Cannabinoid Suppression of Noxious Heat-Evoked Activity in Wide Dynamic Range Neurons in the Lumbar Dorsal Horn of the Rat

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Hohmann, Andrea G., Kang Tsou, and J. Michael Walker. Cannabinoid suppression of noxious heat-evoked activity in wide dynamic range neurons in the lumbar dorsal horn of the rat. J. Neurophysiol. 81: 575–583, 1999. The effects of cannabinoid agonists on noxious heat-evoked firing of 62 spinal wide dynamic range (WDR) neurons were examined in urethan-anesthetized rats (1 cell/animal). Noxious thermal stimulation was applied with a Peltier device to the receptive fields in the ipsilateral hindpaw of isolated WDR neurons. To assess the site of action, cannabinoids were administered systemically in intact and spinalis transected rats and intraventricularly. Both the aminoalkylindole cannabinoid WIN55,212-2 (125 μg/kg iv) and the bicyclic cannabinoid CP55,940 (125 μg/kg iv) suppressed noxious heat-evoked activity. Responses evoked by mild pressure in nonnociceptive neurons were not altered by CP55,940 (125 μg/kg iv), consistent with previous observations with another cannabinoid agonist, WIN55,212-2. The cannabinoid induced-suppression of noxious heat-evoked activity was blocked by pretreatment with SR141716A (1 mg/kg iv), a competitive antagonist for central cannabinoid CB1 receptors. By contrast, intravenous administration of either vehicle or the receptor-inactive enantiomer WIN55,212-3 (125 μg/kg) failed to alter noxious heat-evoked activity. The suppression of noxious heat-evoked activity induced by WIN55,212-2 in the lumbar dorsal horn of intact animals was markedly attenuated in spinal rats. Moreover, intraventricular administration of WIN55,212-2 suppressed noxious heat-evoked activity in spinal WDR neurons. By contrast, both vehicle and enantiomer were inactive. These findings suggest that cannabinoids selectively modulate the activity of nociceptive neurons in the spinal dorsal horn by actions at CB1 receptors. This modulation represents a suppression of pain neurotransmission because the inhibitory effects are selective for pain-sensitive neurons and are observed with different modalities of noxious stimulation. The data also provide converging lines of evidence for a role for descending antinociceptive mechanisms in cannabinoid modulation of spinal nociceptive processing.

INTRODUCTION

The cloning of a specific G-protein coupled cannabinoid receptor (Matsuda et al. 1990) and the discovery of endogenous cannabinoids (Devane et al. 1992; Mechoulam et al. 1995) established the existence of a cannabinergic neurotransmitter system in the CNS. Anandamide, an endogenous cannabinoid, binds with relatively high affinity to cannabinoid receptors (Ki = 40–50 nM) (Devane et al. 1992), and its pharmacological activity (Fride and Mechoulam 1993) and signal transduction mechanisms (Mackie et al. 1993; Vogel et al. 1993) are similar to those of other cannabinoid agonists. Anandamide is synthesized and inactivated by neurons (Beltrom et al. 1997; Di Marzo et al. 1994) thus fulfilling some of the requirements of a neurotransmitter, but more work is needed to establish its role in neurotransmission.

Cannabinoid receptors are found within anatomic regions implicated in pain modulation, including the spinal dorsal horn and periaqueductal gray (Herkenham et al. 1991; Hohmann and Herkenham 1998, 1999; Tsou et al. 1998). Behavioral and neurochemical studies (Calignano et al. 1998; Richardson et al. 1998a; Strangman et al. 1998) also suggest that endogenous cannabinoids modulate pain.

Cannabinoids suppress pain reactivity in behavioral tests employing different stimulus modalities, including thermal (Bicher and Mechoulam 1968; Bloom et al. 1977; Buxbaum 1972; Jacob et al. 1981; Lichtman and Martin 1991a; Martin et al. 1991, 1993; Yaksh 1981), mechanical (Sofia et al. 1973), and chemical (Calignano et al. 1998; Moss and Johnson 1980; Richardson 1998b,c; Sofia et al. 1973; Tsou et al. 1996) pain. Until recently, however, a role for cannabinoid receptors in pain modulation was not established beyond doubt because the profound motor effects of cannabinoids (e.g., Gough and Olley 1977; Loewe 1946; Ueki 1980) complicate interpretation of behavioral studies assessing motor reactions to noxious stimuli (Cartmell et al. 1991). Therefore it was not clear whether the antinociceptive effects of cannabinoids represented true analgesia (i.e., suppression of nociceptive neurotransmission) or artifacts of motor impairment.

