

Differential Blockade of the Antinociceptive Effects of Centrally Administered Cannabinoids by SR141716A¹

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ABSTRACT

We evaluated *delta*-9 tetrahydrocannabinol (Δ^9 -THC), *delta*-8 tetrahydrocannabinol (Δ^8 -THC), CP55,940 (CP55), 1-deoxy-11-hydroxy- Δ^8 -THC-dimethylheptyl (deoxy-HU210, a CB2-selective cannabinoid that also binds the CB1 receptor) and the endogenous cannabinoid anandamide (ANA) *via* i.c.v. and/or intrathecal (i.t.) routes of administration, alone and in combination with SR141716A (SR), a CB1 antagonist, using the tail-flick test. Our studies were performed in order better to characterize potential diversity in interactions of the cannabinoids with the cannabinoid (CB1) receptor. When SR was administered i.c.v. or i.p. before Δ^9 -THC, Δ^8 -THC or CP55 (i.c.v. or i.t.), SR was a potent antagonist and the blockade was complete ($AD_{50} \leq 8.1 \mu\text{g}/\text{mouse}$ i.c.v. or $AD_{50} \leq 1.4 \text{ mg}/\text{kg}$ i.p.). The AD_{50} values (dose of antagonist that produced a 50% antagonism of agonist effects) for blockade of Δ^9 -THC, Δ^8 -THC, CP55,940 (i.c.v. or i.t.) by SR (i.c.v. or i.p.) differed significantly for only two combinations [Δ^8 -THC/SR, both i.c.v. and CP55 (i.t.)/SR (i.p.)]. Conversely, SR (i.t.) produced an incomplete block of the an-

tinociceptive effects of i.t. Δ^9 -THC, Δ^8 -THC and CP55 ($AD_{50} = 28.6, 50.2$ and $20.9 \mu\text{g}/\text{mouse}$, respectively). Blockade of the deoxy-HU210 (i.c.v.) by SR (either i.c.v. or i.p.) was incomplete and AD_{50} values could not be calculated. Although the maximal blockade of deoxy-HU210 (i.t.) by SR (i.t.) was only 50%, SR administered i.p. before deoxy-HU210 (i.t.) produced a potent and complete blockade ($AD_{50} = 0.4 \text{ mg}/\text{kg}$). The effects of SR on ANA-induced antinociception were mixed. The maximal attenuation of the ANA (i.t.) by SR (i.t.) was 38%. SR (i.p.) blockade of ANA was complete, but the AD_{50} was $15.4 \text{ mg}/\text{kg}$, greater than 15-fold higher than that required to block Δ^9 -THC, Δ^8 -THC, CP55 or deoxy-HU210. In addition, SR (i.p. or i.t.) failed to block the hypothermic effects of ANA (i.t.), while completely reversing the hypothermic effects of Δ^9 -THC (i.t.). These data indicate that SR has a much greater efficacy at supraspinal than at spinal sites. Alternatively, such data suggest either a differential interaction of the cannabinoids at the CB1 receptor or the existence of subtypes of the CB1 receptor.

Cannabinoids produce antinociceptive effects at spinal sites when injected i.t. (Yaksh, 1981; Gilbert, 1981; Lichtman and Martin, 1991a and b; Welch and Stevens, 1992; Welch *et al.*, 1995a and b; Pugh *et al.*, 1996; Welch, 1997). Intrathecally administered cannabinoids appear to act at predominantly spinal sites in the production of antinociception (Smith and Martin, 1992). The mechanisms by which the cannabinoids produce antinociception are as yet unclear. Two distinct cannabinoid receptors have been cloned: the CB1 receptor, which is predominantly located in the CNS (Matsuda *et al.*, 1990), and the CB2 receptor, which is found on immune cells and on peripheral tissues (Munro *et al.*, 1993). In addition, a splice variant of the CB1 receptor

termed the CB1A receptor has been identified (Shire *et al.*, 1995). When the sequence for the cannabinoid receptor was published, Gérard *et al.* (1990) reported that they had isolated the human homolog of this receptor. The discovery of the cannabinoid antagonist SR (Rinaldi-Carmona *et al.*, 1994) and the discovery of an endogenous cannabinoid-like ligand, anandamide, (Devane *et al.*, 1992) have greatly facilitated work with the cannabinoids and complement the discovery and cloning of the cannabinoid receptors.

We have accumulated evidence indicating that cannabinoids produce antinociception by indirect interaction with *kappa* opioids in the spinal cord after i.t. administration (Smith *et al.*, 1994b). The *kappa* antagonist nor-binaltorphimine (nor-BNI) and dynorphin antisera block Δ^9 -THC-induced (THC i.t.) antinociception but do not block THC-induced catalepsy, hypothermia or hypoactivity (Smith *et al.*, 1994a; Pugh *et al.*, 1996; Welch, 1993). In addition, the discovery of the bidirectional cross-tolerance of Δ^9 -THC and

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ABBREVIATIONS: AD_{50} , dose of antagonist producing 50% blockade of agonist response; i.t., intrathecally; CP55, CP55,940; %MPE, percent maximal possible effect; ED_{50} or ED_{80} , effective dose in 50% or 80% of the animals, respectively; CL, 95% confidence limits; ANA, anandamide; SR, SR141716A; deoxy-HU210, 1-deoxy-11-hydroxy- Δ^8 -THC-dimethylheptyl; Δ^9 -THC, *delta*-9 tetrahydrocannabinol; Δ^8 -THC, *delta*-8 tetrahydrocannabinol; DMSO, dimethyl sulfoxide.

CP55 to κ agonists using the tail-flick test (Smith *et al.*, 1994a) and to dynorphin A (Welch, 1997), indicates that cannabinoids interact in a yet-to-be-determined manner with κ opioids. The attenuation of the antinociceptive effects of THC by antisense to the κ -1 receptor further implicates the release of endogenous κ opioids in the mechanism of action of the cannabinoids (Pugh *et al.*, 1995). In addition, dynorphin antibodies block cannabinoid-induced antinociception, and prevention of the metabolism of dynorphin A (1–17) to dynorphin (1–8) or to leucine enkephalin prevents the enhancement of morphine-induced antinociception by the Δ^9 -THC (Pugh *et al.*, 1996).

