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;pinal and Supraspinal Components of Cannabinoid-Induced \ntinociception[1]

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.BSTRACT

he purpose of this study was to investigate whether cannabi-oids produce antinociception spinal and supraspinal sites of ction. The antinociceptive effect of Δ^9-tetrahydrocannabinol (3 r 10 mg/kg), a naturally occurring cannabinoid, and CP-55,940).1, 0.3, or 0.5 mg/kg), a potent synthetic cannabinoid, were ssessed in spinally transected and intact rats. Each drug, dministered i.v., produced a potent, long-lasting elevation of ail-flick latencies in the intact animals. This antinociception was gnificantly attenuated by spinal transection. Administration of ach cannabinoid (i.t.) to the lumbar region of the spinal cord roduced a weak, but long-enduring antinociceptive effect. In ontrast, spinal administration of CP-55,940 to the upper tho-acic region failed to elevate tail-flick latencies above base-line alues. Additionally, i.t. administration of CP-55,940 (30 or 100 g) continued to have a weak antinociceptive effect in spinal rats. In contrast, i.t. administration of CP-56,667, the (+)-enantiomer of CP-55,940, failed to elevate tail-flick latencies above base line at a dose of 1000 μg, thus indicating stereoselectivity. Finally, the biodisposition of ^{3}H-Δ^9-tetrahydrocannabinol after either i.v. or i.t. administration to spinal and intact rats was also assessed. The levels of radioactivity did not differ between spinal and intact animals in either whole brain, spinal cord, or plasma when the drug was administered i.v. When the drug was administered i.t., however, surgical transection of the spinal cord led to a decreased concentration of labeled substances in the whole brain and plasma. These converging lines of evidence indicate that cannabinoids produce antinociception through multiple mechanisms at the spinal and supraspinal levels of the central nervous system.

Δ^9-THC, a naturally occurring psychoactive chemical present n marijuana, as well as other naturally occurring and synthetic annabinoids, has been demonstrated to produce potent anti-iociception when administered i.v. in mice (Martin, 1985, 1986; .ittle *et al.*, 1988). Converging evidence supports the hypothesis hat cannabinoids produce their effects through a receptor nechanism. First, SAR studies examining synthetic and natu-ally occurring cannabinoids indicate that subtle changes in tructure can greatly affect pharmacological potency (Razdan, 986; Little *et al.*, 1988). SAR studies have also revealed the existence of behaviorally active and inactive cannabinoid ster-oisomers. The fact that these stereoisomers have approxi-nately equal lipophilicity (Thomas *et al.*, 1990) further indi-ates that cannabinoids do not produce their effects through nonspecific effects such as disrupting membrane fluidity. Additionally, it has been hypothesized that cannabinoids produce their effects through the inhibition of cyclic AMP (Howlett *et al.*, 1988). Finally, the recent characterization of a putative cannabinoid receptor (Devane *et al.*, 1988), its localization in the CNS (Herkenham *et al.*, 1990) and its cloning (Matsuda *et al.*, 1990) provide compelling support for a receptor-like mechanism.

Despite the multitude of studies examining the SAR as well as the cellular mechanisms of the cannabinoids, relatively little is known about the neuroanatomical mechanisms that mediate cannabinoid-induced antinociception. In the tail-flick assay, drugs can produce antinociception either *via* direct spinal mechanisms (Yaksh and Rudy, 1977; Yaksh, 1985), at brain sites that in turn activate descending antinociceptive pathways (Yaksh, 1979; Sagen and Proudfit, 1981; Hammond and Yaksh, 1984; Hammond, 1986), or through a synergistic action at spinal and supraspinal sites (Yeung and Rudy, 1980). Although few studies have addressed the issue of site of action, there is some indication that cannabinoids can produce antinociception at the spinal level (Yaksh, 1981). Specifically, i.t. administration of the cannabinoid analog levonantradol produced potent antinociception in rats, suggesting a spinal mechanism of action. Alternatively, the drug may have diffused rostrally and thereby produced its effects at the supraspinal level. Such has been the case for other i.t. administered drugs (Tang and Schoenfeld, 1978), thus rendering delineation of segmental and supraspinal

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ABBREVIATIONS: Δ^9-THC, Δ^9-tetrahydrocannabinol; ANOVA, analysis of variance; CNS, central nervous system; % MPE, percentage of maximum possible effects; SAR, structure-activity relationship.

contribution difficult to assess. Nonetheless, Yaksh (1981) noted only minimal signs of supraspinal activity after i.t. administration of levonantradol.