Biochemical (Tsou et al. 1996) and electrophysiological (Hohmann et al. 1995; Martin et al. 1996) experiments first established the ability of cannabinoids to suppress processing of nociceptive processing within the nervous system. A potent synthetic cannabinoid, WIN55,212-2 (D’Ambra et al. 1992), suppressed noxious stimulus-evoked c-fos expression in spinal dorsal horn (Tsou et al. 1996) and the electrical responses of spinal (Hohmann et al. 1995) and thalamic (Martin et al. 1996) wide dynamic range (WDR) neurons, which encode the strength of noxious and nonnoxious stimuli (Coghill et al. 1993; Giesler et al. 1976; Maixner et al. 1986; Mendell 1966; see Price and Dubner 1977). In contrast, the responses of low-threshold mechanosensitive cells to mild pressure were not altered. The level of processing at which the suppression of nociceptive input occurs remained unknown because cannabinoids were administered systemically in all of the previous studies.

Experiments were conducted to extend this investigation to another modality of noxious stimulation (thermal), to examine...
a second cannabinoid agonist (CP55,950), and to determine the site(s) of action. Both the bicyclic cannabinoid CP55,940 (Compton et al. 1992) and the aminoalkylindole WIN55,212-2 (Kuster et al. 1993) bind to brain cannabinoid receptors with high affinity. The ligands differ in their affinities for the central (CB1) and peripheral (CB2) (Munro et al. 1993) subtypes of cannabinoid receptors in vitro; CP55,940 exhibits equal affinity for CB1 and CB2 receptors, whereas WIN55,212-2 exhibits a preferential affinity for CB2 receptors (Felder et al. 1995). In tests of analgesia in mice, CP 55,940 exhibited approximately four times the potency of WIN55,212-2 (Aboud and Martin 1992).

Receptor specificity was demonstrated in this work with 1) pretreatment with the competitive CB1 cannabinoid receptor antagonist SR141716A (Rinaldi-Carmona et al. 1994), 2) examination of WIN55,212-3, a cannabinoid receptor-inactive enantiomer of WIN55,212-2, and 3) different ligands (WIN55,212-2 and CP55,940).

To assess the site(s) of action, separate studies examined the electrophysiological effects of cannabinoids on spinal nociceptive neurons after intraventricular administration in spinalized rats and intraventricular administration in intact rats. Cannabinoids were administered systemically in spinal animals to determine whether cannabinoid modulation of spinal nociceptive neurons is dependent on a supraspinal mechanism. Cannabinoids were administered intraventricularly to determine whether cannabinoid modulation of spinal nociceptive neurons occurs through descending mechanisms. These experiments were carried out to determine whether cannabinoids alter spinal nociceptive processing directly at the spinal level or indirectly by activation of descending antinociceptive systems or both.

**METHODS**

**Subjects**

A total of 72 male Sprague-Dawley (Charles River, Boston MA) rats (250–330 g) were used in the experiments reported herein. Extracellular recordings were obtained from 1 neuron per animal in 66 animals, and 6 were used to determine biodistribution of intraventricularly administered [3H]-WIN55,212-2. The experimental protocols were approved by the Brown University Institutional Animal Care and Use Committee.

**Drug preparation**

WIN55,212-2 mesylate was obtained from Research Biochemicals (Natick, MA). WIN55,212-3 mesylate, CP55,940 and SR141716A were gifts from Sterling-Winthrop (Rensselaer, NY), Pfizer Central Research (Groton, CT), and Sanofi Recherche (Montpellier, France), respectively. For systemic administration, drugs were dispersed in a vehicle solution of emulphor:ethanol:saline (1:1:18) and administered through the lateral tail vein in a volume of 1 ml/kg. For intraventricular administration, WIN55,212-2 and WIN55,212-3 (20 μg) were dissolved in a 60% solution of dimethyl sulfoxide, a vehicle previously shown to be inactive in tests of behavioral analgesia (Martin et al. 1993). Intraventricular doses were selected on the basis of previous work (e.g., Martin et al. 1993, 1996), which revealed antinociception in the tail-flick (D’Amour and Smith 1941) test. The systemic doses of WIN55,212-2 were based on previous reports of its efficacy in suppressing noxious pressure-evoked activity in spinal dorsal horn and thalamic neurons (Hohmann et al. 1995; Martin et al. 1996).