The potent, synthetic cannabinoid CP55 was instrumental in demonstrating that cannabinoid binding sites are present in the substantia gelatinosa, an area involved with the transmission of pain signals (Herkenham *et al.*, 1990). In addition, CP55 produces many of the behavioral and physiologic effects characteristic of THC. Despite these similarities, we have found that THC and CP55 differ in their interaction with morphine in the spinal cord (Welch and Stevens, 1992). Pretreatment of mice with CP55 (i.t.) does not enhance the antinociceptive effects of morphine (i.t.), whereas pretreatment with THC produces a 10-fold decrease in the morphine ED₅₀. Our data indicate that THC enhances the antinociception of morphine through the release of endogenous dynorphin A (Pugh *et al.*, 1996); CP55 appears to release dynorphin B (Pugh *et al.*, 1997).

The endogenous cannabinoid anandamide appears to differ from Δ^9 -THC in its lack of interactions with dynorphinergic systems (Smith *et al.*, 1994a; Welch, 1997). Anandamide is but one of a family of arachidonic acid derivatives that have cannabinoid-like effects (Fride, 1995; Pertwee *et al.*, 1994; Mechoulam *et al.*, 1994), interacting with a G_i protein, modulating cAMP levels in cells (Welch, 1993; Felder *et al.*, 1993) and inhibiting "N-type" calcium channels (Felder *et al.*, 1993; Mackie *et al.*, 1993). Anandamide is a partial agonist at the "N-type" calcium channels, whereas the other cannabinoids are full agonists. Anandamide at low, inactive doses has been shown to attenuate the effects of Δ^9 -THC in a variety of behaviors, including antinociception and catalepsy (Fride *et al.*, 1995; Welch *et al.*, 1995a). Anandamide competitively inhibits the specific binding of [³H] HU-243, a radiolabeled cannabinoid probe, to synaptosomal membranes and produces a dose-dependent inhibition of the electrically evoked twitch response in the mouse vas deferens (Devane *et al.*, 1992). It has also been shown to displace [³H] CP55,940 binding in brain (Smith *et al.*, 1994a) and spinal cord (Welch *et al.*, 1995a). Despite similarities in the profile of action to classic cannabinoids, distinct differences between anandamide and other cannabinoids in terms of behavioral effects have been reported (Smith *et al.*, 1994a; Welch *et al.*, 1995a; Pugh *et al.*, 1996; Welch, 1997).

Given the aforementioned diversity in the antinociceptive effects of various cannabinoids, we evaluated the ability of the cannabinoid CB1 antagonist SR to attenuate the antinociceptive effects of several cannabinoids in two test systems: the tail-flick test for antinociception and rectal temperature evaluation for hypothermic effects commonly observed with cannabinoids. We evaluated the effects of SR *via* i.t., i.c.v. and i.p. routes of administration *vs.* the cannabinoids administered either i.t. or i.c.v. The cannabinoids evaluated included Δ^9 -THC and Δ^8 -THC, which have been shown to in-

teract with dynorphin A systems (Welch *et al.*, 1995a; Pugh *et al.*, 1996); CP55, which has been shown to release dynorphin B; deoxy-HU210, which has been shown to have nearly a 38-fold selectivity for the CB2 receptor (Huffman *et al.*, 1996) and anandamide, which has been shown to fail to interact with dynorphinergic systems (Welch, 1997). Our initial goal was to determine the pA₂ values for SR *vs.* the various cannabinoids in order to obtain some indication of potential subtypes of the CB1 receptor. However, it soon became apparent that we would not be able to perform full shifts of curves for anandamide or other cannabinoids as a consequence of only partial antagonism by SR. We have therefore presented the data as the differential AD₅₀ values for SR *vs.* the various cannabinoids as an indicator of potential differences in binding of the cannabinoids at the CB1 receptor.

Materials and Methods

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

Intrathecal injections. Intrathecal injections were performed according to the protocol of Hylden and Wilcox (1983). Unanesthetized mice were injected between the L5 and L6 areas of the spinal cord with a 30-gauge, 1/2-inch needle. Injection volumes of 5 μ l were administered. Cannabinoids and SR were prepared in 100% DMSO. DMSO vehicle produced scratching behavior in mice that lasted 2 min after injection. Other vehicles have previously been tested 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.

Intracerebroventricular injections. Intracerebroventricular 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. Mice were administered an injection volume of 5 μ l at a site 2 mm rostral and 2 mm caudal to the bregma at a depth of 2 mm. The cannabinoids and SR were prepared in 1:1:18 (emulphor/ethanol/saline) for i.c.v. administration. Comparison of vehicles for the cannabinoids by the i.c.v. route of administration indicated that 1:1:18 (emulphor/ethanol/saline) vehicle was devoid of antinociceptive effects (less than 10% MPE). The DMSO vehicle, which proved inactive (less than 15% MPE) upon i.t. administration, had variable effects upon i.c.v. administration (between 10% and 25% MPE) and was therefore not used for the i.c.v. route of administration.

Intraperitoneal administration of SR. SR was dissolved in 1:1:18 (emulphor/ethanol/saline) for i.p. administration. The use of DMSO i.p. in animals leads to a long duration of abdominal irritation and abdominal scratching that interferes with the testing procedure. The 1:1:18 (emulphor/ethanol/saline) vehicle has a long history of use by many laboratories for solubilization of cannabinoids and is devoid of antinociceptive effects in our test systems.