The purpose of the present investigation was to elucidate the relative contribution of spinal and supraspinal components of cannabinoid-induced antinociception by examining the antinociceptive activity of the prototypical cannabinoid, Δ^9-THC, as well as the more potent synthetic compound, CP-55,940, in both spinally transected and intact rats (see fig. 1 for chemical structures). Of consequence, CP-55,940 has been used *in vitro* to characterize the putative cannabinoid receptor (Devane *et al.*, 1988) and to localize cannabinoid binding sites in the CNS through autoradiography (Herkenham *et al.*, 1990). The degree to which spinal ligation attenuated antinociception produced by i.v. administered cannabinoids was used to infer the relative contribution of supraspinal structures, because direct neural communication between the brain and spinal cord was completely severed. Conversely, any residual antinociception in the spinal animals was attributed to spinal action. Additionally, the impact of i.t. administration of both compounds as well as CP-56,667, the inactive (+)-enantiomer of CP-55,940, on nociception was also studied. Two methodologies were used to insure that the i.t. administered drugs produced their effects through direct spinal mechanisms and not simply because of rostral diffusion to the brain. First, the effect of i.t. administered CP-55,940 was assessed in spinal and intact rats. If the drug acted solely at supraspinal sites, spinal transection would completely block its antinociceptive effect. Second, CP-55,940 was administered to either the lumbar or upper thoracic region of the spinal cord. A drug acting at the spinal level should be more potent when it is administered at the lumbar level than when administered to the rostral thoracic segments of the spinal cord (Tang and Schoenfeld, 1978). Finally, to determine the access of cannabinoids to the brain and spinal cord, [^{3}H] Δ^9-THC was administered either i.v. or i.t. and total radioactivity was assessed.

Materials and Methods

Subjects

Male Sprague-Dawley rats (Dominion Labs, Dublin, VA) with a mean body weight of approximately 350 g served as subjects. Each rat was individually housed, and Prolab 3000 Animal Chow and tap water were continuously available. All experiments were conducted during the light portion of a 14:10 hr light/dark cycle.

Drug Preparation and Administration

CP-55,940 and CP-56,667 were obtained from Pfizer Pharmaceuticals (Groton, CT), and Δ^9-THC was provided by the National Institute on Drug Abuse (Rockville, MD). For i.v. administration, each drug was dissolved in a 1:1 mixture of ethanol and emulphor. Isotonic saline was then added to yield a final vehicle of 1:1:18 (ethanol-emulphor-saline) for the preparation of CP-55,940. Because of solubility constraints, Δ^9-THC was mixed in a vehicle of 1:1:8 (ethanol-emulphor-saline). Each subject was placed in a restraining chamber, and the tail was immersed in 37°C water to dilate the veins for the i.v. injection. Injections were administered *via* the tail vein in a volume of 0.5 ml/kg.

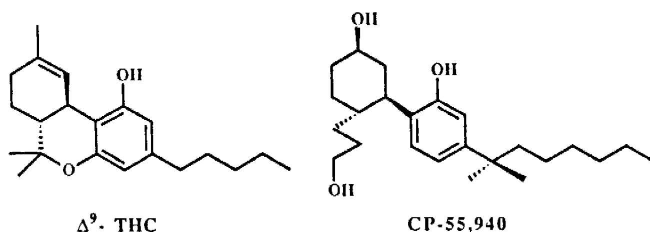

Fig. 1. Chemical structures of Δ^9-THC and CP-55,940.

In order to obtain a highly concentrated solution of cannabinoids for the i.t. injections, dimethyl sulfoxide was used as the vehicle. Unless otherwise indicated, drugs were administered in 10 μl of dimethyl sulfoxide, followed by a 10 μl flush of isotonic saline over a period of 1 min. A cap approximately 2 cm long made from PE50 tubing was then fit over the exposed catheter to prevent leakage. Any subject in which drug leaked from the catheter was removed from the experiment.

Surgery

Subjects were anesthetized with a pentobarbital (50 mg/kg, i.p.) and supplemented with halothane as needed.

Spinal catheter implantation. After induction of anesthesia, each subject was implanted with an i.t. catheter (PE10, approximately 0.7? mm diameter) through an incision in the atlanto-occipital membrane into the subarachnoid space (Yaksh and Rudy, 1976). The catheters extended 8.5 cm in length, with the tip extending just rostral to the lumbar enlargement. An additional group of animals was implanted with catheters that were 3.5 cm in length, extending to the upper thoracic segments of the cord. Immediately before surgery, each catheter was flushed with sterile saline, and its external end was heat sealed. Approximately 20% of the subjects exhibited signs of motor dysfunction and were immediately eliminated from the study. All subjects were given at least 1 week to recover before testing. At the conclusion of the study, subjects were euthanized with pentobarbitol (100 mg/kg, i.p.), black Indian ink was injected into the catheter, and the internal end was examined to verify its viability. Although all catheters remained viable, many of them were encapsulated with scar tissue as reported by Durant and Yaksh (1986).

