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Spinal cannabinoids are anti-allodynic in rats with persistent inflammation

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Abstract

Cannabinoid receptor (CB1) agonists strongly inhibit behavioral responses to acute noxious stimuli, but their effects on behavioral responses in persistent pain states are less clear. Here, we examined the effects of intrathecal (i.t.) administration of a CB1 agonist, WIN55,212-2, on mechanical allodynia (decreased withdrawal threshold) produced by injections of complete Freund's adjuvant (CFA) in the plantar surface of the rat hindpaw. We measured mechanical thresholds with calibrated von Frey filaments before and after CFA and used Fos expression as a marker of the activity of spinal cord neurons during inflammation and in response to a CB1 antagonist. One day post CFA-induced injury, mechanical sensitivity was significantly increased in the hindpaw ipsilateral to the CFA injection, as was the number of neurons that express Fos. Intrathecal injection of WIN55,212-2, significantly, reversed the allodynia at doses that had no effect on the mechanical threshold of the contralateral paw of CFA-treated or the withdrawal thresholds in naive animals. This effect was blocked by coadministration of the CB1 antagonist, SR141716A, with WIN55212-2. By itself, SR141716A, had no effect on mechanical thresholds in normal animals. In inflamed animals, SR141716A did not further reduce mechanical thresholds in the inflamed paw, but it significantly enhanced mechanical sensitivity 'contralateral' to the inflammation. Furthermore, i.t. injection of SR141716A increased Fos expression in both normal and inflamed animals, to a different extent in different laminae. In normal animals, the increase was primarily in laminae V–VI and in the ventral horn; in animals with persistent inflammation SR141716A increased the number of Fos neurons in laminae I–II and V–VI. These results demonstrate that WIN55212-2 reverses inflammation-induced allodynia at doses that do not produce analgesia and that SR141716A differentially affects the pattern of Fos expression in the spinal cord, depending on the presence or absence of inflammation. Taken together, these results suggest that the CB1 receptor system is tonically active in the spinal cord under normal conditions and that its activity is increased in response to injury. © 1999 International Association for the Study of Pain. Published by Elsevier Science B.V.

Keywords: Spinal cannabinoids; Allodynia; Inflammation

1. Introduction

The identification and cloning of CB1 (Matsuda et al., 1990) and CB2 (Munro et al., 1993) cannabinoid receptors, their localization in central and peripheral tissues that are implicated in nociceptive processing (Herkenham et al., 1991; Tsou et al., 1998) and the discovery of putative endogenous cannabinoids (Devane et al., 1992; Di Marzo et al., 1994) suggest that an important function of the cannabinoid receptor system is to modulate pain. There is, in fact, now considerable evidence in support of this function. For example, cannabinoids reduce the behavioral responses to noxious thermal (Buxbaum, 1972; Sofia et al., 1973; Bloom et al., 1977; Jacob et al., 1981; Lichtman and Martin, 1991), mechanical (Sofia et al., 1973; Martin et al., 1996; Smith et al., 1998), visceral (Welburn et al., 1976) and chemical (Moss and Johnson, 1980; Tsou et al., 1996) stimuli. Furthermore, these antinociceptive actions are associated with cannabinoid-induced modulation of noxious-stimulus induced activity of neurons in the spinal cord (Hohmann et al., 1995), brainstem (Meng et al., 1998) and thalamus (Martin et al., 1996).

Several studies have also assessed the contribution of endogenous cannabinoids to pain modulation. Anandamide, a naturally occurring endogenous ligand for the CB1 receptor, produces analgesia in the hot-plate (Fride and Mechoulam, 1993), tail-flick (Smith et al., 1994) and formalin (Calignano et al., 1998) tests and reduces capsaicin-evoked calcitonin gene-related peptide (CGRP) release from isolated rat spinal cord (Richardson et al., 1998b). Furthermore, administration of the CB1 receptor antagonist, SR141716A, inhibits the anandamide-induced analgesia and, in the absence of agonist stimulation, blockade of

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CB1 receptors by SR141716A exacerbates the behavioral response to formalin (Calignano et al., 1998). Other studies found that spinal administration of SR141716A (Richardson et al., 1997) or reduction of the number of CB1 receptors by antisense treatment (Richardson et al., 1998a) lowers nociceptive thresholds to noxious thermal stimuli. Taken together, these results indicate that CB1 receptors modulate acute nociceptive processing and that this system is tonically active.

In addition to the tonic activation of the cannabinoid receptor system, several reports indicate that cannabinoids also modulate nociceptive processing after peripheral injury. Consistent with this notion, cannabinoids reduce the pain behavior associated with nerve (Herzberg et al., 1997) and tissue injury (Richardson et al., 1998c). Moreover, anandamide modulates thermal nociceptive thresholds more effectively during carrageenan-induced inflammation (Richardson et al., 1998b). This suggests that activity of the cannabinoid receptor system is increased after injury. If this is indeed the case, then the decreased nociceptive threshold that occurs in the setting of injury may partly be due to 'loss' of a tonic cannabinoid activity. It follows that cannabinoids may alleviate the allodynia and pain that is triggered by injury.