SR time course. A time course study of SR (i.p., i.t. and i.c.v.) block of Δ^9 -THC-induced (i.t. and i.c.v.) antinociception was evaluated. In all cases the peak time-point for blockade was at 1 hr. An example of one study of SR (i.p.) *vs.* Δ^9 -THC (i.t.) is shown in figure 1. A similar study was performed using SR *vs.* anandamide, and again the peak blockade of anandamide by SR was at 1 hr. Thus the 1 hr time-point was chosen for all subsequent studies of SR in combination with the cannabinoids. Δ^9 -THC, Δ^8 -THC, deoxy-HU210 and CP55 or DMSO vehicle (i.t.) were administered 15 min before determination of the response latency of the mice in the tail-flick

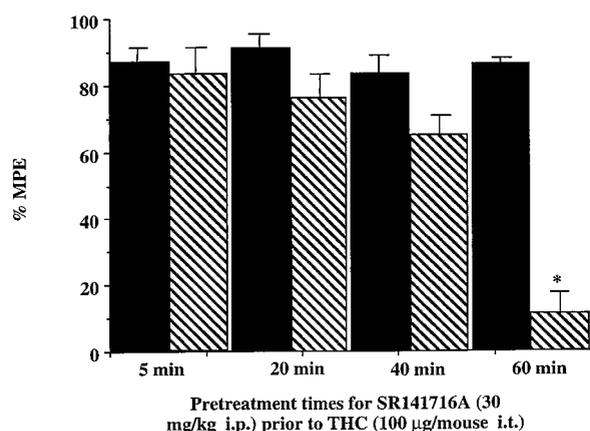


Fig. 1. Time course of SR administered i.p. before Δ^9 -THC (i.t.). SR was administered i.p. at 5, 20, 40 and 60 min before THC (i.t., 100 μ g/mouse). Eight mice were used per treatment group. The average %MPE was calculated for each group and compared statistically using ANOVA followed by Dunnett's *t* test. * indicates that the effect was significant at the $P < .05$ level.

test. This time-point represents the peak effect of the compounds as determined in our laboratory in numerous previous studies. Anandamide in 100% DMSO was administered 3 min before testing, the time of peak antinociception (Smith *et al.*, 1994a).

SR blockade of cannabinoids. The effects of SR on cannabinoid antinociception were evaluated using ED_{80} doses of the cannabinoids in combination with SR. Thus in all cases, the AD_{50} for SR blockade of cannabinoid-induced antinociception represents the dose of SR that blocks equally efficacious doses of the cannabinoids. Although the data are not shown, the SR (i.p.)-induced shifts of the dose-effect curves for Δ^9 -THC (i.t.), CP55 (i.t.) and Δ^8 -THC (i.t.) were parallel rightward shifts as evaluated by the method of Tallarida and Murray (1987). For the reference of the reader, the ED_{50} values CLs for Δ^9 -THC, CP55 and Δ^8 -THC (all i.t.) are 44.97 (22.96–88.09), 2.28 (0.006–8.59) and 72.07 (36.06–144), respectively. The ED_{50} values plus CLs for Δ^9 -THC, CP55 and Δ^8 -THC (all i.c.v.) are 16.4 (11–24.8), 2.89 (1.6–5.1) and 125.8 (65–244), respectively. ED_{50} values for the drugs administered i.t. do not differ significantly from the ED_{50} values after i.c.v. administration (Welch *et al.*, 1995b).

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 cutoff time of 10 sec were used. Antinociception was quantified as the %MPE as developed by Harris and Pierson (1964) using the following formula:

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

We calculated %MPE for each mouse using at least six mice per dose. By using the %MPE for each mouse, we calculated the mean effect and S.E.M. for each dose. Dose-response curves were generated using at least three doses of drug. ED_{50} values were determined by log-probit analysis and CLs were determined using the method of Tallarida and Murray (1987) for graded dose-response curves, omitting doses that produced 0% effect or 100% effect. The percent antagonism of an ED_{80} dose of drug by SR was determined using the following formula:

%Antagonism

$$= 100 \times \frac{[average \%MPE \text{ with SR pretreatment}]}{[average \%MPE \text{ with vehicle pretreatment}]}$$

We calculated %MPE for each mouse using at least six mice per dose. The %MPE for each mouse pretreated with SR was divided by the average %MPE for a group of six mice pretreated with vehicle. The percent antagonism for each mouse pretreated with SR was calcu-

lated. Each experiment was replicated at least twice. AD_{50} values were generated using at least three doses of SR by log-probit analysis, and CLs were determined with the method of Tallarida and Murray (1987) as described above, using the percent antagonism for each mouse and at least 12 mice per dose of SR. Significant differences between AD_{50} values were determined as a lack of overlap of the CLs for the AD_{50} values.

Hypothermia. Base-line rectal temperatures were determined before drug or vehicle injection with a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH) and a thermistor probe inserted to 25 mm. Rectal temperatures were measured again after the injection. The difference between values before and after injection was calculated for each animal. Statistical analysis of all hypothermia data was performed using ANOVA with Dunnett's *t* test for comparison with vehicle or Dunnett's *t* test for comparisons among all groups (Dunnett, 1955).

Results

SR (i.t.) produced an incomplete block of the antinociceptive effects of i.t. Δ^9 -THC, Δ^8 -THC and CP55 ($AD_{50} = 28.6$, 50.2 and 20.9 μ g/mouse, respectively) (fig. 2). The AD_{50} values and CLs for all studies are summarized in table 1. Doses of the drugs tested are listed in table 1 and represent nearly equivalent antinociceptive effects (approximate ED_{80} doses). Because of the partial blockade of the cannabinoids by SR (i.t.), the CLs about the AD_{50} values are larger than for other drug administrations in which a complete block by SR was observed. Increasing the dose of SR to 100 μ g/mouse failed to produce any greater blockade than that observed with 50 μ g/mouse. In addition, the solubility of the drug at greater than 100 μ g/mouse (20 mg/ml) was poor. SR at any dose tested failed to produce either antinociceptive or hyperalgesic effects in this test system. The maximal attenuation of the ANA ED_{80} (i.t.) by SR (i.t.) was 38% (fig. 2). The AD_{50} for SR in the presence of ANA could not be calculated, although the effects of SR led to a significant blockade of ANA.

