

Cannabinoid WIN 55,212-2 Inhibits the Activity-Dependent Facilitation of Spinal Nociceptive Responses

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Strangman, Nicole M. and J. Michael Walker. Cannabinoid WIN 55,212-2 inhibits the activity-dependent facilitation of spinal nociceptive responses. *J. Neurophysiol.* 81: 472–477, 1999. Cannabinoids suppress nociceptive processing of acute stimuli, but little is known about their effects on processes that lead to hyperexcitability of nociceptive neurons following prolonged noxious stimulation. Wind-up, the increasingly strong response of spinal nociceptive neurons to repetitive noxious electrical stimuli, results from a fast-rising cumulative depolarization and increase in intracellular calcium concentration. These processes produce central sensitization, the increased excitability of spinal nociceptive neurons that contributes to the hyperalgesia and allodynia associated with chronic pain. Intravenous injection of the potent, synthetic cannabinoid agonist WIN 55, 212-2, but not the inactive enantiomer, WIN 55,212-3, dose-dependently decreased the wind-up of spinal wide dynamic range and nociceptive-specific neurons independent of acute responses to activation of low- and high-threshold primary afferents. This is the first direct evidence that cannabinoids inhibit the activity-dependent facilitation of spinal nociceptive responses.

INTRODUCTION

Cannabinoids dampen the pain behavior evoked by a variety of different acute, noxious stimuli (Buxbaum 1972; Martin et al. 1993; Sofia et al. 1973). Consistent with these behavioral findings, cannabinoids inhibit the responses of nociceptive neurons in the spinal dorsal horn and ventral posterolateral nucleus of the thalamus to acute noxious pressure and heat (Hohmann et al. 1995, 1999; Martin et al. 1996). Together, these data provide strong evidence that cannabinoids disrupt the transmission of brief, nociceptive stimuli that lead to acute pain.

Less well characterized are the effects of cannabinoids on reactions to prolonged nociceptive stimulation. Whether cannabinoids modulate responses to prolonged noxious stimuli is a question of great importance because chronic pain involves mechanisms additional to those involved in acute pain. Koserky et al. (1973) found that Δ^9 -tetrahydrocannabinol (Δ^9 -THC) raised the threshold for pressure-induced vocalization in rats whose hindpaws had been inflamed by yeast or carrageenin. Moss and Johnson (1980) and Tsou et al. (1996) reported that behavioral responses to a noxious chemical stimulus in the formalin test were significantly reduced by Δ^9 -THC and WIN 55,212-2, respectively. In the latter study, the cannabinoid markedly attenuated formalin-induced *c-fos* expression in the

spinal cord as well. More recently, Herzberg et al. (1997) reported that intraperitoneal injection of WIN 55, 212-2 decreased thermal hyperalgesia and mechanical allodynia in rats with chronic constriction injury of the sciatic nerve. These findings suggest that cannabinoids suppress responses to prolonged noxious stimulation as effectively as they do responses to acute noxious stimulation. However, the mechanisms by which cannabinoids produce these effects are unknown.

Cutaneous tissue injury leads to a constellation of changes in spinal excitability, which includes elevated spontaneous firing, increased response amplitude and duration, decreased threshold, enhanced afterdischarge to repeated stimuli, and expanded receptive fields (Woolf 1983). The persistence of these changes, which are collectively termed central sensitization, appears to be fundamental to the prolonged enhancement of pain sensitivity definitive of chronic pain. Physiological changes similar to those associated with central sensitization are produced in spinal nociceptive neurons by repetitive, low-frequency, noxious electrical stimulation of C-fibers (Cook et al. 1987; Ren et al. 1992; Wall and Woolf 1986; Woolf and Wall 1986). This type of stimulation also produces wind-up, a centrally mediated increase in both the frequency and duration of spinal nociceptive responses (Mendell 1966). Wind-up and central sensitization exhibit similar pharmacological susceptibilities (Woolf and Thompson 1991; Xu et al. 1992), a reflection of their common reliance on synaptic processes that elevate levels of intracellular calcium (Sivilotti et al. 1993; Thompson et al. 1990; Woolf et al. 1988). As discussed by Woolf (1996), it is not clear whether wind-up is an essential process in the etiology of the hyperalgesia and allodynia associated with chronic pain syndromes. However, the synaptic processes that produce wind-up are sufficient to produce central sensitization, which appears to be an important component of hyperalgesia and allodynia. In this study, we examined the effect of the potent, synthetic cannabinoid agonist WIN 55,212-2 on wind-up of spinal dorsal horn neurons.