Spinal ligations. After anesthesia was inducted, each subject received a laminectomy between the sixth and seventh thoracic vertebrae. The spinal cord was severed with micro dissecting scissors, a 0.5 cm piece of spinal cord was excavated by aspiration and gelfoam was placed in the empty space to promote clotting. In animals implanted with chronic spinal catheters, the spinal cord was severed with micro dissecting forceps to avoid cutting the catheter. The underlying muscles were then sutured and the skin was closed with wounds clips. Each lesioned rat was housed in a tub with wood chip bedding that was placed on top of a heating pad. Throughout the 5-day recovery period their bladders were voided twice daily. Necropsy verified that approximately 0.5-cm section of spinal cord was removed. Control animals received a laminectomy, but the spinal cord was left intact.

Antinociceptive Test

The tail-flick response to radiant heat (D'Amour and Smith, 1941) as modified by Dewey *et al.* (1970) was used to assess antinociception. An automatic 8-sec cut-off was used to prevent tissue damage. Data were transformed to the % MPE by the following equation (Harris and Pierson, 1964): % MPE = [(test latency − control latency) / (8 − control latency)] × 100; where 8 represents the cut-off criterion and the control latency was the mean of the four base-line tests.

Procedure

Each subject was handled for about 1 min/day for at least 1 week before the experiment to reduce the degree of stress. On the test day each subject was given four base-line tail-flick tests before administration of drug or vehicle. Subjects administered i.v. cannabinoids were assigned to one of three groups. They either had their spinal cords surgically ligated, received a sham surgery, or were not subjected to any surgical procedure. As the sham animals did not differ from the control animals, data from these groups were pooled.

Rats tested after i.t. administration of CP-55,940 were given a 1 week rest period and then administered either vehicle or Δ^9-THC (100, 300 or 1000 μg). Subjects were counterbalanced with respect to the dose of CP-55,940 that had previously been administered. Similarly,

the surgery control animals that were tested after i.t. injections of CP-55,940 were given a 1-week recovery period and then administered i.t. CP-55,940 or CP-56,667. Both drug groups were counterbalanced with respect to their previous treatment.

Biodisposition of [^{3}H]Δ^9-THC

Intact and spinal rats were injected with [^{3}H]Δ^9-THC either i.v. (10 mg/kg) or i.t. (300 μg). The specific activity of the [^{3}H]Δ^9-THC was adjusted so that all animals received 250 μCi/kg (approximately 100 μCi per rat) by i.v. administration and 100 μCi by i.t. administration. Fifteen min after either i.v. or i.t. injection, each animal was decapitated. Blood from the cervical wound was collected in a heparinized tube and centrifuged at 3000 × g for 10 min to obtain plasma. The whole brains were removed, weighed and a volume of phosphate buffer 3 times the brain volume was added to each sample. The tissue was then homogenized for 30 sec. The spinal cord was dissected from T6 to the cauda equina and homogenized in 0.75 ml of the phosphate buffer. Each sample was neutralized with 50 μl of glacial acetic acid. Radioactivity of each sample was quantified by solubilizing 150 μl of each sample in 10 ml of scintillation counting cocktail before liquid scintillation spectrometry. Quench was corrected by external standardization. Data were expressed as Δ^9-THC equivalents in which each equivalent represents 1 ng of Δ^9-THC and its metabolites per 1 mg of tissue.

Results

The antinociceptive effect of i.v. Δ^9-THC in spinally transected and intact rats. The mean control tail-flick latency of the spinal animals, 2.6 ± 0.1 sec, was slightly but significantly lower than that of the intact animals, 3.5 ± 0.1 sec, $F(1,41) = 23.5$, $p < 0.05$. In figure 2, the mean % MPE 15 min after an i.v. injection of drug or vehicle in both spinal and intact rats is shown. ANOVA revealed that Δ^9-THC produced a significant dose-dependent increase in tail-flick latency ($F(1,37) = 57$, $P < .05$). More important, i.v. administration of Δ^9-THC had a differential effect between the spinal and intact animals, as indicated by a significant surgery-by-dose interaction ($F(2,37) = 4.71$, $P < .05$). *Post hoc* analysis revealed that spinal transection decreased the antinociceptive effect of 10 mg/kg of Δ^9-THC, Newman-Keuls, $P < .05$, but not in the 3 mg/kg and vehicle control groups. The tail-flick latencies of the intact and spinal animals receiving the 10 mg/kg dose were 7.6 ± 0.4 and 4.8 ± 0.6 sec, respectively. Finally, the antinociception in both spinal and intact rats was of long duration because tail-flick latencies did not significantly decrease during the 2-hr test (data not shown).