Deoxy-HU210 had not previously been tested after i.t. or i.c.v. administration. The effects of deoxy-HU210 were dose-related after both routes of administration (fig. 3) upon a peak time of testing at 15 min after administration. The ED_{50} values for the drug were 4.9 μ g/mouse (1.5–16.4) after i.t.

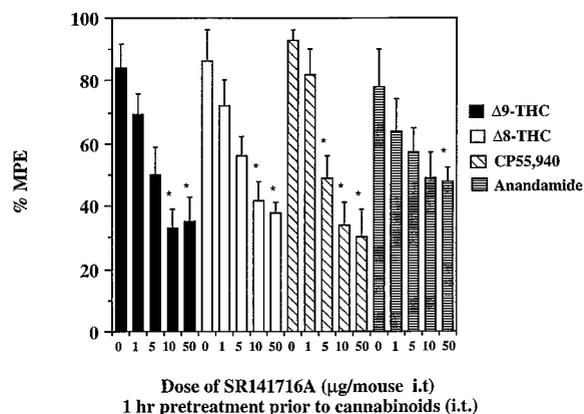


Fig. 2. Antagonism of the effects of cannabinoids administered i.t. by SR administered i.t. SR was administered i.t. at 1 hr before the ED_{80} doses of the cannabinoids (as given in table 1) or vehicle (i.t.) in mice. At 15 min later the mice were tested for antinociception using the tail-flick test, with the exception of anandamide, which was tested at 3 min after administration. Eight mice were used per treatment group. The average %MPE was calculated with the vehicle control using ANOVA followed by Dunnett's *t* test. * indicates significance at the $P < .05$ level.

TABLE 1

AD₅₀ values for SR blockade of cannabinoid-induced antinociceptive effects after administration to mice

Mice were injected with SR (i.p., i.c.v. or i.t.) or vehicle (DMSO for i.t. administration; 1:1:18, emulphor/ethanol/saline, for i.c.v. and i.p. administration). One hour later the mice were administered approximate ED₈₀ doses of various cannabinoids i.t. and/or i.c.v. The mice were tested 15 min later using the tail-flick test. At least 12 mice were used per treatment group. The % inhibition of antinociception in the presence of SR was determined as described in "Materials and Methods."

	SR AD ₅₀ Values (± CLs)		
	i.p. (mg/kg)	i.c.v. (μg/mouse)	i.t. (μg/mouse)
i.c.v. (μg/mouse)			
Δ ⁹ -THC (50)	1.4 (1.1–2.3)	4.2 (3.4–5.4)	NT
Δ ⁸ -THC (150)	0.47 (0.2–1.6)	8.1 (5.9–9.9)**	NT
CP55,940 (5)	0.88 (0.52–1.3)	2.8 (1.2–6.6)	NT
Anandamide	—	—	—
Deoxy-HU210 (20)	blocked ^a	blocked ^a	NT
i.t. (μg/mouse)			
Δ ⁹ -THC (50)	0.76 (0.61–1.8)	NT	28.6 (20.2–40.5) ^a
Δ ⁸ -THC (150)	0.9 (0.5–2.2)	NT	50.2 (40.4–63) ^a
CP55,940 (5)	0.1 (0.08–0.26)***	NT	20.9 (19–50.2) ^a
Anandamide (100)	15.4 (13.8–28.6)*	NT	blocked ^b
Deoxy-HU210 (20)	0.4 (0.3–0.8)	NT	blocked ^b

* significantly different from Δ⁹-THC, Δ⁸-THC and CP55,940 (all i.t.); ** significantly different from Δ⁹-THC and CP55,940 (all i.c.v.); *** significantly different from Δ⁹-THC and Δ⁸-THC (both i.t.).

^a indicates that block by SR was incomplete (<70%) up to 100 μg/mouse i.t.

^b The AD₅₀ could not be determined where maximal blockade was less than 50%.

NT indicates that the combination was not tested.

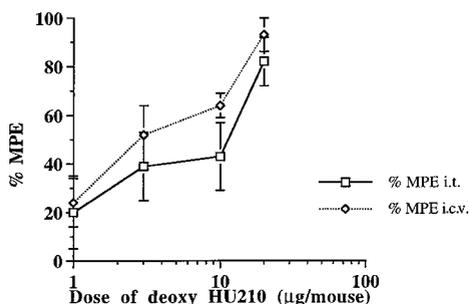


Fig. 3. Dose-response curves of deoxy-HU210 administered either i.t. or i.c.v. to mice. Deoxy-HU210 was administered either i.t. (■) or i.c.v. (○) to mice. At 15 min later the mice were tested for antinociception using the tail-flick test. Eight mice were used per treatment group. The average %MPE was calculated for each treatment group (± S.E.M.), and the ED₅₀ was determined using the method of Litchfield and Wilcoxon (1949).

administration and 3.1 μg/mouse (1.6–8.2) after i.c.v. administration. The maximal attenuation of the deoxy-HU210 i.t. ED₈₀ by SR (i.t.) was 50% attenuation (fig. 4). The AD₅₀ for SR i.t. vs. deoxy-HU210 could not be calculated, although the effects of SR led to a significant blockade of the drug.

However, when SR was administered i.c.v. before the cannabinoids [Δ⁹-THC, Δ⁸-THC or CP55 (i.c.v.)], SR was a potent antagonist and the blockade was complete and dose-related (fig. 5). The AD₅₀ values for SR (i.c.v.) vs. these cannabinoids (i.c.v.) ranged from 2.4 μg/mouse to 8.1 μg/mouse (table 1). The only significant difference in AD₅₀ was observed for SR (i.c.v.) in combination with Δ⁸-THC, where more than 2-fold more SR was required to block Δ⁸-THC. Anandamide is not active after i.c.v. administration (Smith *et al.*, 1994a) and thus could not be tested i.c.v. in combination with SR (i.c.v.). A study of deoxy-HU210 (20 μg/mouse, i.c.v.) in combination with SR (30 μg/mouse, i.c.v.) was performed (fig. 4). The effect of SR was significant, but an incomplete block resulted; higher doses of SR failed to block deoxy-HU210 completely. Interestingly, SR at 10 and 20 μg/mouse failed to alter the antinociceptive effects of deoxy-HU210 significantly [data not shown because the effect does not differ from (i.c.v.) deoxy-HU210 alone in fig. 4]. Thus we were unable to calculate an AD₅₀ for SR (i.c.v.) vs. deoxy-HU210 (i.c.v.).