In the present study, we tested the hypothesis that spinal administration of a cannabinoid agonist normalizes nociceptive thresholds in the setting of tissue injury. In addition to monitoring behavior, we assessed the effect of administering a CB1 antagonist on the magnitude of inflammation-induced Fos expression, a marker of activity of neurons in the dorsal horn. We hypothesized that blockade of CB1 receptors would inhibit the actions of endogenous cannabinoids released after injury and that this loss of antinociception would be manifest as an increase in inflammation-induced Fos expression in the spinal cord.

2. Materials and methods

2.1. Experimental animals

All experiments were reviewed and approved by the Institutional Care and Animal Use Committee at UCSF and conform to the guidelines on the study of pain in awake animals established by the International Association for the Study of Pain (Zimmermann, 1983). Unilateral inflammation was induced in male Sprague–Dawley rats weighing 240–300 g (Bantin and Kingman, Freemont, CA). Under brief halothane anesthesia, we injected 0.2 ml of complete Freund's adjuvant (CFA: *Mycobacterium tuberculosis*, Sigma; suspended in an oil/saline (1:1) emulsion; 0.5 mg *Mycobacterium*/ml) subcutaneously in the plantar surface of the left hindpaw. Animals were housed in pairs in plastic cages lined with sawdust and were tested 24 h after injection of CFA.

2.2. Drug preparation and administration

Since previous studies indicated that the duration of anandamide-induced antinociception is less than 20 min after intrathecal administration (Smith et al., 1994), we chose to use the high affinity cannabinoid receptor agonist WIN55,212-2 mesylate ((D'Ambra et al., 1992) RBI, Natick, MA) to examine anti-allodynia. Both WIN55212-2 and SR141716A (NIDA), a potent CB1 receptor antagonist (Rinaldi-Carmona et al., 1994), were dissolved in a 1:1:18 mixture of ethanol: emulphor (Alkamuls EL-620, Rhone–Poulenc, Cranbury, NJ): saline. WIN 55,212-2 was administered in concentrations of 10, 30 and 100 μg. SR141716A was prepared in concentrations of 1, 10, 30 and 100 μg. In pilot experiments, we found that the 100 μg dose of SR141716A produced a caudally directed scratching behavior that prevented evaluation of mechanical thresholds. For this reason, we did not use doses higher than 30 μg. Drugs were administered intrathecally via a direct injection method (Mestre et al., 1994) in a volume of 20 μl, 10–15 min prior to behavioral testing.

2.3. Behavior

Tactile allodynia was assessed with calibrated von Frey filaments using an up-down paradigm (Chaplan et al., 1994). Briefly, animals were placed in plastic cages with a wire mesh floor and allowed to acclimate for 15 min before each test session. To determine the 50% response threshold, the von Frey filaments were applied (over a range of intensities from 0.4–28.8 g) to the mid-plantar surface for 8 s or until a withdrawal response occurred. When a positive response was noted, we tested a weaker stimulus. If there was no response to a stimulus, then a stronger stimulus was presented. After the initial threshold crossing, this procedure was repeated for four stimulus presentations per animal per test session. Only animals that exhibited at least a 30% reduction in mechanical threshold in the paw ipsilateral to the CFA injection were included in the subsequent analyses.

2.4. Immunocytochemistry

Ninety minutes after injection of either vehicle or drug(s) the animals were deeply anaesthetized with sodium pentobarbital (100 mg/kg, i.p.) and perfused intracardially with 200 ml of 0.1 M phosphate-buffered saline (PBS), followed by 500 ml of 10% formalin in 0.1 M phosphate buffer. Spinal cords were removed, postfixed in the same fixative for 4 h and cryoprotected in 30% sucrose overnight. The tissue was sectioned in the transverse plane at 40 μm on a freezing microtome and collected for immunocytochemical analysis.

Sections were immunostained for Fos according to the avidin–biotin peroxidase method (Hsu and Raine, 1981). Sections were blocked in a Tris–PBS (TPBS) solution containing 10% normal goat serum for 1 h. The sections