**Noxious thermal stimulation**

A Peltier device similar to that described by Wilcox and Giesler (1984) was used to apply thermal stimuli to the receptive fields located on the ipsilateral hindpaw of isolated neurons. The thermode (3 × 3 mm) was used to rapidly heat the center of the receptive field and then cool it to a baseline temperature (30°C). It was positioned in contact with the hindpaw throughout the recording so that the thermal-evoked response would not be confounded with mechanical stimulation. After adaptation to the baseline temperature (2.5 min), stimuli were applied in 20-s pulses at 1.5-min intervals for nonnoxious temperatures (34, 38, and 42°C) and at 2.5-min intervals for noxious temperatures (46, 50, and/or 54°C). A single noxious temperature (50 or 54°C) was used for evaluation of drug effects. After establishing stable baseline responses to the noxious thermal stimulus during five successive stimulation trials, drug or vehicle was administered. Neuronal responsiveness was reassessed at 2.5-min intervals.

**Electrophysiological methods**

Electrophysiological methods were described (Hohmann et al. 1995). Briefly, animals were anesthetized with urethan (1.5 g/kg ip) and prepared for spinal recordings; body temperature was maintained at 37°C. Stainless steel microelectrodes (FHC, Brunswick, ME) were used. Neurons that responded with increasing firing rates to stimuli ranging from mild to noxious (brush, pressure, pinch, and heat) were classified as WDR neurons. In general, these neurons exhibited little spontaneous activity.

Data were quantified by computer acquisition of the time of occurrence of each action potential, the generation of peristimulus time histograms, raster plots, and data reduction for statistical analyses. Stimulation trials consisted of a 1-s prestimulus interval, 20 s of noxious thermal stimulation, and 9 s after stimulus offset. Recording sites were marked by iron deposition (Hohmann et al. 1995) and were localized to both superficial and deep laminae.

**Effects of WIN55,212-2 and CP55,940 on WDR neurons after systemic administration**

WIN55,212-2 (125 μg/kg; n = 7), CP55,940 (125 μg/kg; n = 4), WIN55,212-3 (125 μg/kg; n = 7), or vehicle (n = 6) was administered through the lateral tail vein. SR141716A (1 mg/kg iv; n = 8) was administered to separate rats 10.5 min before the agonist. After establishing stable baseline responses to the noxious thermal stimulus, SR141716A was administered, and the response to the stimulus was examined five times. Then either WIN55,212-2 (n = 5, 125 μg/kg iv) or CP55,940 (n = 3, 125 μg/kg iv) was administered. Response to the stimulus was reexamined over 40 min.

**Effects of CP55,940 on nonnociceptive mechanosensitive cells**

The effect of CP55,940 on activity evoked in nonnociceptive mechanosensitive cells (n = 4) was examined to extend previous work with WIN55,212-2 (Hohmann et al. 1995; Martin et al. 1996). These cells were recorded in the vicinity of nociceptive neurons in the lumbar dorsal horn. Neuronal responses to cutaneous stimuli ranging from nonnoxious to noxious (e.g., brush, pressure, pinch, and heat) were assessed. These cells were characterized as nonnociceptive based on 1) lack of a differential response to noxious over nonnoxious pressure, 2) absence of afterdischarge firing after the termination of noxious pressure (3 kg/cm2), and 3) failure to respond to noxious heat.