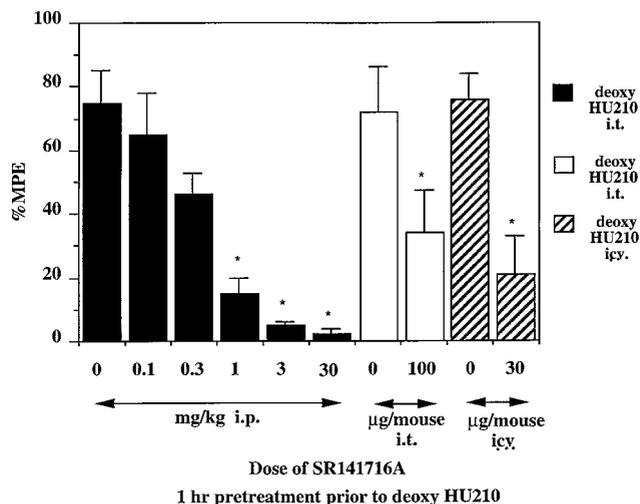


Fig. 4. Antagonism of the effects of deoxy-HU210 administered (i.t. or i.c.v.) by SR (i.p., i.t. or i.c.v.). SR was administered by all routes at 1 hr before the ED₈₀ dose of deoxy-HU210 (as given in table 1) or vehicle in mice. At 15 min later the mice were tested for antinociception using the tail-flick test. Eight mice were used per treatment group. The average %MPE was calculated for each treatment group (± S.E.M.) and compared with the vehicle control using ANOVA followed by Dunnett's *t* test. * indicates significance at the *P* < .05 level.

The AD₅₀ values for blockade of Δ⁹-THC, Δ⁸-THC and CP55 (i.c.v.) by SR (i.p.) also did not differ significantly (fig. 6; table 1). The AD₅₀ values ranged from 0.47 mg/kg to 1.4 mg/kg, and the blockade by SR was complete. The blockade of deoxy-HU210 (i.c.v.) by SR (i.p.) was incomplete (48% blockade) using 50 μg/mouse SR. SR (100 μg/mouse) produced no greater effect than the 50 μg/mouse (data not shown).

The AD₅₀ values for blockade of Δ⁹-THC, Δ⁸-THC and deoxy-HU210 (i.t.) by SR (i.p.) did not differ significantly from each other (figs. 4 and 7; table 1) and ranged from 0.4 to 0.9 mg/kg. However, the blockade by SR (i.p.) of CP55 was significantly different in that we generated a 9-fold lower AD₅₀. However, the AD₅₀ for SR (i.p.) blockade of ANA (i.t.) was 15.4 mg/kg, significantly greater than 15-fold higher than that required to block Δ⁹-THC, Δ⁸-THC, CP55 and deoxy-HU210.

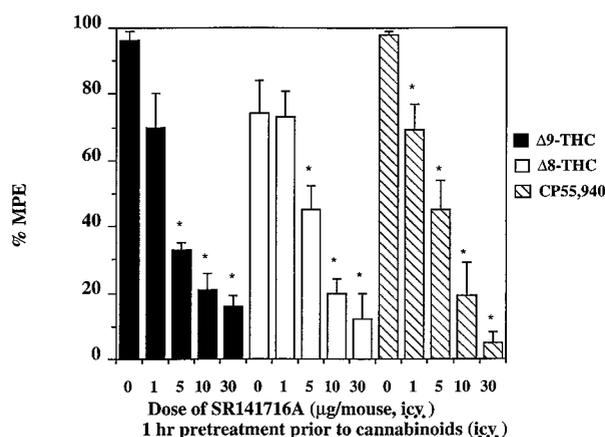


Fig. 5. Antagonism of the effects of cannabinoids administered i.c.v. by SR administered i.c.v. SR was administered i.c.v. at 1 hr before the ED_{50} doses of the cannabinoids (as given in table 1) or vehicle (i.c.v.) in mice. At 15 min later the mice were tested for antinociception using the tail-flick test. Eight mice were used per treatment group. The average %MPE was calculated for each treatment group (\pm S.E.M.) and compared with the vehicle control using ANOVA followed by Dunnett's *t* test. * indicates significance at the $P < .05$ level.

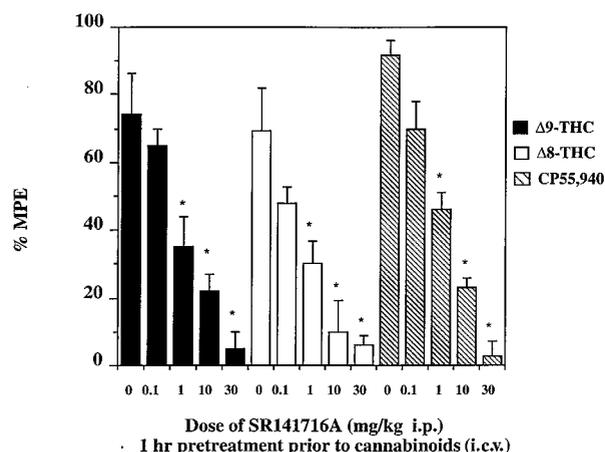


Fig. 6. Antagonism of the effects of cannabinoids administered i.c.v. by SR administered i.p. SR was administered i.p. at 1 hr before the ED_{50} doses of the cannabinoids (as given in table 1) or vehicle (i.c.v.) in mice. At 15 min later the mice were tested for antinociception using the tail-flick test. Eight mice were used per treatment group. The average %MPE was calculated for each treatment group (\pm S.E.M.) and compared with the vehicle control using ANOVA followed by Dunnett's *t* test. * indicates significance at the $P < .05$ level.