METHODS

All protocols were reviewed and approved by the Brown University Institutional Animal Care and Use Committee.

Male Sprague-Dawley rats (Charles River, Boston, MA) were anesthetized by urethan (25%) injection (1.5 g/kg ip) and mounted in a stereotaxic frame. A laminectomy was performed at the T₁₂–L₁ vertebrae, and the spinal cord was immobilized with spinal clamps and immersed in warm mineral oil. Extracellular, single-unit responses were recorded from 22 wide dynamic range and 8 nociceptive-specific neurons in the lumbar spinal dorsal horn using 5-M Ω

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stainless steel electrodes. Units were characterized based on the pattern of their responses to natural stimulation and the latency of their responses to transcutaneous, C-fiber intensity electrical stimulation applied to the receptive field.

The procedure of Chapman et al. (1994) was used to elicit wind-up. After isolation and characterization of a cell, 16 transcutaneous, electrical stimuli (2 ms in duration, 2 s apart) at 3 times the threshold of the C-fiber response (~ 3 mA) were applied to the receptive field. After a baseline trial, WIN 55,212-2 [0.125, 0.25 or 0.5 mg/kg; dosages selected on the basis of work by Hohmann et al. (1995) and preliminary data], the inactive enantiomer WIN 55,212-3 (0.5 mg/kg), or vehicle (5:5:90, ethanol/emulphor/0.9% saline) was injected intravenously, and subsequent trials were performed at 5-min intervals. Seven cells were lost after 25–75 min of recording. All others were tested for a total of 20 trials.

Unit responses to transcutaneous electrical stimulation were subdivided into A β - (0–20 ms), A δ - (20–90 ms), and C- (90–800 ms) fiber-mediated components. The acute response to high- or low-threshold primary afferent activation (the input) was calculated by tallying the total number of A β -, A δ -, or C-fiber latency action potentials that occurred in response to the first stimulus of a trial. Wind-up was calculated as

$$W = \sum t_1 \dots t_{16} - 16(t_1)$$

where W is the wind-up score and t is the number of C-fiber latency action potentials that occurred in response to 1 of 16 stimuli within a trial.

At the end of each experiment, the recording site was marked by iron deposition (2 μ A for 20–30 s) and perfusion with Prussian Blue solution. Spinal cord sections were stained with Neutral Red, and recording sites were determined microscopically using the stereotaxic atlas of Paxinos and Watson (1986).

Scores were collapsed across postinjection trials 1–5, determined in previous experiments by Hohmann et al. (1995) to encompass the period of maximal effect of WIN 55,212-2. Drug effects on acute responses and wind-up were statistically compared using the Student's t -test and the Bonferroni adjustment for multiple planned comparisons. $P < 0.05$ was considered statistically significant. Multiple linear regression using the initial A β -, A δ -, and C-fiber latency responses as predictor variables was used to examine the relationship between the acute response and wind-up based on data from the control and 0.5 mg/kg WIN 55,212-2 groups.