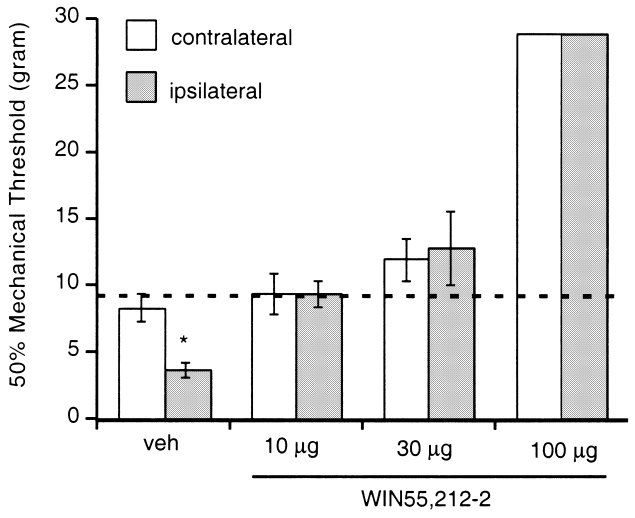

Fig. 1. Mechanical thresholds contralateral (open bars) and ipsilateral (shaded bars) to inflammation. Twenty-four hours after CFA, vehicle ($n = 13$), or WIN55,212-2 (10 μg: $n = 9$; 30 μg ($n = 7$); 100 μg: $n = 4$) were administered intrathecally. Vehicle treatment had no effect on mechanical thresholds. However, the 10 μg dose of WIN55,212-2 significantly reversed the allodynia in the inflamed paw, without affecting the mechanical threshold in the non-inflamed paw. Although higher doses of WIN55,212-2 also increased the mechanical threshold in the inflamed paw, these doses also significantly increased the mechanical threshold in the non-inflamed paw. The dashed line refers to the mean pre-injection mechanical thresholds. *$P = 0.01$, compared to vehicle.

were then incubated overnight at room temperature in rabbit anti-Fos antiserum diluted 1:30 000 (kindly provided by Dr. D. Slamon, UCLA). Triton X-100 was used in all incubation steps. The primary antibody was removed, the sections washed and then incubated in biotinylated goat anti-rabbit IgG and avidin-biotin-peroxidase complex (Elite Kit, Vector Labs, Burlingame, CA). The horseradish peroxidase immunoreaction product was localized with a nickel-intensified diaminobenzidine protocol with glucose oxidase (Llewellyn-Smith and Minson, 1992). Reacted sections were mounted on gelatin-coated slides, dried, dehydrated and coverslipped with DPX.

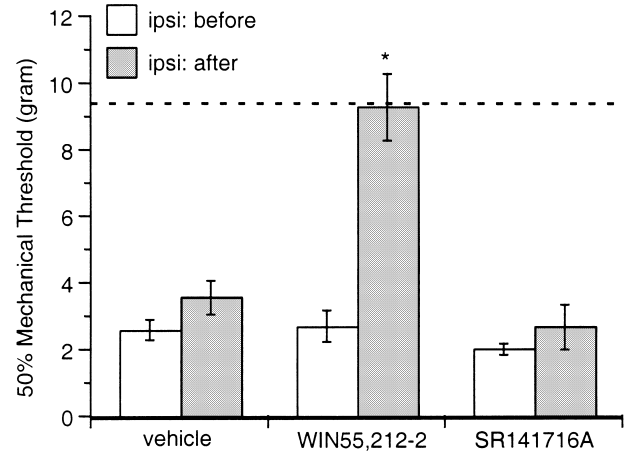

Fig. 2. CB1 receptor-dependent reversal of allodynia by WIN55,212-2. Mechanical thresholds in the inflamed paw before (open bars) and after (shaded bars) spinal administration of vehicle ($n = 13$), WIN55,212-2 (10 μg: $n = 9$) or co-administration of a 30 μg dose of SR141716A with 10 μg of WIN55,212-2 ($n = 8$). The dashed line refers to the mean pre-injection mechanical thresholds. *$P < 0.01$, compared with vehicle.

2.5. Quantification and statistical analysis

We used a Wilcoxon signed rank test to analyze the changes in mechanical thresholds after CFA. To examine the effect of treatment, we used Mann–Whitney tests. For Fos analysis, lumbar spinal cord sections were photographed at 4 × power with Kodak Technical Pan film on a Nikon Microphot-FXA microscope. Individual sections of the L4 and L5 segments of the spinal cord were divided into four regions: (i) the superficial laminae (I–II); (ii) the nucleus proprius (III–IV); (iii) the neck of the dorsal horn (laminae V–VI); and (iv) the ventral horn (laminae VII, VIII, IX, X). Fos-immunoreactive (Fos-ir) neurons were identified and counted in each of these regions by an individual blind to experimental treatment. The number of Fos-ir neurons was counted in six sections per animal, averaged to generate a regional mean per animal and analyzed by factorial ANOVA. Post hoc comparisons were made with Fisher's PLSD test.

