensitive to calcium modulation and thus may not directly involve calcium modulation.

Conversely, the icv. administered cannabinoids were sensitive to blockade by calcium icv. and the AD_{50} values for calcium icv. *vs.* the cannabinoids (icv.) did not differ significantly from that previously shown for calcium blockade of morphine icv. (Harris *et al.*, 1975; Chapman and Way, 1980; Guerrero-Munoz and Fearon, 1982; Vocci *et al.*, 1980). Thapsigargin which increases intracellular calcium (Koshiyama and Tashjian, 1991) and stimulates calcium influx into cells (for a review see Irvine, 1992), also blocked the antinociception produced by Δ^9-THC and CP 55,940. Non-antinociceptive doses of BAY K 8644 (icv.), which increases calcium entry to cells *via* the voltage-gated "L"-type calcium channel, as well as *via* nonspecific mechanisms (Bouchelouche *et al.*, 1989), did not alter cannabinoid antinociception.

It is likely that the calcium-induced blockade of the cannabinoids is due to calcium entry through voltage-gated calcium channels because ω-conotoxin reversed the calcium-induced blockade (fig. 4), although ω-conotoxin alone did not alter cannabinoid-induced antinociception. Thus, it appears that the entry of calcium to neurons must be stimulated by the administration of calcium to observe effects of omega conotoxin. Using a similar logic we attempted to reverse the calcium-induced blockade of cannabinoids with both verapamil and nimodipine, which block "L"-type calcium channels, and found no reversal of the effects of icv. calcium (data not shown). Thus, the calcium-induced blockade of cannabinoid-induced antinociception seems to be due to calcium entry through the "N"-type calcium channel. These results are consistent with reported effects observed *in vitro* (Mackie and Hille, 1992). The blockade of cannabinoid-induced antinociception by thapsigargin, which raises intracellular calcium levels, indicates that the cannabinoids may decrease calcium entry to neurons which is consistent with the *in vitro* work of Harris and Stokes (1982) and Martin *et al.* (1988). In addition, the stimulation of calcium entry to neurons after thapsigargin treatment would further increase calcium concentrations intracellularly, an effect insensitive to blockade by classic calcium channel blockers (for review, see Irvine, 1992; Jackson *et al.*, 1988; Takemura *et al.*, 1991) and consistent with our data. Our data therefore suggest that cannabinoids produce antinociception at supraspinal, but not spinal, sites by decreasing intracellular calcium levels *via* blockade of calcium entry through "N"-type calcium channels.

Consistent with the report of Mackie and Hills (1992) *in vitro*, antinociception resulting from supraspinal administration of the cannabinoids seems to involve interaction with a G_i or G_o protein and be independent of changes in cAMP, due to the blockade of antinociception by pertussis toxin pretreatment which inactivates G_i and G_o proteins *via* ADP-ribosylation (for a review, see Brown and Birnbaumer, 1990). Our data agree with studies indicating an interaction of the cannabinoids with G_i proteins, but indicating a lack of involvement of cAMP in the brain in the modulation of cannabinoid-induced antinociception or other behavioral effects of the cannabinoids (Hillard and Bloom, 1983; Little and Martin, 1991; Dolby and Kleinsmith, 1974; Dolby and Kleinsmith, 1977; Askew and Ho, 1974). *In vitro* studies indicate a somewhat different profile of cannabinoid action. Studies in NG108–15 cells show that cannabinoid-induced inhibition of cAMP formation follows a structure-activity relationship and stereoselectivity similar to that observed for behavioral measures (Howlett, 1985; Dill and Howlett, 1988; Howlett *et al.*, 1986; Howlett and Fleming, 1984; Howlett, 1987; Howlett *et al.*, 1990; Devane *et al.*, 1986; Bidaut-Russell *et al.*, 1991; Howlett *et al.*, 1992) and is mediated *via* coupling to the G_i