Nociceptive pressure was applied to the region of the receptive field eliciting the maximal response of the cell. The mild pressure stimuli were administered to the hindpaw (3 mm2) with a modification of the computer-controlled miniature air cylinder described by Hohmann et al. (1995). The applied pressure (0.75 kg/cm2) failed to elicit limb
withdrawal in lightly anesthetized rats and was not painful to the experimenter (Hohmann et al. 1995). Pressure was applied to the hindpaw for 3 s and then rapidly removed. After establishing stable responses to the stimulus, CP55940 (125 μg/kg iv) was administered, and the response of the cell to the pressure stimulus was determined at 2-min intervals.

Activity was reliably but transiently evoked at the onset of the pressure stimulus in all cells. Mean firing rates during stimulus onset (200–600 ms) were calculated and compared with spontaneous firing rates for a 1-s prestimulation interval. Repeated-measures analysis of variance (ANOVA) was used to assess changes in evoked and spontaneous firing rate across stimulation trials for 10 min pre- and postdrug.

Systemic administration of cannabinoids in spinally transected rats

A thoracic laminectomy was performed (vertebrae T8/T9). The exposed cord was transected and cauterized. Gelfoam was inserted between the severed ends, and the surface was covered with 37°C mineral oil. A caudal laminectomy was subsequently performed at the level T13–L1 to expose the lumbar spinal cord for electrophysiological recordings.

Extracellular recordings were initiated ≥2 h after transection. Electrophysiological methods and thermal stimulation were as described for intact rats. WIN55,212-2 (125 μg/kg, n = 5, or 250 μg/kg, n = 4), WIN55,212-3 (250 μg/kg, n = 4), or vehicle (n = 5) was administered intravenously. The response of the cell to the stimulus was subsequently evaluated at 2.5-min intervals for 40 min.

Intraventricular administration of cannabinoids

Stainless steel guide cannulae (24-gauge thinwall hypodermic tubing; Small Parts, Miami, FL) were implanted in the left or right lateral ventricle according to stereotaxic coordinates (Paxinos and Watson 1986) (=1.0 mm AP, 1.5 mm LAT, and –4.3 mm DV from bregma, the midline suture, and the skull surface, respectively). Cannulae were secured to the skull with dental acrylic and stainless steel screws. Animals were subsequently prepared for spinal recordings. Microinjection needles were constructed from 31-gauge stainless steel hypodermic tubing so that the tips extended 1 mm beyond the cannulae. WIN55,212-2 (20 μg; n = 3), WIN55,212-3 (20 μg; n = 4), or vehicle (n = 5) was administered in a volume of 10 μl over 60 s manually or with an infusion pump. Microinjection needles were left in place throughout the recording interval. The placement of ventricular injections was verified by postmortem administration of Fast Green (10 μl). Only cells recorded in subjects with dye spread throughout the ventricular system were included in the analysis.

Biodistribution of intraventricularly administered cannabinoids

To ascertain whether intraventricularly administered cannabinoids enter the systemic circulation, plasma levels of [3H]WIN55,212-2 (Specific Activity: 49.6 Ci/mmol; Dupont/NEN, Boston, MA), administered intraventricularly, were assessed in urethane-anesthetized rats (n = 3). [3H]WIN55,212-2 (6.95 μCi per rat) or vehicle (n = 3) was administered together with 20 μg unlabeled WIN55,212-2. At the time of peak analgesic effect (6 min postinjection), a laparotomy was performed, and heart blood was extracted and centrifuged. Levels of radioactivity were calculated in 200-μl plasma samples. Background counts were determined from rats receiving the vehicle.

Statistical methods

Data were analyzed by ANOVA, analysis of covariance (ANCOVA), and t-tests with BMDP statistical software (Los Angeles, CA). The Greenhouse-Geisser (1959) correction was applied to the interaction terms of all repeated factors. Posthoc comparisons were performed with the Tukey test; P < 0.05 was considered significant.

RESULTS

Baseline electrophysiological responses

Neurons assigned to different treatment conditions were similar. There were no differences between vehicle and drug groups in preinjection levels of spontaneous or evoked activity (P > 0.05 for each experiment; overall n = 62). Moreover, spontaneous and evoked firing rates did not vary across baseline trials, suggesting that the stimulus parameters employed were appropriate for determination of drug effects. Noxious heat reliably increased firing rates in all groups relative to preinjection levels of spontaneous activity (P < 0.0001 for all experiments).