The antinociceptive effect of i.v. CP-55,940 in spinally transected and intact rats. The control tail-flick latencies among the intact animals was 2.6 ± 0.1 sec and for the spinal animals was 2.2 ± 0.1 sec ($F(1,56) = 22.6$, $P < .05$). At 15 min after i.v. administration of CP-55,940 (0.3 or 0.5 mg/kg), the respective tail-flick latencies for the intact rats were 6.7 ± 0.6 and 6.7 ± 0.8 sec and for the spinal animals they were 3.9 ± 0.3 and 4.9 ± 0.5 sec. The % MPE in both spinal and intact rats which received an i.v. injection of CP-55,940 is depicted in figure 3. CP-55,940 produced a significant dose-dependent increase in tail-flick latency ($F(3,49) = 33$, $P < .05$), which did not significantly decrease during the 1-hr test period. Spinal transection attenuated the antinociceptive effect of the drug as revealed by a significant surgery-by-dose interaction ($F(3,49) = 3.11$, $P < .05$. Specifically, spinal ligation decreased the antinociceptive effect of both 0.3 and 0.5 mg/kg of CP-55,940 (Newman-Keuls, $P < .05$), but not in the 0.1 mg/kg or vehicle control groups.

The antinociceptive effect of CP-55,940 and Δ^9-THC administered i.t. The control tail-flick latencies ranged between 2.6 and 2.9 sec. The antinociceptive effect of i.t. CP-55,940 during a 4-hr test period is illustrated in figure 4. CP-55,940 significantly elevated tail-flick latencies ($F(4,27) = 4.07$, $P < .05$). Furthermore, subjects receiving 30, 60 and 100 μg doses differed from those animals administered 10 μg and vehicle (Newman-Keuls, $P < .05$), but did not significantly differ from one another at any time point. Similarly, as illustrated in figure 5, i.t. Δ^9-THC produced a weak but significant antinociceptive effect ($F(3,20) = 3.12$, $P < .05$). Although inspection of the data indicates less antinociception after 1000 μg than after 300 μg of Δ^9-THC, these groups did not significantly differ.

To examine whether the drug was merely producing its effects because of rostral diffusion to the brain, CP-55,940 (100 μg, i.t.) was administered to either the upper thoracic region or the lumbar level (see fig. 6). To prevent drug spread, CP-55,940

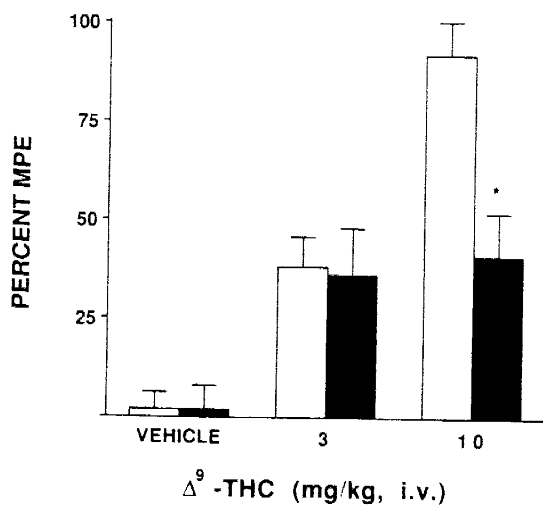

Fig. 2. Antinociceptive activity of Δ^9-THC in intact (□) and spinal (■) rats. The tail-flick response was measured 15 min after an i.v. injection, and the results are presented as means ± S.E.M. (n = 7 per group) of % MPE. *Significantly different from the intact group.

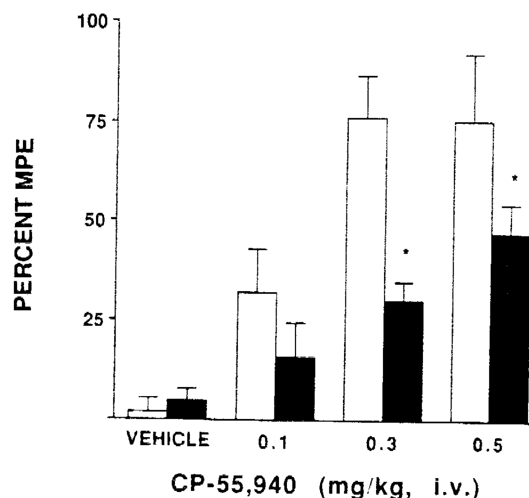

Fig. 3. Antinociceptive activity of CP-55,940 in intact (□) and spinal (■) rats. The tail-flick response was measured 15 min after an i.v. injection, and the results are presented as means ± S.E.M. (n = 6–8 per group) of % MPE. *Significantly different from the intact group.