Evaluation of the hypothermic effects of Δ^9 -THC *vs.* anandamide indicated that SR (i.t. [fig. 8, panel A] or i.p. [fig. 8, panel B]) failed to block the significant hypothermic effects of the opioids (Welch and Stevens, 1992). The focus of this manuscript is the interaction of the cannabinoids with the CB1 receptor as quantitated by the actions of the highly CB1-selective antagonist SR (Rinaldi-Carmona *et al.*, 1994; Felder *et al.*, 1995; Showalter *et al.*, 1996) to attenuate such antinociceptive effects. SR has been extensively studied in a variety of systems and appears to be selective for the CB1 receptor (Rinaldi-Carmona, 1995; Rinaldi-Carmona *et al.*, 1996b; Showalter *et al.*, 1996; Felder *et al.*, 1995). Compton *et al.* (1996) have evaluated the effects of SR-induced blockade of a tetrad of traditional cannabinoid behaviors, in addition to the p-phenylquinone (PPQ) test for antinociception, using both i.v. and i.p. administration of SR *vs.* i.v. administration of Δ^9 -THC. They did not evaluate the effects of SR *vs.* any cannabinoid administered centrally. Although the time course of effects of SR (i.v.) observed by Compton *et al.* (1996) differs from those observed in our study (i.p., i.c.v. and i.t.), such an effect is to be expected given the differences in routes of administration of the drug. However, their AD_{50} for SR (i.v.) blockade of the antinociceptive effects of Δ^9 -THC (i.v.) [0.16 mg/kg in the tail-flick test] is within the range observed in our study for SR (i.p.) block of Δ^9 -THC, Δ^8 -THC and CP55 or deoxy-HU210 (all i.t.) (table 1). Similarly, the AD_{50} values generated for SR (i.p.) by Compton *et al.* (1996) [0.38 mg/kg in the tail-flick test and 2.7 mg/kg in the PPQ test] are also in the range of those shown in our study to block Δ^9 -THC, Δ^8 -THC and CP55 (i.c.v.). These data indicate that the efficacy and potency of SR (i.p.) are similar to those of SR administered i.v. In addition, the AD_{50} values for SR (i.p. and i.v.) are similar to those for peripherally or centrally administered cannabinoids.

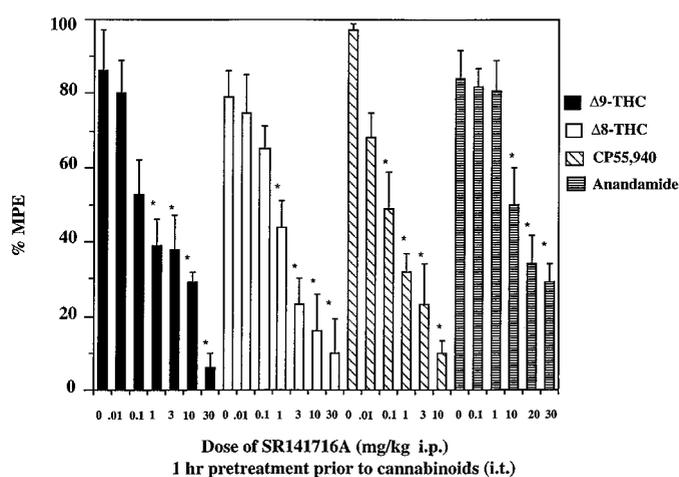


Fig. 7. Antagonism of the effects of cannabinoids administered i.t. by SR administered i.p. SR was administered i.p. at 1 hr before the ED_{50} doses of the cannabinoids (as given in table 1) or vehicle (i.t.) in mice. At 15 min later the mice were tested for antinociception using the tail-flick test, with the exception of anandamide, which was tested at 3 min after administration. Eight mice were used per treatment group. The average %MPE was calculated for each treatment group (\pm S.E.M.) and compared with the vehicle control using ANOVA followed by Dunnett's *t* test. * indicates significance at the $P < .05$ level.

Discussion

However, Compton *et al.* (1996) did not evaluate the block by SR of diverse cannabinoids. One major difference between the cannabinoids tested in our study was that SR (i.p.) was at