3. Results

3.1. Behavior

Prior to injection of CFA, the mean mechanical threshold for withdrawal was 9.1 ± 0.7 g and there was no difference between right and left paws. Twenty-four hours after CFA injection, we observed a profound mechanical allodynia that was manifest as a significant decrease in the withdrawal threshold in the inflamed paw, to 2.5 ± 0.1 g ($P < 0.0001$). There was no significant change in mechanical thresholds in the paw contralateral to the inflammation. Intrathecal (i.t.) administration of the cannabinoid agonist, WIN55, 212-2 (10–100 μg), dose-dependently increased mechanical thresholds (Fig. 1). However, only the 10 μg dose of WIN55,212-2 significantly reversed the allodynia in the inflamed paw (9.2 ± 1.0 g) without altering the response to mechanical stimuli in the non-inflamed paw (9.2 ± 1.5 g) or in non-inflamed animals ($n = 4$). By contrast, the 30 μg dose, significantly increased mechanical thresholds both ipsilateral and contralateral to the inflammation. Finally, the highest dose of WIN55,212-2 (100 μg) that we tested completely blocked the response in both paws to the maximum (28.8 g) von Frey filament.

Administration of the CB1 antagonist, SR141716A (1.0-30 μg, i.t.), had no effect on mechanical thresholds in normal animals ($n = 20$). In inflamed animals, however, SR141716A (30 μg i.t.) significantly reduced mechanical thresholds, by 63%, in the paw contralateral to the inflam-

Table 1
Total Fos expression in L4–L5 spinal segments in normal and CFA-treated rats

	No CFA		CFA			
	Vehicle	SR141716A (30 μg)	Vehicle	SR141716A (30 μg)	WIN55212-2 (10 μg)	WIN + SR (10 + 30 μg)
Ipsilateral						
Dorsal horn	79.4 ± 4.3	98.0 ± 7.6*	117.3 ± 8.6	153.0 ± 10.3*	115.1 ± 14.7	146.9 ± 12.7*
Ventral horn	19.9 ± 1.5	27.6 ± 2.5*	36.1 ± 4.2	47.6 ± 4.6	32.8 ± 3.5	40.1 ± 2.5
Contralateral						
Dorsal horn	n.a.[a]	n.a.	79.0 ± 5.2	106.9 ± 10.5*	68.3 ± 11.4	103.4 ± 6.3*
Ventral horn	n.a.	n.a.	16.3 ± 1.5	21.2 ± 3.8	14.4 ± 2.3	18.4 ± 1.0

[a] Since there is no ipsi or contralateral in untreated rats, the counts for non-inflamed animals represent the mean number of cells from the right and left sides of the spinal cord. *$P < 0.05$ compared with vehicle.

mation ($P < 0.05$; $n = 9$), but did not further reduce thresholds in the inflamed paw (2.35 ± 0.4 vs. 2.17 ± 0.1). When the same dose of SR141716A was co-administered with WIN55,212-2 (10 μg), it completely abolished the anti-allodynic effect produced by the cannabinoid agonist (2.6 ± 0.7 g; Fig. 2) but, in the presence of the agonist, it did not affect mechanical sensitivity contralateral to the inflamed paw (8.5 ± 1.4 g).