protein because pertussis toxin attenuates binding of CP 55,940 (Howlett *et al.*, 1988; Bidaut-Russell and Howlett, 1991). These studies, along with supporting work evaluating the stereoselectivity and the Hill coefficient for binding of the synthetic bicyclic cannabinoid. CP 55,940 (Devane *et al.*, 1988; Howlett *et al.*, 1990), support the hypothesis of a cannabinoid receptor linked through a G_i protein to the modulation of cAMP. Although our studies indicate that modulation of cAMP is not directly involved in the production of antinociception, we can not rule out the possibility that c-AMP indirectly alters icv. cannabinoid-induced antinociception by alteration of calcium entry to neurons *via* phosphorylation of calcium channels (Brown and Birnbaumer, 1990; Schultz *et al.*, 1990). By decreasing cAMP, cannabinoids could decrease calcium entry to neurons.

In the spinal cord, the cannabinoids seem to produce antinociception *via* a G_i or G_o protein in conjunction with the modulation of cAMP because the antinociceptive effects of i.t. cannabinoids are blocked by pertussis toxin, forskolin and Cl-cAMP. The lack of blockade of antinociception by db-cAMP may be due to the lack of penetration of the db-cAMP to the site of action. The effects of cAMP on antinociception are the opposite in the brain *vs.* in the spinal cord, which is similar to the opposite effects of calcium observed in brain *vs.* spinal cord (Lux *et al.*, 1988). Thus, differences in the involvement of cAMP in cannabinoid-induced antinociception following icv. *vs.* i.t. administration may reflect differences in the function of cAMP in the brain *vs.* in the spinal cord in the modulation of antinociception.

Modulation of potassium. Four basic classes of potassium channels exist in the central nervous system (for a review, see Aronsen, 1992). This classification is clearly a generalization and the drugs discussed often interact with more than one of the channels. In general these potassium channels are either voltage-activated, calcium-gated, ATP-gated or ligand-activated. The voltage-gated potassium channels are either opened or closed in response to membrane electrical activity and are blocked pharmacologically by TEA and 4-AP, as well as several toxins (North, 1992; Aronsen, 1992). The calcium-gated potassium channels are activated by alteration in intracellular calcium and are thought to be coupled to G proteins or other second messenger systems. Calcium-gated potassium channels have been studied using the bee venom peptide, apamin, which blocks the small (low) conductance (SK or LK; $I_{K(Ca)s}$) channel (Hugues *et al.*, 1982; Castle *et al.*, 1989; Strong, 1990). An endogenous ligand for the apamin receptor has been found in brain (Fosset *et al.*, 1987). Charybdotoxin, a scorpion venom, blocks predominantly the large (or fast) conductance (BK; $I_{K(Ca)f}$) channel in neuronal preparations (Smith *et al.*, 1986; Gimenez-Gallego *et al.*, 1988) by insertion into the mouth of the channel (Anderson *et al.*, 1988). Charybdotoxin does not interact with apamin-sensitive sites in rat brain (Vazquez *et al.*, 1990). Morphine has been shown to modulate charybdotoxin-sensitive calcium-gated potassium channels in peripheral sensory neurons (Stretton *et al.*, 1992). The ATP-gated potassium channels are gated by changes in intracellular ATP/ADP ratios. High ATP levels close these channels leading to a less negative membrane potential and depolarization. Opening of these channels leads to hyperpolarization. Pharmacologically, these channels are blocked by the oral hypoglycemic agents, such as glyburide, which stimulate the entry of calcium into the cells of both the pancreas and the brain (Fosset *et al.*, 1988; Gaines *et al.*, 1988; Panten *et al.*, 1989; DeWeille and Lazdunski, 1990). ATP-gated potassium channels have been found in the brain, with particularly high levels in the substantia nigra, hippocampus, basal ganglia and thalamus, and to a lesser degree in the spinal cord, although the function of such channels remains unknown (Mourre *et al.*, 1990). Anatomically, these channels exist in areas where high levels of opiate binding occur (Yaksh *et al.*, 1988). Opiates also interact with ligand-activated potassium channels, a diverse collection of channels which are modulated by a variety of neurotransmitters and drug classes. These channels are most often coupled to a G protein (Brown and Birnbaumer, 1990).