Effects of systemically administered WIN55,212-2 and CP55,940 in intact rats

Cannabinoid agonist administration decreased noxious heat-evoked activity in lumbar dorsal horns in intact rats. Systemic administration of cannabinoids decreased noxious heat-evoked firing of wide dynamic range (WDR) neurons compared with treatment with vehicle (P < 0.05) and the receptor-inactive enantiomer (P < 0.01). The effects of WIN55,212-2 and CP55,940 did not differ from each other. Treatment with vehicle did not differ from treatment with the enantiomer WIN55,212-3.

Values are means ± SE for the first 5 postinjection trials. Mean preinjection evoked firing rates did not differ before administration of drug or vehicle (22.6 ± 3.8, 26.2 ± 4.2, 24.0 ± 4.9, 20.9 ± 3.2 Hz for vehicle, WIN55,212-2, WIN55,212-3 and CP55,940 treated groups, respectively).

CA): The Greenhouse-Geisser (1959) correction was applied to the interaction terms of all repeated factors. Posthoc comparisons were performed with the Tukey test; P < 0.05 was considered significant.
an effect; both agonists produced a greater effect than WIN55,212-3 (P < 0.01 for both comparisons, Fig. 1), and firing rate after treatment with the enantiomer did not differ from that observed following vehicle. An example neuron shows the suppression of noxious heat-evoked activity by WIN55,212-2 (Fig. 2).

Further evidence for mediation by cannabinoid receptors was suggested by antagonism of this action by SR141716A. The antagonist alone did not reliably alter either spontaneous (mean ± SE, 4.95 ± 4.6 and 7.2 ± 2.6 Hz pre- and postdrug, respectively) or evoked (22.34 ± 3.27 and 19.9 ± 4.69 Hz pre- and postdrug, respectively) firing rates. Noxious heat-evoked firing was compared in antagonist-pretreated animals receiving WIN55,212-2 or CP55,940 and animals receiving either agonist alone with ANCOVA. The mean rate of evoked activity before agonist administration (i.e., after administration of SR141716A) was used as a covariate. Pretreatment with SR141716A blocked suppression induced by both the aminoalkylindole and bicyclic cannabinoid agonists (Figs. 3 and 4). Noxious heat-evoked firing was higher in the antagonist pretreatment groups compared with groups receiving agonist alone (F < 8.74, P < 0.05 and F = 6.14, P < 0.04 for CP55,940 and WIN55,212-2, respectively; Figs. 3 and 4).

**Effects of CP55,940 on nonnociceptive mechanosensitive cells**

CP55,940 did not alter activity evoked by mild pressure in nonnociceptive mechanosensitive cells. ANOVA failed to reveal a reliable effect of cannabinoid treatment on spontaneous or evoked firing rates (Fig. 5). Evoked firing after administration of CP55,940 did not differ from preinjection levels (49.9 ± 20.0 and 56.1 ± 14.0 Hz for pre- and postinjection levels, respectively). Nonnoxious pressure evoked activity in these neurons (F = 12.88, P < 0.04, ANOVA on 10 consecutive trials).

**Effects of intravenous administration of cannabinoids in spinalized rats**

In spinalized rats, WIN55,212-2 failed to suppress noxious heat-evoked activity relative to vehicle (Table 1 and Fig. 6). The cannabinoid failed to alter either spontaneous or noxious thermal-evoked firing rates. After administration of drug or vehicle, noxious heat increased firing rates (F = 57.28, P < 0.0001).

The effects of cannabinoid administration on noxious heat-evoked activity in spinalized rats were compared with that observed in intact rats (Fig. 6). Because pre- and postdrug levels of spontaneous or evoked firing did not differ in spinalized rats treated with either dose of WIN55,212-2 (125 or 250 µg/kg iv), the groups were pooled for comparison with intact rats receiving the low dose. Evoked firing differed in intact and spinal rats after drug administration (ANOVA on means of the first 5 pre- and postdrug trials, F = 5.98, P < 0.03); postinjection levels of evoked firing were higher in the spinal group relative to the intact group (F = 4.76, P < 0.05 by Tukey test), indicating that the suppressive effects of cannabinoids were attenuated in the spinal rats. Spontaneous firing was