RESULTS

The composition of each group was as follows: the WIN 55,212-3 group was composed of two wide dynamic range neurons and two nociceptive-specific neurons; the WIN 55,212-2 0.125 mg/kg group was composed of four wide dynamic range and three nociceptive-specific neurons; the vehicle and WIN 55,212-2 0.25 mg/kg groups each contained six wide dynamic range neurons and one nociceptive-specific neuron; and the WIN 55,212-2 0.5 mg/kg group was composed of three wide dynamic range neurons and one nociceptive-specific neuron. One wide dynamic range neuron that showed erratic responses to stimulation was excluded as an outlier. Recording sites could be microscopically determined for 26 of 29 cells. In 25 cases the recording site was localized to the deep laminae III–V of the lumbar spinal dorsal horn. The two remaining recording sites were located in the most superficial laminae, I and II.

Wind-up stimulation produced a gradual increase in the frequency and duration of the C-fiber discharge and afterdischarge of spinal nociceptive neurons, an effect that was highly

repeatable over time (Fig. 1). Treatments with vehicle or the cannabinoid inactive enantiomer WIN 55,212-3 were virtually identical in their failure to produce a significant change in wind-up, the C-fiber response, or the acute A β response; therefore these two groups were pooled into a single control group for all subsequent analyses.

Before drug injection, the control group did not differ from the WIN 55,212-2 groups in measures of wind-up or acute responses to A β - or C-fiber activation. WIN 55,212-2 0.25 mg/kg and WIN 55,212-2 0.5 mg/kg significantly inhibited wind-up as compared with the control group (Figs. 1–3, $F(1, 16) = 2.92$, $F(1, 13) = 3.78$), whereas treatment with WIN 55,212-2 0.125 mg/kg failed to produce any effects on wind-up (Fig. 2). Treatment with WIN 55,212-2 at the dose of 0.5 mg/kg but not 0.125 mg/kg or 0.25 mg/kg significantly decreased the acute C-fiber response as compared with control [Fig. 2, $F(1, 13) = 3.03$]. However, all doses of WIN 55,212-2 failed to alter the acute response to A β -fiber and A δ -fiber activation.

As noted above, WIN 55,212-2 suppressed acute C-fiber responses in dorsal horn neurons when administered at the dose of 0.5 mg/kg. A more detailed examination of the data revealed that even at this dose, it is possible to dissociate the effects on the acute C-fiber response from those on wind-up. Thus wind-up score and the initial C-fiber latency response (but not the initial A β or A δ latency response) were correlated (Fig. 3, $r = 0.71$, $P < 0.0005$), but they were frequently dissociated. For example, even with an acute response of 0 action potentials, a neuron recorded in an animal that received vehicle showed a wind-up response of 180, ranging 3–180 times higher than that of neurons recorded in animals that received WIN 55,212-2 (0.5 mg/kg) and also showed zero acute response to the stimulation. At identical levels of acute response, wind-up of control cells was much greater than that of WIN 55,212-2 cells. In five cases WIN 55,212-2 (0.5 mg/kg) cells did not show any wind-up, and in three additional cases the wind-up score did not exceed four action potentials. However, in all 9 cases where the acute C-fiber response of vehicle and WIN 55,212-3 cells was equal to or less than that of WIN 55,212-2 (0.5 mg/kg) cells, these control cells showed wind-up of 36 action potentials or greater. The mean wind-up score for neurons that responded with two action potentials to the first stimulus was 40.3 ± 23.9 (mean \pm SE) for WIN 55,212-2 (0.5 mg/kg) cells ($n = 4$) and 123.3 ± 43.7 for vehicle cells ($n = 3$). Taken together, these observations indicate that the suppression of the acute C-fiber response by the highest dose of the cannabinoid agonist can be dissociated from its effect on wind-up.