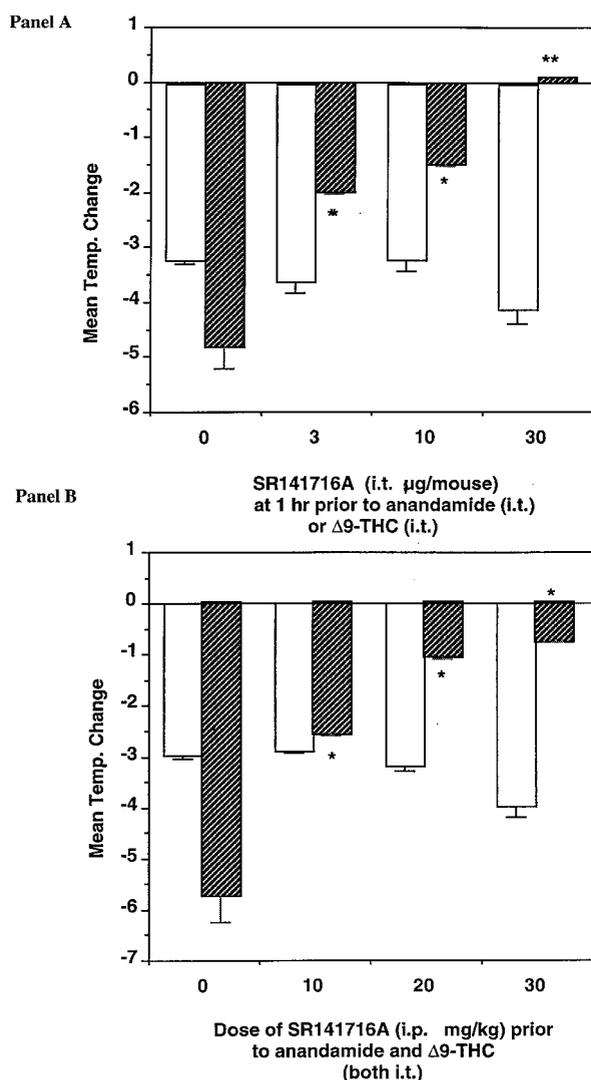


Fig. 8. Antagonism of the hypothermic effects of anandamide and Δ^9 -THC administered i.t. by SR administered i.t. (panel A) or i.p. (panel B). SR was administered i.t. or i.p. at 1 hr before the ED_{50} doses of the cannabinoids (as given in table 1) or vehicle (i.t.) in mice. At 15 min later the THC-pretreated mice (filled bars) were tested for hypothermia. Mice pretreated with anandamide (white bars) were tested at 3 min after administration. Eight mice were used per treatment group. The average change in body temperature from that of naive mice was calculated for each treatment group (\pm S.E.M.) and compared with the vehicle control using ANOVA followed by Dunnett's *t* test. * indicates significance at the $P < .05$ level.

least 15-fold less effective in blocking the effects of anandamide administered i.t. than in blocking the other classic cannabinoids. Because anandamide has been shown to have somewhat higher affinity for the CB1 receptor (Showalter *et al.*, 1996) and to displace [3 H]-SR binding with a K_i similar to that of Δ^9 -THC (Hirst *et al.*, 1996), such a difference was unexpected and may represent some differences in the binding of anandamide to the CB1 receptor. We were not able to generate a pA_2 value for SR block of anandamide, so we cannot provide evidence of a different CB1 receptor subtype for anandamide binding, although such a possibility cannot be ruled out. Judging by the lack of interaction of anandamide with the dynorphinergic system in the production of antinociception and tolerance, it appears reasonable to speculate on the existence of potential subtypes of the CB1

receptor. The CB1_A receptor has been cloned (Shire *et al.*, 1995) and characterized in cell lines (Rinaldi-Carmona *et al.*, 1996a), but it differs only slightly from the CB1 receptor in the events mediated by activation of the CB1_A receptor. SR has about 10-fold lower affinity at the CB1_A receptor than at the CB1 receptor. Anandamide has nearly equal affinity for both isoforms of the CB1 receptor. Given such data, the potential for other isoforms of the receptor cannot be ruled out, nor can the potential for differences in SR binding at such putative new CB1 receptor subtypes.

A similar difference between cannabinoids that we tested was observed with the CB2-selective drug deoxy-HU210 (Huffman *et al.*, 1996). The i.p. administration of SR only partially attenuated the antinociceptive effects of deoxy-HU210 (i.c.v.). Deoxy-HU210 has high affinity at the CB1 receptor as well as at the CB2 receptor. The AD_{50} for SR (i.p.) *vs.* deoxy-HU210 (i.t.) did not differ from other cannabinoids. Thus it was surprising that the effects of the drug in combination with SR differed from other cannabinoids upon i.c.v. administration. Because at spinal sites deoxy-HU210 appears to interact with the CB1 receptor, the lack of efficacy of SR (i.c.v. or i.p.) in blocking the drug's effects after supraspinal administration of deoxy-HU210 (i.c.v.) may simply reflect some pharmacokinetic interaction with SR. Alternatively, the data may indicate that the binding of deoxy-HU210 to the CB1 receptor supraspinally differs from that spinally or that subtypes of the CB1 receptor exist. We have no data to indicate why such a diversity in the effect of SR *vs.* deoxy-HU210 is observed.

Unlike work in the myenteric plexus of the guinea pig ileum, where SR was less potent in blocking contractile inhibition induced by CP55 *vs.* Δ^9 -THC (Pertwee *et al.*, 1996), we found few differences among Δ^9 -THC, Δ^8 -THC and CP55 in the AD_{50} values for blockade by SR *via* any route of administration. Our data indicated that SR (i.p.) was more potent in blocking CP55 (i.t.) and less potent in blocking Δ^8 -THC when both drugs were administered i.c.v. The biological relevance of such differences is not apparent, because such differences were observed as two random events and were not consistent across all the data. It is possible but unlikely that the pA_2 values for such blockade differ significantly. Our data indicate that, unlike the suggestion of different cannabinoid receptors for Δ^9 -THC *vs.* CP55 in the guinea pig ileum (Pertwee *et al.*, 1996), in our system we have only the above evidence based on the SR data to indicate differences in binding sites for Δ^9 -THC *vs.* CP55 in the spinal cord. However, we have demonstrated differential release of dynorphin A *vs.* dynorphin B by Δ^9 -THC *vs.* CP55, respectively (Pugh *et al.*, 1997). It is difficult to envision such diverse dynorphin release profiles for the drugs if they exert their effects through actions at one receptor subtype. The mechanisms underlying the differential release of dynorphins by Δ^9 -THC *vs.* CP55 thus remain unknown.