![Image](http://jn.physiology.org/)

**FIG. 2.** Example of inhibition of noxious heat-evoked activity in a WDR neuron by the cannabinoid WIN55,212-2. The responses of the neuron to a 50°C stimulus were examined during 16 stimulus trials. A: noxious stimulus, illustrated by the temperature waveform (top middle), was administered at 2.5-min intervals. Trials 1–5 constituted baseline trials; after trial 5 WIN55,212-2 (125 µg/kg iv) was administered. The black peristimulus time histogram represents baseline firing before injection, whereas the shaded peristimulus time histogram represents the firing rate for the first 5 postinjection trials. B: comparison of the mean firing rate during the stimulus for the 5 baseline trials to the firing rate during the stimulus for the first 5 postinjection trials illustrating, approximately, a 75% decrease in responsiveness.

**FIG. 3.** Pretreatment with SR141716A, a competitive antagonist for CB1 cannabinoid receptors, blocks the suppression of noxious heat-evoked activity by (A) the aminoalkylindole cannabinoid WIN55,212-2 (125 µg/kg iv) and (B) the bicyclic cannabinoid CP55,940 (125 µg/kg iv). Mean preinjection evoked firing rates were 24.0 ± 4.9 and 23.5 ± 4.9 Hz for groups receiving WIN55,212-2 alone and SR141716A + WIN55,212-2 and 20.9 ± 3.2 and 20.3 ± 4.0 Hz for groups receiving CP55,940 alone and SR141716A + CP55,940, respectively (*P < 0.05 compared with vehicle or CP55,940 + SR141716A).
higher in the spinal group than in the intact group (F_{1,14} = 7.31, P < 0.02; Fig. 6). These effects did not differ pre- or postdrug. A neuron illustrating the failure of WIN55,212-2 to suppress noxious heat-evoked activity after spinal transection is shown in Fig. 7.

In some cells recorded in spinal animals, a modest suppression of evoked firing rate was observed after cannabinoid administration, although this suppression was not reliable.

**Effects of intraventricular administration of cannabinoids**

Intraventricular administration of WIN55,212-2 suppressed noxious heat-evoked firing in WDR neurons (Fig. 8). Evoked and spontaneous firing did not differ between groups receiving WIN55,212-3 or vehicle pre- or postinjection; these groups were pooled for comparison with the group receiving WIN55,212-2. Intraventricular administration of WIN55,212-2 suppressed noxious heat-evoked activity (ANOVA on 5 postinjection trials, F_{1,10} = 9.29, P < 0.02) but failed to alter spontaneous activity. This suppression was mediated by cannabinoid receptors because evoked firing was lower after treatment with the active enantiomer compared with the inactive enantiomer (3.7 ± 2.7 and 14.9 ± 3.1 Hz for WIN55,212-2 and WIN55,212-3, respectively, t = 3.05, df = 4, P < 0.04). Plasma levels of [3H]WIN55,212-2 (12,162 ± 1,389 dpm/ml whole blood), administered intraventricularly, were <0.08% of that injected intraventricularly.

### TABLE 1. WIN55,212-2 fails to alter noxious heat-evoked activity in lumbar dorsal horn neurons in spinal rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Preinjection</th>
<th>Postinjection</th>
</tr>
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<tbody>
<tr>
<td>Vehicle (n = 5)</td>
<td>46.2 ± 13.2</td>
<td>44.0 ± 13.6</td>
</tr>
<tr>
<td>WIN55,212-2 (125 μg/kg; n = 5)</td>
<td>29.3 ± 6.9</td>
<td>24.2 ± 8.6</td>
</tr>
<tr>
<td>WIN55,212-2 (250 mg/kg; n = 4)</td>
<td>24.3 ± 6.5</td>
<td>26.7 ± 7.1</td>
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Data are expressed as mean evoked firing rate ± SE (Hz). There were no significant differences between treatment conditions prior to or after injection of drug or vehicle.
DISCUSSION

Potent and selective suppression of spinal nociceptive processing by cannabinoid agonists