DISCUSSION

The cannabinoid agonist WIN 55,212-2 inhibited the wind-up of spinal wide dynamic range and nociceptive-specific neurons in response to repeated noxious stimulation. The dose dependency of this effect and the lack of efficacy of the inactive enantiomer WIN 55,212-3 indicate that the suppression was mediated by cannabinoid receptors. The inhibition of wind-up was independent of any reduction in the acute response to C-fiber activation. At the dose of 0.25 mg/kg, WIN 55,212-2 suppressed wind-up without altering the acute C-fiber response. Although the highest dose of WIN 55,212-2 (0.5

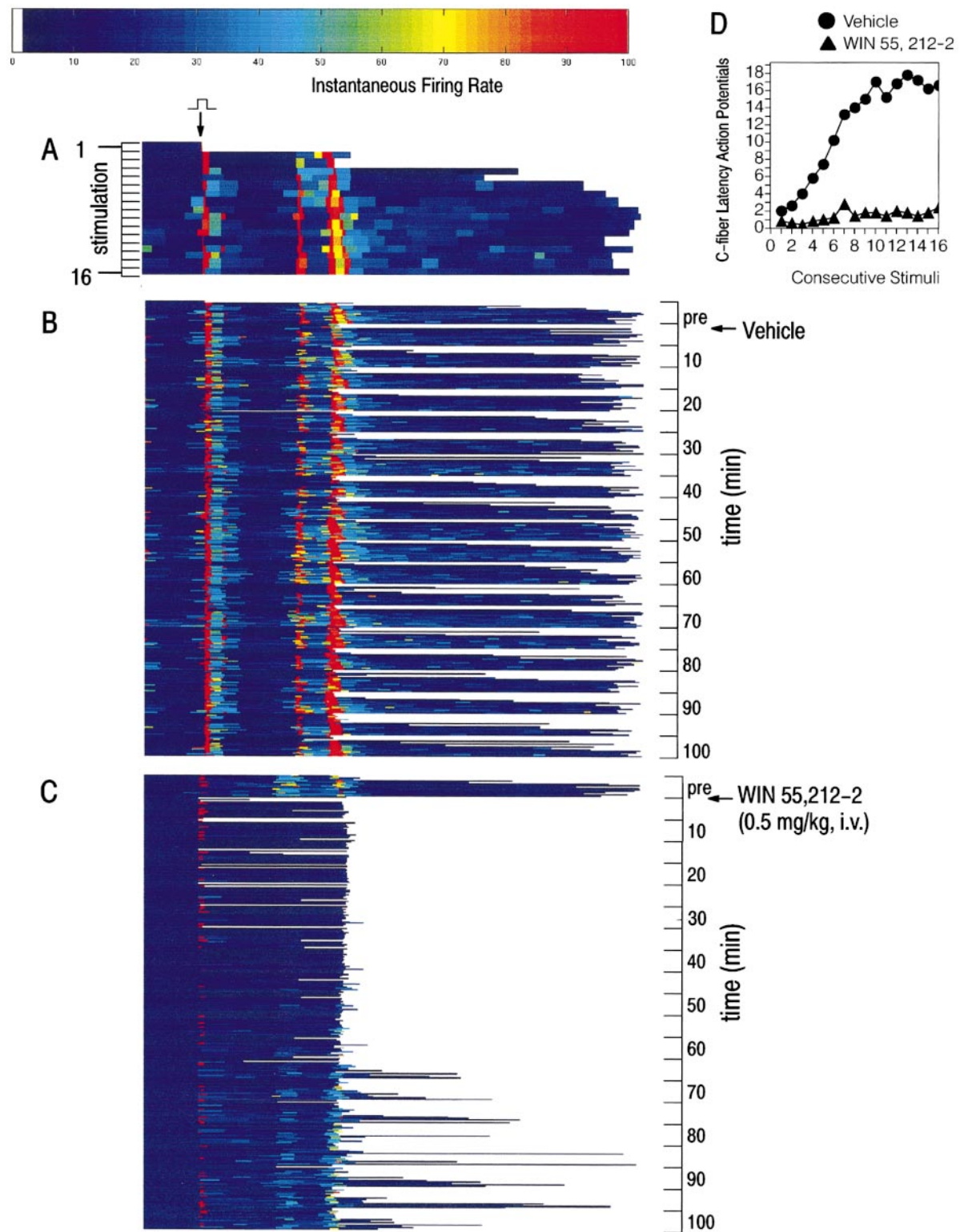


FIG. 1. Effects of vehicle and WIN 55,212-2 on the wind-up of 2 spinal wide dynamic range neurons. Colors represent the instantaneous firing rate of the cell calculated as the reciprocal of the interspike interval and colorized (backward) to the preceding spike. Each row represents the response to a single transcutaneous stimulus over an 800-ms period following stimulus onset (arrow). Sixteen stimuli (3 mA, 2 ms, 0.5 Hz) were presented per trial. Horizontal calibration bar, 100 ms. *A*: single wind-up trial under baseline conditions showing the increasing response of a neuron to successive C-fiber strength electrical stimuli. *B*: lack of effect of vehicle (injected intravenously after the 1st trial) on wind-up over a 1.5-h period. Trials were performed at 5-min intervals. *C*: WIN 55,212-2 (0.5 mg/kg), injected intravenously after the 1st trial, suppressed wind-up. The cell began to recover 65 min after drug injection. *D*: mean number of C-fiber latency action potentials per stimulus over the 1st 5 postinjection trials for the vehicle and WIN 55,212-2 (0.5 mg/kg) cell. The cell from a vehicle-treated rat showed an increasing number of C-fiber latency action potentials across the 1st 10 stimuli, whereupon the response plateaued at an exaggerated level of response as compared with the 1st stimulus. The wind-up of a cell from a WIN 55,212-2-treated rat was suppressed.

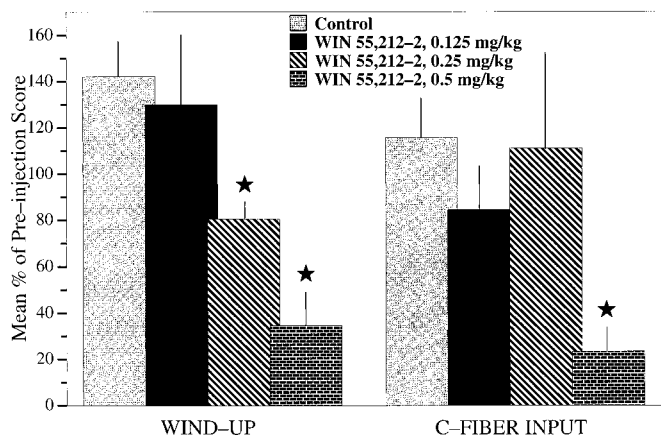