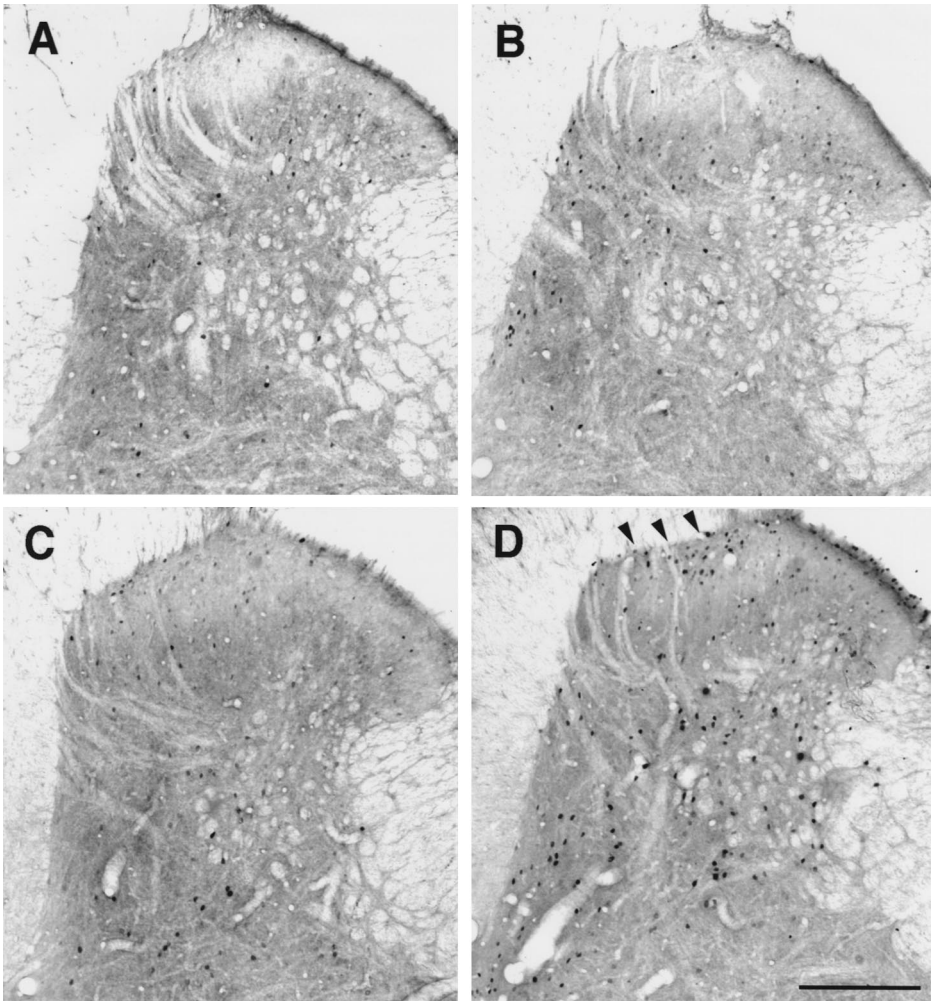

Fig. 3. Photomicrographs illustrating Fos expression in the dorsal horn of the lumbar spinal cord in the absence of inflammation after i.t. treatment with (A) vehicle ($n = 14$) or (B) SR141716A (30 μg; $n = 12$), or in the presence of inflammation after i.t. treatment with (C) vehicle ($n = 6$) or (D) SR141716A (30 μg; $n = 5$). Twenty-four hours after CFA treatment, there is an increase in the number of Fos-immunoreactive neurons in the deep dorsal horn (A vs. C). Spinal administration of SR141716A not only increased the number of Fos-ir neurons in the deep dorsal horn, but also in the superficial (black arrowheads) dorsal horn (B vs. D). Calibration bar = 250 μm.

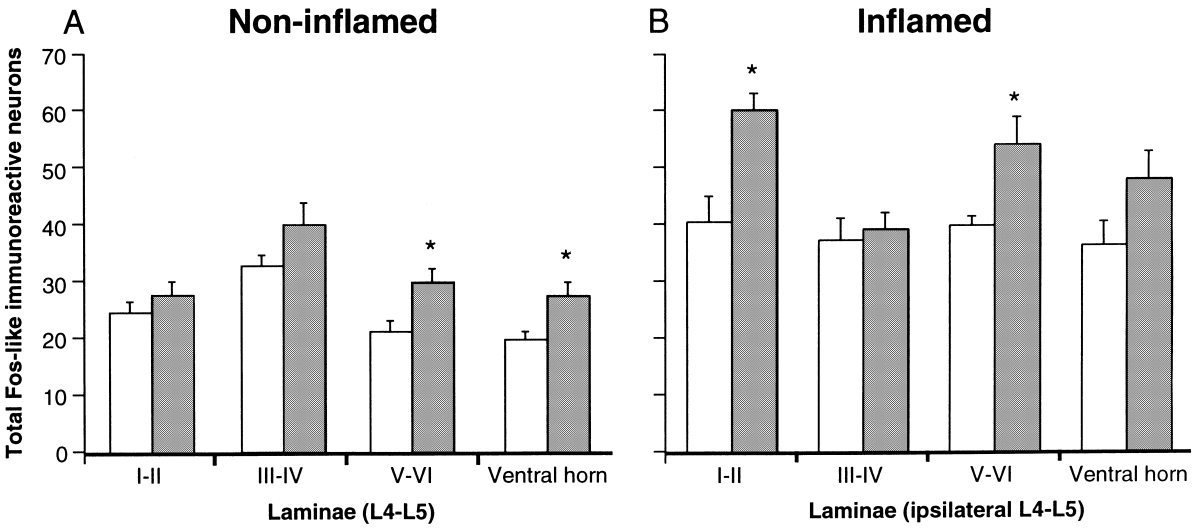

Fig. 4. The distribution of Fos-immunoreactive cell bodies per 40 μm section in the L4–L5 segments of the spinal cord in the (A) absence or (B) or presence of inflammation and after spinal administration of vehicle (open bars) or SR141716A (30 μg: shaded bars). In non-inflamed animals, SR141716A significantly increased the number of Fos-immunoreactive neurons in laminae V-VI of the dorsal horn and in the ventral horn. By contrast, SR141716A significantly increased the number of Fos-immunoreactive neurons in laminae I–II and laminae V–VI of the dorsal horn in inflamed animals. *$P < 0.05$, treatment by laminae.

3.2. Fos immunocytochemistry

We used Fos immunocytochemistry to monitor the activity of neurons in the spinal cord in the absence or presence of inflammation. Twenty-four hours after CFA injection, we recorded a 44% increase in the number of spinal cord neurons expressing Fos compared with non-inflamed animals ($F_{1,72} = 6.90$; $P < 0.01$; Table 1; Fig.3). These increases were observed in different laminae of the spinal cord ($F_{3,72} = 2.99$; $P < 0.05$; Fig.4). Post hoc analyses revealed Fos expression increased significantly in laminae V/VI (122%) and in the ventral horn (125%); there was no significant difference in laminae I–II or III–IV.