Systemically administered cannabinoids induced a CB1 receptor-mediated suppression of noxious heat-evoked firing in spinal WDR neurons. This effect occurred at low doses; >60% suppression of heat-evoked firing was observed at the dose of 125 μg/kg of either agonist. The doses of cannabinoids required to suppress nociceptive responses of WDR neurons are an order of magnitude lower than those required to produce similar effects with morphine (1–2 mg/kg ip) (Douglass and Carstens 1997). This finding is consistent with our previous observations with pressure stimuli (Martin et al. 1996). These data together with the cannabinoid-induced suppression of noxious pressure-evoked activity in spinal dorsal horn (Hohmann et al. 1995) and thalamic (Martin et al. 1996) neurons suggest that cannabinoids modulate the activity of nociceptive neurons in the spinothalamic tract. The electrophysiological data are consistent with previous work suggesting that cannabinoids suppress reactivity to multiple modalities of painful stimulation (Bloom et al. 1977; Buxbaum 1972; Moss and Johnson 1980; Richardson et al. 1998b,c; Sofia et al. 1973; Tsou et al. 1996).

The observed suppression of noxious heat-evoked activity is unlikely to be an artifact of a cannabinoid-induced fall in blood pressure (Lake et al. 1997; Varga et al. 1995, 1996). Intracerebroventricular administration of cannabinoids failed to lower...
blood pressure (Vollmer et al. 1974) but did suppress noxious heat-evoked firing in the lumbar dorsal horn in the current work and produced antinociception in a previous study (Martin et al. 1993). Furthermore, cannabinoids suppressed activity in WDR neurons (Hohmann et al. 1995; Martin et al. 1996) evoked by noxious pressure, a stimulus modality that is less sensitive to changes in autonomic function than is thermal stimulation (Thurston and Randich 1995). Our previous work demonstrated dose-dependent suppressions of pressure-evoked firing rates in WDR neurons by a cannabinoid that correlated with the antinociceptive effects of the same doses \( r = 0.99 \) (Martin et al. 1996). These data collectively suggest that cannabinoid modulation of nociceptive processing is independent of the hemodynamic effects of cannabinoids. Nonetheless, because sympathetic outflow is also controlled by descending pathways, further work is necessary to ensure that changes in blood flow/pressure do not contribute to reduced responses to thermal stimulation.

The suppression of noxious heat-evoked activity is mediated by actions at cannabinoid receptors. First, noxious heat-evoked firing is suppressed by cannabinoids from different chemical classes. Therefore any nonreceptor-mediated effects of one compound (e.g., Felder et al. 1992) would be unlikely to occur with the other compound. Second, pretreatment with SR141716A blocked the suppressive effects of both the bicyclic and the aminooxylindolone cannabinoid agonists. Because this antagonist is highly selective for CB1, we may conclude that cannabinoid analgesia is mediated by this receptor subtype. Third, WIN55,212-3, the receptor-inactive enantiomer, failed to suppress noxious heat-evoked activity. The rapid onset and reversibility of the electrophysiological effects are also consistent with receptor-mediated effects. Despite differences in the affinities of the agonists for CB1 and CB2 receptors, the trend toward greater suppression after CP55,940 than WIN55,212-2 (mean postinjection evoked firing rate \( 5.9 \pm 2.5 \) Hz vs. \( 10.4 \pm 3.5 \) Hz for CP55,940 and WIN55,212-2) was not statistically significant perhaps because of a floor effect or the sample size.

Although hyperalgesic effects of SR141716A were noted in behavior studies (Calignano et al. 1998; Richardson et al. 1998a; Strangman et al. 1998), the antagonist alone did not reliably alter evoked or spontaneous firing rates in our neuronal sample. It is possible that such effects would be revealed with different doses of the antagonist, different anesthetics, lower intensities of noxious stimulation, or a different population of nociceptive neurons. It is also possible that a slight agonist effect may be produced by the relatively high dose of SR141716A employed, as observed with naltrexone (Calvillo et al. 1974). This could mask hyperalgesic effects mediated by blocking actions of an endogenous cannabinoid.