FIG. 2. Differences in wind-up and the acute C-fiber response between WIN 55,212-2 and control groups during the 1st 5 postinjection trials (*, $P < 0.05$). There were no significant differences between the groups' wind-up or acute C-fiber responses during the preinjection period. However, intravenous WIN 55,212-2, at the dose of 0.25 mg/kg ($n = 7$) and 0.5 mg/kg ($n = 4$) but not 0.125 mg/kg ($n = 7$), significantly decreased the mean wind-up score as compared with control. Only the highest dose of WIN 55,212-2, 0.5 mg/kg, significantly reduced the acute response to C-fiber activation as compared with controls.

mg/kg) suppressed both wind-up and the acute response to C-fiber activation, close examination of the data revealed a dissociation between these two measures. These findings are consistent with an action of the cannabinoid on synaptic processes that produce increased spinal excitability upon repeated C-fiber activation. This study thus provides evidence that cannabinoids suppress the facilitation of spinal responses following repeated noxious stimulation independent of acute responses to low- and high-threshold primary afferents.

The effect of WIN 55,212-2 is distinct from that of morphine, which inhibits wind-up only at doses that also suppress the acute response to C-fiber activation (Dickenson and Sullivan 1986), suggesting that opiates may act relatively selectively to suppress acute nociceptive transmission but making it difficult to judge, based on grouped data, whether there is an independent effect on the synaptic processes that lead to wind-up. We suggest that a cell-by-cell comparison of the acute C-fiber response and wind-up may be useful in cases such as these for teasing out a drug's relative effects on the acute responses and wind-up. The ability of WIN 55,212-2 to suppress the repetitive stimulus-induced facilitation of spinal excitability, independent of acute responses, may account in part for the higher potency of cannabinoids in behavioral models of chronic pain (Herzberg et al. 1997).