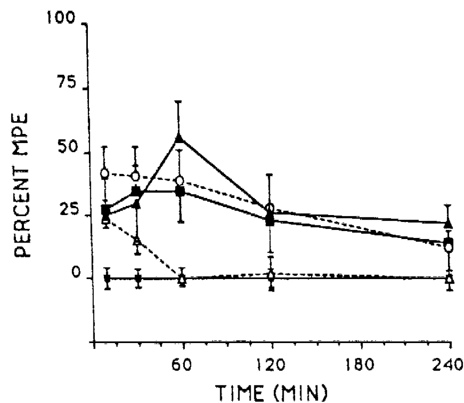

Fig. 4. The time course of the antinociceptive effect of vehicle (×), 10 (△), 30 (■), 60 (○) or 100 μg (▲) of CP-55,940 administered i.t. The means ± S.E.M. (n = 6–7 per group) of the % MPE are presented.

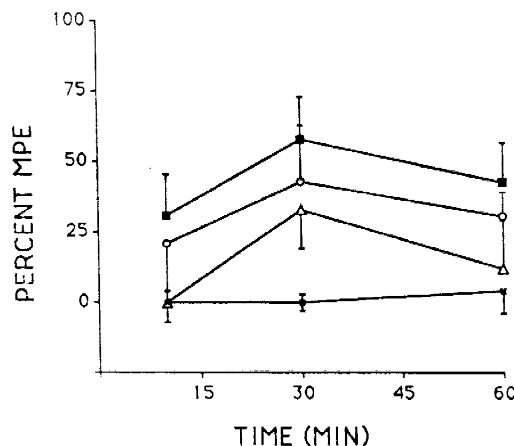

Fig. 5. The time course of the antinociceptive effect of vehicle (×), 100 (△), 300 (■) or 1000 (○) μg of Δ^9-THC administered i.t. The means ± S.E.M. (n = 6 per group) of the % MPE are presented.

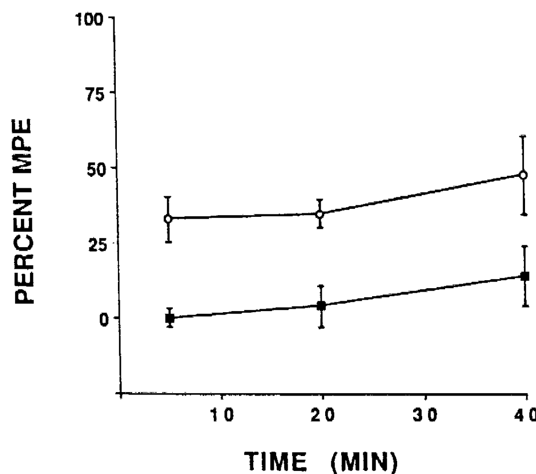

Fig. 6. Antinociceptive effect of CP-55,940 (100 μg, i.t.) administered to either the upper thoracic (■) or lumbar (○) regions of the spinal cord. The results are presented as means ± S.E.M. (n = 6 per group) of % MPE.

was administered in a 1-μl volume followed by either a 4- or 6-μl saline flush, the volume of each respective spinal catheter. Animals receiving drug *via* the lumbar catheters exhibited significantly more antinociception than those receiving the same concentration of drug in the upper thoracic catheters ($F(1,10) = 9.36$, $P < .05$). At 40 min, for example, the % MPE of i.t. administered CP-55,940 was 48 ± 13 in the lumbar group compared to 14 ± 10 in the upper thoracic group.

The antinociceptive effect of i.t. CP-55,940 in spinally transected and intact rats. The mean control tail-flick latency for the intact rats was 2.8 ± 0.1 and for the spinal rats was 1.9 ± 0.1 sec. This difference was statistically significant ($F(1,42) = 85.7$, $P < .05$. As indicated in figure 7, CP-55,940 produced a significant dose-related increase in tail-flick latency ($F(2,38) = 19.8$, $P < .05$. Furthermore, the spinal animals treated with CP-55,940 exhibited less antinociception than the intact group ($F(1,38) = 4.99$, $P < .05$. Although the surgery-by-dose interaction did not approach significance, a t test revealed that spinal ligation decreased the antinociceptive effect of 100 μg of CP-55,940 ($t(38) = 2.16$, $P < .05$), but had no effect in the 30-μg or vehicle control groups. The antinociceptive effect of i.t. administered CP-55,940 did not significantly decrease over the 2-hr test period.