To test the hypothesis that inflammation increases endogenous cannabinoid activity in the spinal cord, we injected the CB1 receptor antagonist, SR141716A (30 μg i.t.), and examined Fos expression. We found that SR141716A (30 μg, i.t.) significantly increased the number of Fos-ir neurons in the spinal cord ($F_{3,120} = 14.9$; $P < 0.0001$) and compared with non-inflamed animals, this increase was significantly greater in the inflamed animals (Table 1; $P < 0.05$). Moreover, we found that the laminar pattern of the increase was dependent on the absence or presence of inflammation. Thus, when SR141716A was administered to animals with ongoing inflammation, there was no change in Fos-ir in laminae III–IV or in the ventral horn, but there was a significant increase in the number of Fos-ir neurons in laminae I–II (46%) and in laminae V–VI (39%). Interestingly, SR141716A also increased Fos expression in non-inflamed animals ($F_{1,96} = 15.9$; $P < 0.0001$) but post hoc analyses indicated that these increases were limited to laminae V–VI (70%) and the ventral horn (71%). Administration of WIN55,212-2 did not reduce Fos expression in inflamed animals (see below).

4. Discussion

In the present study, we tested the hypothesis that cannabinoids reverse the allodynia produced by peripheral inflammation through an action at CB1 receptors in the spinal cord. We found that a 10 μg dose of WIN55,212-2 raised mechanical thresholds in the inflamed paw to pre-inflammation levels (i.e. normalized nociceptive thresholds) without affecting thresholds in the non-inflamed paw. We also found that the CB1 receptor antagonist, SR141716A, completely blocked the cannabinoid-induced increase in mechanical threshold in the inflamed paw. Higher doses of WIN55,212-2 (30 and 100 μg) increased mechanical thresholds both ipsilateral and contralateral to the inflammation. These results indicate that spinal cannabinoids can produce both, anti-allodynic and analgesic effects, and demonstrate that the anti-allodynic effects of WIN55,212-2 are mediated by CB1 receptors in the spinal cord.

In agreement with a previous study (Smith et al., 1998), SR141716A did not affect mechanical thresholds in normal animals. To determine whether an antinociceptive cannabinoid tone is augmented in the spinal cord in the setting of inflammation we also tested the effect of SR141716A after injection of CFA. We found that blocking CB1 receptors with SR141716A did not further reduce thresholds in the inflamed paw, however, it significantly reduced mechanical thresholds in the contralateral paw. This result establishes that a paw contralateral to one that is inflamed is not equiva-

lent to the paw of a normal animal. This differential effect of the CB1 antagonist indicates that tissue injury indeed modifies cannabinoid activity in the spinal cord.

Consistent with previous findings (Ma and Woolf, 1996), 24 h CFA treatment increased Fos-ir in laminae V–VI (Fig. 4A vs. B). Administration of WIN55,212-2, by itself, did not reduce Fos expression. As the half-life of the Fos protein is at least 2 h, this was expected (Morgan and Curran, 1991). On the other hand, by examining the effect of SR141716A on Fos expression in the spinal cord, we demonstrated that blockade of spinal CB1 receptors evokes a different pattern of Fos expression in the presence and absence of peripheral inflammation. In normal animals, spinal administration of the CB1 receptor antagonist significantly increased Fos expression in laminae V–VI in the dorsal horn and in the ventral horn. The increase in Fos expression without a concomitant change in mechanical withdrawal thresholds underscores the now well documented fact that Fos expression can be used as a marker of activity, but that the absolute number of neurons does not necessarily correlate with behavior. By contrast, in the setting of inflammation, SR141716A increased Fos expression in laminae I–II and V–VI where nociceptive primary afferents terminate (Willis and Coggeshall, 1991) and where the majority of nociceptive neurons are located in the dorsal horn (Besson and Chaouch, 1987). Together, these data provide strong support for the notion that the cannabinoid receptor system is active under basal conditions, as well as, under conditions of injury and further suggests that the absence or presence of injury influences the locus of cannabinoid-mediated modulation influence in the spinal cord.