The suppressive effects of the cannabinoids were selective for pain-sensitive neurons. CP55,940 failed to alter evoked activity in nonnociceptive mechanosensitive cells, consistent with previous observations of this selectivity with WIN55,212-2 (Hohmann et al. 1995; Martin et al. 1996). The cannabinoid-induced suppression of noxious heat-evoked activity in WDR neurons cannot be interpreted as an anesthetic effect because nociceptive neurons are modulated by cannabinoid, but nonnociceptive mechanosensitive cells are unaffected. Purely nonnociceptive neurons are similarly unaffected by morphine (Einspahr and Piercey 1980; Homma et al. 1983). Another indication of selectivity stems from the observation that noxious heat-evoked activity was suppressed, but spontaneous activity was not reliably altered.

**Role of supraspinal influences in cannabinoid antinociception**

Experiments comparing the effects of cannabinoids in spinally transected and intact rats and experiments employing intraventricular administration suggest that cannabinoid modulation of spinal nociceptive processing is mediated in part by a supraspinal component. Spinal transection attenuated the cannabinoid-induced suppression of noxious heat-evoked activity. The dose of the cannabinoid that produced a 60% suppression of evoked firing rates in intact rats and a two-fold higher dose failed to suppress noxious heat-evoked firing in spinal rats. The failure of WIN55,212-2 to suppress evoked activity in spinal animals is unlikely to reflect restricted access of the drug to the lumbar dorsal horn caused by spinal transection. First, spontaneous and evoked firing rates were stable both before and after cannabinoid administration, suggesting that the spinal rats were not compromised physiologically. Moreover, the main arterial supply for the lower thoracic and lumbosacral cord remains intact (e.g., Greene 1935; Scremin 1995) after the spinal transection.

The electrophysiological data from spinalized rats are consistent with the attenuation of cannabinoid-induced antinociception (Lichtman and Martin 1991a) after spinal transection. The observation of a modest but reliable residual analgesia in the spinal preparation was attributed to direct spinal actions of cannabinoids (Lichtman and Martin 1991a). Our data do not preclude the possibility that higher doses of cannabinoids would suppress noxious heat-evoked firing in spinalized rats. Effects of spinally administered cannabinoids on dorsal horn neurons recorded in intact and spinalized rats require further investigation.

Further evidence for a supraspinal site of action stems from the receptor-mediated suppression of noxious heat-evoked activity in spinal WDR neurons after intraventricular administration of WIN55,212-2. The behaviorally effective dose used here was roughly one-half of the systemic dose. Therefore it could be argued that the drug acted systemically to modulate spinal nociceptive processing. However, several findings argue against this possibility. First, the minute blood level of \( [3\text{H}]\text{WIN55},2212-2 \) after intraventricular administration suggests that the drug is not producing analgesia by leakage into the systemic circulation. Second, in a previous study, 99.9% of recovered \( [3\text{H}]\text{WIN55},2212-2 \), administered intraventricularly, was confined to brain, and only 0.06% was found in thoracic to sacral levels of the spinal cord (Martin et al. 1993). Third, the autoradiographic distribution of intraventricularly administered \( [3\text{H}]\text{WIN55},2212-2 \) in brain is largely confined to periventricular sites (Martin et al. 1995). Analgesia is produced by microinjection of WIN55,212-2 into certain periventricular structures labeled by intraventricular injection (Martin et al. 1995), including the periaqueductual gray (Lichtman et al. 1996; Martin et al. 1995) and the dorsal raphe (Martin et al. 1995). Follow-up studies indicated that cannabinoids also act in the rostral ventral medulla to modulate spinal pain transmission (Martin et al. 1998; Meng et al. 1998). These findings and...
the previous literature collectively demonstrate a supraspinal influence in cannabinoid analgesia.

Finally, the results of these experiments suggest a role for endogenous cannabinoids in nonopioid mechanisms of pain suppression. A cannabinoid analgesic system acts in part by modulating nociceptive transmission within a classical ascending pain pathway, the spinthalamic tract. The modulation of spinal nociceptive neurons shares some neuroanatomic and neurochemical substrates (e.g., Lichtman and Martin 1991b; Martin et al. 1995, 1998) with the descending pain inhibitory systems that mediate opioid analgesia. Identification of the endogenous cannabinoids that modulate pain neurotransmission and the physiological conditions under which this occurs remain important directions for future investigations.

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REFERENCES