The effect of i.t. CP-55,940 and CP-56,667 in the inhibition of the tail-flick response. The % MPE after i.t. administration of either CP-55,940 or CP-56,667 is presented in figure 8. Whereas the [−]-isomer produced approximately 60% MPE, the [+]-isomer failed to elevate tail-flick latencies above base line. ANOVA confirmed that the animals treated with CP-55,940 had significantly longer tail-flick latencies than those treated with CP-56,667 ($F(1,12) = 21.8$, $P < .05$. Furthermore, the antinociceptive effect of CP-55,940 increased over time as indicated by a significant drug-by-time interaction ($F(3,36) = 7.0$, $P < .05$.

The biodisposition of i.t. and i.v. administered [^{3}H]Δ^9-THC in spinal and intact rats. The concentration of Δ^9-THC and its metabolites in whole brain, spinal cord and plasma after i.v. and i.t. administration are presented in table 1. Intravenous administration of [^{3}H]Δ^9-THC resulted in equivalent concentrations of radioactivity in both whole brain and spinal cord, whereas the plasma concentration was considerably lower. More important, there were no differences of drug distribution between the spinal and intact rats in any of the three tissue samples. After i.t. drug administration, the spinal animals had significantly less Δ^9-THC equivalents in both brain tissue and plasma than the intact animals (Mann-Whitney U test, $P < .05$), however, the spinal concentrations did not differ. Although

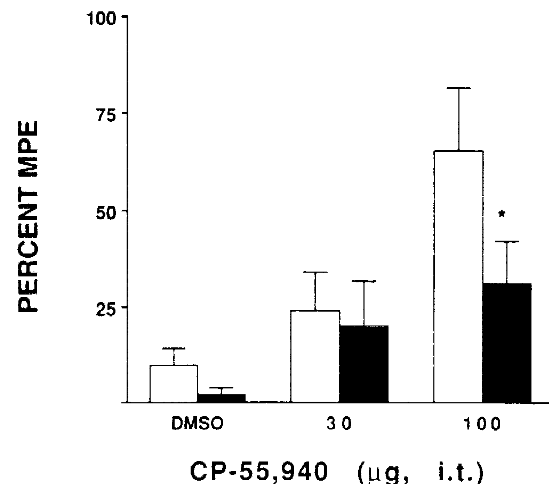

Fig. 7. Antinociceptive effect of i.t. administered CP-55,940 among intact (□) and spinal (■) rats. The results are presented as means ± S.E.M. (n = 6–8 per group) of % MPE. *Significantly different from the intact group.

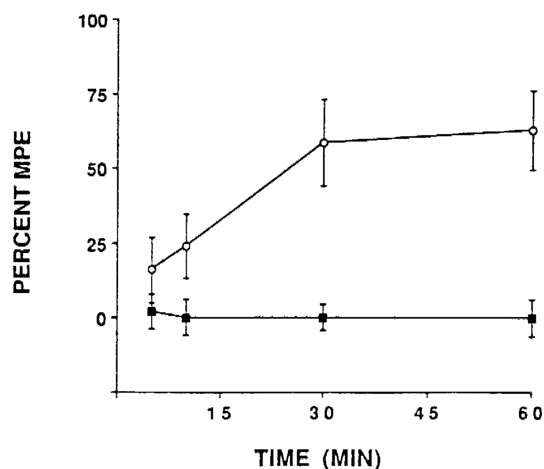

Fig. 8. Comparison of the antinociceptive effects of 100 μg CP-55,940 (O) and 1000 μg of its (+)-enantiomer CP-56,667 (■) after i.t. injections. The results are presented as means ± S.E.M. (n = 7 per group) of % MPE.

TABLE 1

Biodisposition of i.v. and i.t. administered [^{3}H]Δ^9-THC in spinal and intact rats (data are expressed as ng of Δ^9-THC equivalents per mg of tissue)

Tissue Type	Intravenous[a]		Intrathecal[b]	
	Spinal	Intact	Spinal	Intact
Whole brain	20 ± 3	17 ± 2	4 ± 2	17 ± 5
Spinal cord	27 ± 5	23 ± 3	179 ± 36	152 ± 52
Plasma	11 ± 1	10 ± 1	0.2 ± 0.0	1.0 ± 0.1

[a] Five rats decapitated 15 min after 10 mg/kg.
[b] Five rats decapitated 15 min after 300 μg.

the brain concentration of Δ^9-THC equivalents in the intact animals was not affected by the type of drug administration, the spinal concentrations were approximately 5- to 6-fold greater after i.t. injection than the i.v. injection.