The notion that peripheral inflammation increases the release of endogenous cannabinoids in the spinal cord is consistent with several other reports that found that administration of SR141716A lowers nociceptive thresholds to acute noxious thermal stimuli (Richardson et al., 1997; 1998a,b,c) and produces exaggerated behavioral responses to formalin (Calignano et al., 1998). There are reports, however, that SR141716A possesses properties of an inverse agonist (Bouaboula et al., 1997; Landsman et al., 1997; MacLennan et al., 1998), at least in cultured CHO cells. If SR141716A is, in fact, an inverse agonist in vivo, then our results may not reflect an inflammation-induced activation of the cannabinoid system. Although we cannot eliminate this possibility, our results and those from other laboratories argue against this interpretation. For example, Richardson and colleagues observed hyperalgesia with spinal administration of either CB1 receptor antisense, which decreases the number of CB1 receptors, or with SR141617A (Richardson et al., 1997; 1998a). Furthermore, we found that SR141716A produced two distinct patterns of Fos expression, depending on the presence or absence of inflammation. This result suggests that CB1 receptor blockade inhibited actions of endogenous cannabinoids in specific laminae of the spinal cord in response to inflammation.

The mechanisms by which cannabinoids reverse the allodynia produced by peripheral inflammation are not known. Cannabinoid receptors have been localized in the spinal cord (Herkenham et al., 1991; Tsou et al., 1998) and there is evidence that CB1 receptors are present in small TrkA- (Friedel et al., 1997) and substance P or CGRP-expressing neurons in the dorsal root ganglia (Hohmann and Herkenham, 1999), from where they are presumably transported to central and peripheral terminals. Furthermore, anandamide inhibits capsaicin-evoked CGRP release from spinal cord slices (Richardson et al., 1998a). Since the activation of cannabinoid receptors in the hippocampus presynaptically inhibits the release of glutamate (Shen et al., 1996), one possibility is that CB1 receptors on central terminals modulate transmitter release from small diameter DRG cells that terminate in the superficial dorsal horn. On the other hand, dorsal rhizotomy does not completely eliminate binding to CB1 receptors in the dorsal horn (Hohmann et al., 1999). Thus, it is also likely that cannabinoids act postsynaptically to modulate spinal cord activity.

Previous studies suggested that, under conditions of acute nociception, tonic activation of cannabinoid receptors is required to maintain appropriate responses to noxious stimuli (Richardson et al., 1997; 1998a,b; Calignano et al., 1998; Meng et al., 1998) and that anandamide (i.t.) can block the carrageenan-induced reduction of thermal response thresholds (Richardson et al., 1998b). In the present study, we showed that under conditions of persistent inflammation cannabinoids can restore nociceptive thresholds, via an action at CB1 receptors in the spinal cord and that blockade of CB1 receptors activates neurons in laminae I–II. Our results suggest that alterations in the CB1 receptor system contribute to the spinal cord reorganization that accompanies peripheral injury and that these changes are involved in the maintenance of persistent pain conditions.

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References

Besson JM, Chaouch A. Peripheral and spinal mechanisms of nociception. Physiol Rev 1987;67:67–186.

Bloom AS, Dewey WL, Harris LS, Brosius KK. 9-nor-9beta-hydroxyhexahydrocannabinol, a cannabinoid with potent antinociceptive activity: comparisons with morphine, . J Pharmacol Exp Ther 1977;200:263–270.

Bouaboula M, Perrachon S, Milligan L, Canat X, Rinaldi-Carmona M, Portier M, Barth F, Calandra B, Pecceu F, Lupker J, Maffrand JP, Le Fur G, Casellas P. A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated

by insulin or insulin-like growth factor 1.Evidence for a new model of receptor/ligand interactions. J Biol Chem 1997;272:22330–22339.

Buxbaum DM. Analgesic activity of 9-tetrahydrocannabinol in the rat and mouse. Psychopharmacology (Berl) 1972;25:275–280.

Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. Nature 1998;394:277–281.

Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55–63.

D'Ambra TE, Estep KG, Bell MR, Eissenstat MA, Josef KA, Ward SJ, Haycock DA, Baizman ER, Casiano FM, Beglin NC, Chippari SM, Grego JD, Kullnig RK, Daley GT. Conformationally restrained analogues of pravadoline: nanomolar potent, enantioselective, (aminoalkyl)indole agonists of the cannabinoid receptor. J Med Chem 1992; 35:124–135.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor (see comments). Science 1992;258:1946–1949.

Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, Piomelli D. Formation and inactivation of endogenous cannabinoid anandamide in central neurons (see comments). Nature 1994;372: 686–691.

Fride E, Mechoulam R. Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. Eur J Pharmacol 1993;231:313–314.

Friedel RH, Schnurch H, Stubbusch J, Barde YA. Identification of genes differentially expressed by nerve growth factor- and neurotrophin-3-dependent sensory neurons. Proc Natl Acad Sci USA 1997;94:12670–12675.

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 1991;11:563–583.

Herzberg U, Eliav E, Bennett GJ, Kopin IJ. The analgesic effects of R(+)-WIN 55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. Neurosci Lett 1997;221:157–160.

Hohmann AG, Briley EM, Herkenham M. Pre- and postsynaptic distribution of cannabinoid and mu receptors in rat spinal cord. Brain Res 1999;822:17–25.