The failure of WIN 55,212-2 to suppress the responses of spinal wide dynamic range neurons to A β -fiber activation is consistent with the drug's lack of effect on responses of non-nociceptive neurons in the spinal cord and thalamus to non-noxious stimulation (Hohmann et al. 1995; Martin et al. 1996). This observation provides a further indication that cannabinoids produce analgesia rather than anesthesia. The lack of effect of WIN 55,212-2 on acute responses to A δ -fiber activation may seem surprising in light of the profound suppression by this drug of the responses of nociceptive neurons to acute noxious heat and pressure (Hohmann et al. 1995, 1999; Martin et al. 1996). However, this failure to detect an effect of WIN 55,212-2 on acute responses to A δ -fiber activation is likely due

to a floor effect pursuant to the low frequency of the A δ response.

Spinal transection profoundly attenuates the suppression of noxious-heat evoked activity in spinal wide dynamic range neurons by systemically administered WIN 55,212-2 (Hohmann et al. 1996, 1999). Suppression of spinal nociceptive responses can also be produced by intraventricular administration of WIN 55,212-2 (Hohmann and Walker 1999). Together with the behavioral findings that cannabinoids microinjected into the periaqueductal gray and dorsal raphe inhibit the tail-flick reflex (Martin et al. 1995), these data provide strong evidence that cannabinoids act supraspinally to modulate nociceptive processing. However, Hohmann identified a subpopulation of neurons in spinal rats that exhibit moderate suppression following intravenous administration of WIN 55,212-2, and Lichtman and Martin (1991) reported modest inhibition by a cannabinoid of thermal pain sensitivity in spinal animals. Furthermore, topical administration of cannabinoids onto the spinal cord inhibits spinal nociceptive responses (Hohmann et al. 1998). Thus the actions of cannabinoids on a subpopulation of spinal nociceptive neurons may also contribute to the dampening of nociceptive transmission and pain. Recently, cannabinoid (CB1) receptor mRNA was detected in substance P containing neurons in the dorsal root ganglia (Hohmann and Herkenham 1998, 1999). Consistent with the localization of CB1 receptors to the cell bodies of primary