Discussion

It is well documented that antinociceptive drugs can produce their effects directly at the spinal level (Yaksh and Rudy, 1977; Yaksh, 1985), at sites in the brain that activate descending pain inhibitory pathways (Yaksh, 1979; Sagen and Proudfit, 1981; Hammond and Yaksh, 1984; Hammond, 1986) or at both spinal and supraspinal levels (Yeung and Rudy, 1980). In this study, Δ^9-THC and CP-55,940 were administered to spinal and intact rats to ascertain which level of the neural axis mediates cannabinoid-induced antinociception. Because the descending inhibitory neural input from the brain is completely severed by spinal transection, the continued antinociceptive effect of both Δ^9-THC and CP-55,940 indicated a spinal site of action. The partial attenuation caused by spinal transection suggests the involvement of supraspinal mechanisms as well. This pattern of results is similar to the antinociceptive effect of systemically administered opiates in spinal and intact animals. For example, dose-response curves for morphine-induced antinociception were shifted far to the right in spinal rats (Bonneycastle *et al.*, 1953; Irwin *et al.*, 1950) and mice (Dewey *et al.*, 1969).

Another strategy that has widely been used to implicate a spinal site of action is the direct administration of drugs into the subarachnoid space of the lumbar region of the spinal cord. Δ^9-THC, as well as CP-55,940, administered i.t., produced about 50% MPE. Alternatively, the drug may have diffused out of the spinal cord and produced its effects through supraspinal mechanisms. Two approaches were used to examine the possibility of rostral diffusion. First, CP-55,940 was administered either to the upper thoracic level or the lumber segments of the spinal cord. The drug continued to produce about 50% MPE when administered just rostral to the lumbar enlargement. Of importance, it failed to produce any antinociception when administered to the upper thoracic level. Had the drug been acting solely at supraspinal sites, more antinociception would have been expected after administration to the upper thoracic level, where it would have greater accessibility to the brain. The second approach compared the effect of i.t. administered CP-55,940 in spinally transected rats to intact rats. As the brain no longer has any direct neural means of inhibiting the tail-flick reflex after spinal transection, the continued antinociceptive effect produced by i.t. administered cannabinoids in the spinal rats indicates spinal action. The fact that surgical ligation of the spinal cord significantly decreased the MPE of 100 μg of CP-55,940 from 65 to 31%, however, suggests that rostral diffusion accounts for some of its effects. These data taken together provide strong evidence that cannabinoids produce antinociception through both spinal and supraspinal sites of action.

The present study also examined whether spinal transection affected the distribution of drug in the CNS. To address this issue, [^{3}H]Δ^9-THC was administered either i.v. or i.t. to spinal and intact rats and then the amount of radiolabeled substances were assessed in whole brain, spinal cord and plasma. Both spinal and intact groups had equivalent levels of Δ^9-THC and its metabolites in each area sampled after i.v. drug administration. These results indicate that i.v. administered drug has access to the spinal cord despite the ligation. Undoubtedly, the labeled substances reached the spinal cord through the circulatory system, as much of the blood supply to the lumbar and sacral levels of the cord would be left intact after a midthoracic spinal transection (Greene, 1935). Similarly, the spinal levels of labeled substances did not differ between spinal and intact animals after i.t. drug administration. In contrast, i.t. drug administration led to decreased concentrations of Δ^9-THC equivalents in the brains and plasma of the spinal animals. This pattern of results indicates that i.t. administered cannabinoids in intact rats can readily spread to the brain, probably through the CSF.

An issue of concern is whether cannabinoid-induced antinociception is mediated through specific or nonspecific mechanisms. For example, it has been hypothesized that some of the cannabinoid-induced effects may be caused by membrane perturbation. The finding that CP-56,667 (1000 μg dose, i.t.), the (+)-enantiomer of CP-55,940, failed to produce any antinociception, taken together with the observation that both compounds have the same lipophility (Thomas *et al.*, 1990), argues against this explanation. This stereoselectivity is consistent with the hypothesis that cannabinoid-induced antinociception is mediated through a specific site of action. A second issue is whether spinally administered cannabinoids lead to elevations of tail-flick latency because they inhibit noxious afferent input to the CNS (*i.e.*, antinociception) or merely because they interfere with the motor neurons in the cord. Autoradiography, however, has revealed some specific cannabinoid binding in the dorsal but not ventral horn (Herkenham *et al.*, 1990). This pattern of binding tends to argue against the possibility that