SR appears to lack potency when administered at the spinal segmental level. Comparison of the AD_{50} values for SR (i.t.) block of cannabinoids (i.t.) with the AD_{50} values for SR (i.c.v.) block of cannabinoids (i.c.v.) indicates that a 6- to 9-fold increased dose of SR was required at spinal sites. In addition, the block was incomplete in all cases. Thus not only the potency, but also the efficacy, of SR is low when it is administered spinally. The AD_{50} for SR *vs.* deoxy-HU210 and for anandamide (all i.t.) could not be determined. These data

indicate that the predominant effects of SR may be at supraspinal sites. Because the binding of [³H]-CP55 to presumably the CB1 receptor does not appear to differ kinetically at brain *vs.* spinal sites (Smith *et al.*, 1994a; Welch *et al.*, 1995a), and because displacement of SR binding at spinal sites has not been evaluated, there is no evidence for anandamide- or deoxy-HU210-sensitive receptor subtypes. However, this lack of evidence does not rule out such a possibility. In addition, the lack of efficacy of SR in blocking the antinociceptive effects of anandamide might be explained by SR exerting its effects predominantly on antinociception at supraspinal sites, a region where anandamide fails to alter antinociception in mice (Smith *et al.*, 1994a; Welch *et al.*, 1995a) and rats (Lichtman *et al.*, 1996). Anandamide does produce a small but significant hypothermic effect when administered *i.t.*, but not when administered in the rat brain (Lichtman *et al.*, 1996). Presumably, such an effect of anandamide (*i.t.*) would be supraspinally mediated. Δ^9 -THC (*i.t.*) produces a robust hypothermic effect when administered *i.t.* The hypothermic effects of Δ^9 -THC are blocked totally by SR; the hypothermic effects of anandamide (*i.t.*) are not altered by SR. Such data are indicative of differential interactions of the two cannabinoids in temperature regulation. The nature of the differential effect remains to be elucidated, but it is clearly mediated by differences in the binding to the CB1 receptor supraspinally, as evidenced by the lack of blockade of anandamide by SR. Thus anandamide appears to differ from the traditional cannabinoids in that it is not active after *i.c.v.* administration in several behaviors that are characteristic of cannabinoids and is either incompletely blocked or not blocked by SR in quantitation of such behaviors. Other differences between anandamide and Δ^9 -THC have been observed in tasks involving learning and memory (Lichtman *et al.*, 1995), drug discrimination (Wiley *et al.*, 1995) and modulation by agonists and antagonists of classic neurotransmitter systems (Welch *et al.*, 1995b).

It is interesting that the cannabinoids differ in that they generally fall into two categories: those that enhance the antinociceptive effects of morphine only in the spinal cord (Δ^9 -THC, for example) and those that enhance the effects of morphine only in the brain (CP55, for example). We believe that our data indicate that the mechanism by which the cannabinoids produce antinociception involves dynorphin release spinally and that the "greater than additive effects" of the cannabinoids with morphine and the *delta* opioid DPDPE are due to the initial release of dynorphin A peptides and the subsequent breakdown of the dynorphin A to leucine enkephalin (Pugh *et al.*, 1996). We hypothesize that the functional coupling of the *mu/delta* and *mu/kappa* receptors leads to enhanced antinociceptive effects of morphine and DPDPE by the cannabinoids. Several attempts have been made to understand how the cannabinoids produce their pharmacological effects, particularly antinociception. We envision cannabinoid-induced release of dynorphins as an indirect process due to the disinhibition of yet unknown neuronal processes. The localization of the cannabinoid receptors involved in dynorphin release are not known. We hypothesize that in the spinal cord, cannabinoids produce antinociceptive effects *via* the direct interaction of the cannabinoid receptor with Gi/o proteins, resulting in a decreased cAMP production (Welch *et al.*, 1995b), as well as hyperpolarization *via* interaction with specific potassium channels (Deadwyler *et al.*,

1993). Thus the cannabinoids may produce disinhibition by decreasing the release of an inhibitory neurotransmitter in dynorphinergic pathways. The net result of such an effect may be an increase in dynorphin release. The events that precede and follow the release of dynorphin remain unclear. The dynorphin is probably a modulator of other "downstream" systems (possibly substance P release or interaction with NMDA-mediated events) that culminate in antinociception upon administration of cannabinoids. What has proved intriguing is the observation that cannabinoids differ in their interactions with dynorphins (and subsequently with *mu* and *delta* opioids). Δ^9 -THC and Δ^8 -THC appear to interact with the dynorphin A system (Pugh *et al.*, 1996; Welch, 1997), whereas CP55 appears to interact with and release dynorphin B (Pugh *et al.*, 1997), although CP55 is clearly cross-tolerant to Δ^9 -THC (Fan *et al.*, 1994). Δ^9 -THC is not cross-tolerant to dynorphin B but is cross-tolerant to the dynorphins of the "A" type (Welch, 1997).

The most pronounced difference occurs with anandamide, which is neither blocked by the *kappa* antagonist nor-BNI nor cross-tolerant to any dynorphins (Smith *et al.*, 1994a, Welch *et al.*, 1995a, Welch, 1997), although anandamide is cross-tolerant to Δ^9 -THC and CP55 and displaces binding of the traditional cannabinoids (Smith *et al.*, 1994a; Welch *et al.*, 1995a; Devane *et al.*, 1992). Anandamide fails to enhance the activity of any opioid and does not release dynorphin A (Welch *et al.*, 1995a; Pugh *et al.*, 1996; Welch, 1997). Although we have not yet evaluated deoxy-HU210 for dynorphin release, our preliminary data indicate that the drug fails to enhance the activity of *mu*, *delta* or *kappa* opioids (data not shown). However, its antinociceptive effect is blocked by nor-BNI. Such data appear to suggest a release of dynorphin B, rather than dynorphin A based on the work with CP55 (Pugh *et al.*, 1997).

In summary, the CB1 antagonist SR was evaluated systematically after administration by three diverse routes in combination with centrally administered natural and synthetic cannabinoids and the endogenous cannabinoid anandamide. Our data indicate that anandamide and, to a lesser extent, deoxy-HU210 appear to differ from other cannabinoids tested either in that the blockade by SR was partial or in that SR was significantly less potent in such blockade. SR failed to block the hypothermic effects induced by anandamide, while attenuating those of Δ^9 -THC. The potency of SR in blocking Δ^9 -THC did not differ from its potency in blocking CP55, although the drugs exhibit pronounced diversity in the interaction with dynorphinergic systems. Such data suggest either a differential interaction of anandamide *vs.* the classic cannabinoids at the CB1 receptor or the existence of subtypes of the CB1 receptor.

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