We have shown previously that opiate-induced antinociception is blocked by potassium channel blockers and conversely, that potassium channel openers administered i.t. produce antinociception that is blocked by opiate antagonists (Welch and Dunlow, 1993). The administration of apamin, a blocker of calcium-gated potassium channels, produced a complete blockade of morphine-induced antinociception. Evaluation of several potassium channel blockers (icv. and i.t.) in combination with the cannabinoids (icv. and i.t.) indicated that only apamin (i.t.) blocked the antinociceptive effects of the cannabinoids (i.t.), producing parallel rightward shifts in the dose-effect curves of the cannabinoids. Although the parallel shifts could be coincidental and the effects of apamin *in vivo* could be nonspecific, this is unlikely because parallel shifts were observed with three cannabinoids. Thus, it is likely that cannabinoids interact spinally with a calcium-gated, apamin-sensitive potassium channel in the production of antinociception. Cannabinoids administered icv. appear to produce antinociception which does not involve apamin-sensitive potassium channels. Since apamin binding has been shown in the brain (Fosset *et al.*, 1987), the lack of effects of apamin (icv.) on cannabinoid-induced antinociception can not be attributed to lack of binding of the apamin.

Summary. Cannabinoids produce antinociception after administration to both spinal and supraspinal sites; however, modulation of antinociception by calcium, cAMP and potassium seems to differ in the brain *vs.* in the spinal cord. Additionally, the cannabinoid-induced modulation of opiate antinociception seems to differ in the brain and the spinal cord. Based on our data, we hypothesize that in the brain, cannabinoids produce antinociception *via* decreasing calcium levels in neurons through receptor linkage to $G_{i/o}$ proteins involved in the modulation of the function of the "N"-type calcium channel. Cyclic AMP seems to either not be involved or to play an indirect role. In addition, potassium channels seem to not be involved in the production of antinociception induced by icv. administered cannabinoids. In the spinal cord, our data indicate that the cannabinoids produce antinociceptive effects *via* the interaction with $G_{i/o}$ proteins resulting in a decreased cAMP production, and interaction with calcium-gated potassium channels. The involvement of calcium appears to not play a direct role in the antinociceptive effects produced by the cannabinoids, although the interaction with G proteins has the potential to alter both calcium and potassium channel function.

Because acutely administered opiates/opioids have been shown to interact with $G_{i/o}$ protein-coupled receptors (Blume 1978; Childers and Snyder, 1980), decrease calcium entry t

and content of neurons (Harris *et al.*, 1976; Cardenas and Ross, 1976; Welch and Olson, 1991), reduce cAMP levels (Collier and Roy, 1974; Sharma *et al.*, 1975; Blume, 1978) and produce hyperpolarization of neurons *via* both ATP- and apamin-sensitive potassium channels (Welch and Dunlow, 1993; Ocana *et al.*, 1990), these three intracellular systems may be common points of interaction with the cannabinoids. For example, it has been hypothesized that the end result of the modulation of cAMP by cannabinoids and opioids in combination may be the phosphorylation of similar proteins which are proposed to be synapsins I and II which are involved in the release of neurotransmitters (Childers *et al.*, 1992). Finally, the data suggest that cannabinoids interact spinally with nor-BNI-sensitive receptors. This could indicate some interaction of the cannabinoids directly with opiate-sensitive pathways. *Kappa* receptor-selective opioids (Vaysse *et al.*, 1987) and *delta* opioids (Devane *et al.*, 1986) have not been shown to be displaced by the cannabinoids. Thus, although we can hypothesize as to the points of interaction of cannabinoids and opiates based on the *in vivo* mechanisms of action of the drugs indicated by this work and that of others, clearly much more research needs to be done to delineate conclusively the mechanisms underlying the greater than additive effects observed using cannabinoids and opiates in combination.

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