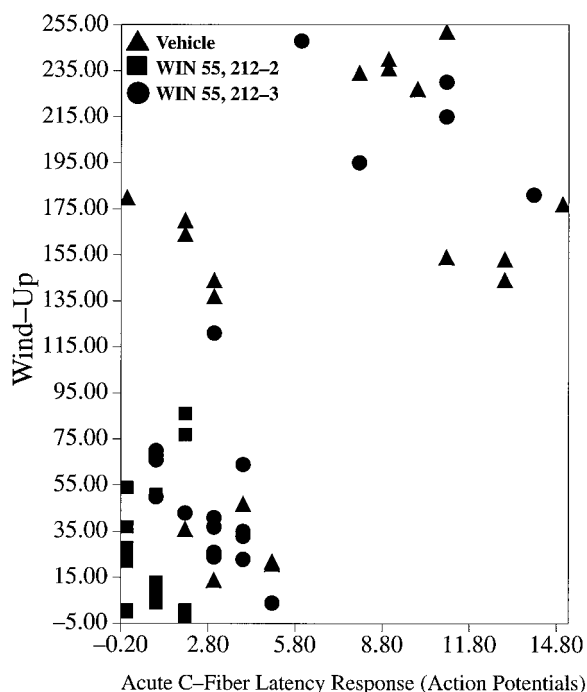


FIG. 3. Scatter plot showing group distributions with respect to initial C-fiber latency responses and wind-up. Linear regression indicated a significant overall relationship between the initial C-fiber latency response and wind-up ($R = 0.71$, $P < 0.0005$). However, frequent dissociations are apparent. In 5 cases WIN 55,212-2 cells did not show any wind-up, and in 3 additional cases the wind-up score did not exceed 4 action potentials. However, all vehicle or WIN 55,212-3 neurons with acute responses identical to WIN 55,212-2 cells showed wind-up. At identical levels of acute response, wind-up of control cells was much greater than that of WIN 55,212-2 cells. For example, the wind-up score for a vehicle cell with zero acute response was 180, 3–180 times greater than scores of WIN 55,212-2 cells with no acute response.

afferent neurons, a dose of anandamide that lacked analgesic efficacy when administered systemically, inhibited the development of carrageenan-induced thermal hyperalgesia when administered peripherally (Richardson et al. 1998). These data raise the possibility that cannabinoids might modulate nociception by decreasing neurotransmitter release from primary afferents. Because WIN 55,212-2 was delivered by intravenous route in the present study, we cannot ascertain whether the drug's site of action was spinal, supraspinal, peripheral, or some combination of the three. Further work on this question would undoubtedly provide important insights into the mechanisms of cannabinoid analgesia.

The role of wind-up in the development of central sensitization and the consequent hyperalgesia and allodynia in chronic pain is uncertain. Wind-up relies on the production of a fast-rising cumulative depolarization that increases the level of intracellular calcium (Sivilotti et al. 1993; Thompson et al. 1990, 1993; Urban and Randic 1984; Woolf et al. 1988). Likewise, experimental manipulations that increase intracellular calcium produce central sensitization (Woolf and Wiesenfeld-Hallin 1986). However, central sensitization may occur in the absence of action potential production in spinal nociceptive neurons (Liu and Sandkühler 1997; Magerl et al. 1998). For example, depolarizations subthreshold for wind-up can produce heterosynaptic facilitation, a phenomenon analogous to central sensitization (Thompson et al. 1993). Therefore, as discussed by Woolf (1996), it is not clear that wind-up is necessary for central sensitization, but it is clear that wind-up stimulation activates synaptic processes that are sufficient to produce central sensitization (Cook et al. 1987; Ren et al. 1992; Wall and Woolf 1986; Woolf and Wall 1986).

In light of the above, it is notable that the suppression of wind-up by WIN 55,212-2 is likely due to inhibition of calcium entry into spinal neurons. Cannabinoids interfere with several components of the calcium signal cascade including the influx of calcium through N- and Q-type Ca^{2+} channels (Felder et al. 1993, 1995; Mackie and Hille 1992), the release of calcium from intracellular stores (Filipeanu et al. 1997), and the activation of protein kinase C (De Petrocellis et al. 1995; Hillard and Auchampach 1994). The central cannabinoid receptor is also coupled to the G_i protein, and inhibition of adenylate cyclase might also underlie this effect (Howlett 1985; Howlett et al. 1987).

If the suppression of wind-up by cannabinoids is due to inhibition of calcium entry, then one would expect cannabinoids to be effective inhibitors of central sensitization produced by a variety of mechanisms. In this regard, it is relevant to note that cannabinoids eliminated hyperalgesia and allodynia in the Bennett model of neuropathic pain at doses that produced no observable side effects (Herzberg et al. 1997). In fact, the potency of WIN 55,212-2 in this study was markedly higher than in models of acute pain. Likewise, cannabinoids inhibit hyperalgesia due to inflammation (Kosersky et al. 1973; Moss and Johnson 1980; Richardson et al. 1998; Sofia et al. 1973; Tsou et al. 1996). The observations that cannabinoids inhibit calcium entry, wind-up, and the allodynia and hyperalgesia following nerve injury or inflammation, suggest the possibility that cannabinoids may act as general inhibitors of central sensitization by inhibiting calcium entry. Studies aimed at determining the effects of cannabinoids on intracellular

calcium following inflammation and nerve injury are needed to examine this possibility.

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