Hohmann AG, Herkenham M. Localization of cannabinoid receptor (CB1) mRNA in neuronal subpopulations of rat dorsal root ganglia: a double-label in situ hybridization study. Neuroscience 1999;90:923–931.

Hohmann AG, Martin WJ, Tsou K, Walker JM. Inhibition of noxious stimulus-evoked activity of spinal cord dorsal horn neurons by the cannabinoid WIN 55,212-2. Life Sci 1995;56:2111–2118.

Hsu SM, Raine L. Protein A, avidin, and biotin in immunocytochemistry. J Histochem Cytochem 1981;29:1349–1353.

Jacob JJ, Ramabadran K, Campos-Medeiros M. A pharmacological analysis of levonantradol antinociception in mice. J Clin Pharmacol 1981;21: 327S–333S.

Landsman RS, Burkey TH, Consroe P, Roeske WR, Yamamura HI. SR141716A is an inverse agonist at the human cannabinoid CB1 receptor. Eur J Pharmacol 1997;334:R1–R2.

Lichtman AH, Martin BR. Spinal and supraspinal components of cannabinoid-induced antinociception. J Pharmacol Exp Ther 1991;258:517–523.

Llewellyn-Smith IJ, Minson JB. Complete penetration of antibodies into vibratome sections after glutaraldehyde fixation and ethanol treatment: light and electron microscopy for neuropeptides. J Histochem Cytochem 1992;40:1741–1749.

Ma QP, Woolf CJ. Basal and touch-evoked fos-like immunoreactivity during experimental inflammation in the rat. Pain 1996;67:307–316.

MacLennan SJ, Reynen PH, Kwan J, Bonhaus DW. Evidence for inverse agonism of SR141716A at human recombinant cannabinoid CB1 and CB2 receptors (in process citation). Br J Pharmacol 1998;124:619–622.

Martin WJ, Hohmann AG, Walker JM. Suppression of noxious stimulus-evoked activity in the ventral posterolateral nucleus of the thalamus by a cannabinoid agonist: correlation between electrophysiological and antinociceptive effects, . J Neurosci 1996;16:6601–6611.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA (see comments). Nature 1990;346:561–564.

Meng ID, Manning BH, Martin WJ, Fields HL. An analgesia circuit activated by cannabinoids. Nature 1998;395:381–383.

Mestre C, Pelissier T, Fialip J, Wilcox G, Eschalier A. A method to perform direct transcutaneous intrathecal injections in rats. J Pharmacol Toxicol Methods 1994;32:197–200.

Morgan JI, Curran T. Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. Ann Rev Neurosci 1991;14:421–451.

Moss DE, Johnson RL. Tonic analgesic effects of delta 9-tetrahydrocannabinol as measured with the formalin test. Eur J Pharmacol 1980;61:313–315.

Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids (see comments). Nature 1993;365:61–65.

Richardson JD, Aanonsen L, Hargreaves KM. SR 141716A, a cannabinoid receptor antagonist, produces hyperalgesia in untreated mice. Eur J Pharmacol 1997;319:R3–R4.

Richardson JD, Aanonsen L, Hargreaves KM. Hypoactivity of the spinal cannabinoid system results in NMDA-dependent hyperalgesia. J Neurosci 1998a;18:451–457.

Richardson JD, Aanonsen L, Hargreaves KM. Antihyperalgesic effects of spinal cannabinoids. Eur J Pharmacol 1998b;345:145–153.

Richardson JD, Kilo S, Hargreaves KM. Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB1 receptors. Pain 1998c;75:111–119.

Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D, et al. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 1994;350:240–244.

Shen M, Piser TM, Seybold VS, Thayer SA. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J Neurosci 1996;16:4322–4334.

Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R, Martin BR. The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. J Pharmacol Exp Ther 1994;270:219–227.

Smith FL, Fujimori K, Lowe J, Welch SP. Characterization of delta9-tetrahydrocannabinol and anandamide antinociception in nonarthritic and arthritic rats. Pharmacol Biochem Behav 1998;60:183–191.

Sofia RD, Nalepa SD, Harakal JJ, Vassar HB. Anti-edema and analgesic properties of delta9-tetrahydrocannabinol (THC). J Pharmacol Exp Ther 1973;186:646–655.

Tsou K, Lowitz KA, Hohmann AG, Martin WJ, Hathaway CB, Bereiter DA, Walker JM. Suppression of noxious stimulus-evoked expression of fos protein-like immunoreactivity in rat spinal cord by a selective cannabinoid agonist. Neuroscience 1996;70:791–798.

Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 1998;83:393–411.

Welburn PJ, Starmer GA, Chesher GB, Jackson DM. Effects of cannabinoids on the abdominal constriction response in mice: within cannabinoid interactions. Psychopharmacology (Berl) 1976;46:83–85.

Willis WD, Coggeshall RE. Sensory mechanisms of the spinal cord, 2nd edn., New York: Plenum Press, 1991.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–110.