cannabinoids interfere with the efferent side of the response because the dorsal horn plays an important role in the modulation of noxious input (Ruda, 1986), whereas the ventral horn plays a major role in motor output (Molander *et al.*, 1984). Finally, on a behavioral level, the apparent correlation between cannabinoid-induced antinociception with catalepsy and decreases in locomotor activity (Little *et al.*, 1988) raises the question of whether increases in tail-flick latencies arise as a consequence of sedation. The occurrence of robust tail-flick responses in anesthetized (Maier, 1989) as well as in spinal rats (Irwin *et al.*, 1950) argues against this possibility.

The potency of i.t. administered cannabinoids found here was weaker than that previously reported by Yaksh (1981). Whereas i.t. administered Δ^9-THC and CP-55,940 elevated tail-flick latencies to about 50% MPE, Yaksh (1981) reported approximately 100% MPE after a 20 μg i.t. injection of levonantradol. We have also found that levonantradol (40 μg, i.t.) produces potent antinociception, specifically 86 ± 14% MPE in intact rats (data not presented). The finding that levonantradol is more potent than CP-55,940 by i.t. route of administration is surprising because it is only one-third as potent as CP-55,940 after i.v. administration in mice (Little *et al.*, 1988). The disproportionately weak action of CP-55,940 and Δ^9-THC compared to levonantradol after i.t. administration may result, in part, from the differing lipophilicities of these compounds. In particular, Dickenson's group has proposed that the antinociceptive potency of spinally administered drugs is inversely related to lipophilicity (McQuay *et al.*, 1989; Dickenson *et al.*, 1990). The highly lipophilic nature of the cannabinoids might increase the likelihood of either its diffusion out of the subarachnoid space and into the vasculature or its absorption into the heavily myelinated fibers of passage in the spinal cord as well as the pia mater and arachnoid membrane. The low levels of radioactive drug detected in plasma and high concentrations in spinal tissue are consistent with the latter explanation. The fact that the lipophilicity of levonantradol is less than half that of CP-55,940 and approximately one-twentieth that of Δ^9-THC (Thomas *et al.*, 1990) may allow it to reach the putative active sites most readily.

Although the data presented here indicate a potent supraspinal component to cannabinoid-induced antinociception, the specific brain sites involved are yet to be determined. Dense cannabinoid binding in the CNS has been shown to occur in the basal ganglia substantia nigra, globus palidus, hippocampus and cerebellum (Herkenham *et al.*, 1990). Of consequence, cannabinoids bind in the periaqueductal gray, an area that has been strongly implicated in the centrifugal modulation of antinociception (Yaksh, 1979; Hammond, 1986). Several studies have examined the pharmacology underlying cannabinoid-induced antinociception. In general, cannabinoids appear to produce antinociception through nonopioid mechanisms because opiate antagonists fail to block their antinociceptive effect (Jacob *et al.*, 1981; Yaksh, 1981; Martin, 1984; Ferri *et al.*, 1986). Nonetheless, they may activate monoaminergic pain inhibitory systems that are common for both opioid and nonopioid forms of antinociception (Yaksh, 1979, 1985; Barbaro *et al.*, 1985; Wigdor and Wilcox, 1987). However, the *alpha*-1 noradrenergic antagonist phenoxybenzamine (Jacob *et al.*, 1981) failed to block antinociception produced by levonantradol. Central injections of either the serotonergic neurotoxin 5,7-dihydroxytryptamine (Jacob *et al.*, 1981) or the dopaminergic neurotoxin 6-hydroxydopamine (Ferri *et al.*, 1986) also attenuated cannabinoid-induced antinociception, however, subjects in neither study were pretreated with a noradrenergic uptake inhibitor to prevent a concomitant destruction of noradrenergic cell bodies. Therefore, the attenuation could also be attributed to the depletion of norepinephrine. Regardless, it is unlikely that serotonin plays a critical role in cannabinoid-induced antinociception because serotonergic depletion by *p*-chlorophenylalanine failed to prevent antinociception elicited from levonantradol (Jacob *et al.*, 1981).

In conclusion, relatively little is known about the neuroanatomical substrates underlying cannabinoid-induced antinociception. This study provides a first step in addressing the issue of site of action by examining the effect of cannabinoids in intact and spinal rats after i.v. and i.t. routes of administration. The results reported here indicate that cannabinoids produce antinociception through both spinal and supraspinal mechanisms.

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